The role of thrombin and antithrombin-therapy in interventional cardiology

De rol van thrombine en antithrombine-therapie in interventie cardiologie

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Cover
Angiographic observation of a post-stenotic intracoronary thrombus.

Thrombin (blue) and argatroban (red) separately. The pronounced residues in the thrombin molecule represent the active binding site.

Argatroban-thrombin complex. The small argatroban molecule exactly fits the active site and blocks the action of thrombin. (By Takao Matsuzaki, Mitsubishi Chemical Corporation, Tokyo, Japan).

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door
Johannes Paulus Remigius Herrman
geboren te Beuningen

For
my mother Bep Herrman †

For her undying love and faith in me.

For
Pleun and Hilde

The child is father to the man.
(William Wordsworth, 1807)

Contents

Introduction

Thrombin and thrombus. The plasma coagulation system is activated in response to vascular injury and, within several minutes, thrombin is generated by a series of linked proteolytic reactions that take place on cell surfaces. These reactions are balanced by naturally occurring inhibitory and procoagulant molecules. Thrombin has three major actions which are it's powerfull stimulus for platelet aggregation and activation; fibrinogen conversion to insoluble fibrin strands in the final common pathway for strengthening of the platelet plug; and it can activate factor XIII which leads to crosslinking and stabilisation of the fibrin clot. In addition, thrombin regulates many postthrombotic cellular events like chemoattraction of inflammatory cells and growth regulation of endothelial and vascular cells. Thus, thrombin is involved in both acute coronary occlusion and late restenosis following coronary angioplasty. Although the haemostatic system has evolved to minimise blood loss from injured vessels, there is little actual difference between the physiological process of normal haemostasis and the pathological events leading to coronary thrombosis and related events. Because of this similarity, thrombosis has been described as haemostasis occurring in the wrong place at the wrong time. The triggering event is the interaction of normal blood components with the abnormal vessel wall surface as in a diseased and atherosclerotic vessel or after successful coronary angioplasty.

Antithrombotic therapy Antithrombotic therapy has changed little during the past few decades. Despite their limitations aspirin, heparin and warfarin have been the mainstays in the treatment of the majority of cardiovascular and cerebrovascular thrombotic diseases.

The effects of acetyl salicylic acid (an acetylated substrate from the salix alba) were first described in the 'Corpus Hypocraticum'. In this document the drug was indicated to relief fever, pain during labour and it was indicated for use in reumatoid inflammatory processes. Felix Hoffman developed the chemical method to refine the active substance and ultimately he patented aspirin in 1899. The antithrombotic properties of acetyl salicylic acid were discovered by K. Breddin. This expanded the indications for its use. The drug has been marketed for almost one century already! It has the ability to inhibit platelet activation by irreversibly blocking the conversion of arachidonic acid to PGG2 as a precursor for thromboxane A2 and B2. However, in the endothelial cell it also results in blockade of prostaglandin PGI2 generation. Thromboxane A2 is a powerful platelet aggregator and vasoconstrictor, but since the biologic action of TxA2 and PGI2 are opposing, acetyl salicylic acid is an antagonist of itself. Although acetyl salicylic acid can be easily administered, it cannot be titrated to any marker of therapeutic efficacy.

The antithrombotic capacities of heparins (discovered by McLean and colleagues in 1916) are achieved by the reversible binding with the natural circulating anticoagulant antithrombin III. The binding is responsible for a 1000-fold acceleration of antithrombin III – heparin binding to factor IIa. The effectivity of heparins is inhibited by fibrin II monomers, thrombospondin and platelet factor 4 which is present in the platelet rich thrombus. The inaccessability of the large

heparin-antithrombin III complex to clot bound thrombin or factor Xa in the prothrombinase complex is an additional major limitation. Heparin is a mixture of heterogenous sized depolymerized heparin molecules and is therefore, subject to lot to lot variety. The mechanism of action is moderated by antithrombin III, and heparin is therefore ineffective in patients having insufficient antithrombin III-levels.
Dicumarols' anticoagulant effect was discovered by accident, by Link in 1930. Its action does not assail the extrinsic pathway, but on the other hand does hinder several commonly used drugs. Furthermore, there is a delay between administration and therapeutic effect.
Another drawback to conventional antithrombotic therapy is the available tests we use to assess their therapeutic efficacy. Tests are performed in a static sample of whole blood which may not be relevant to an actual site of arterial injury past which blood is constantly flowing and to which activated platelets are adhering.

The limitations of conventional antithrombotic therapy in clinical cardiology comprise thrombolysis, percutaneous transluminal coronary angioplasty and surgery which may be marked by resistant thrombotic complications. After thrombolysis, early reperfusion of the infarct-related artery is not achieved in 15 to 20% of treatedpatients [1, 2] and a similar proportion of patients achieve only incomplete perfusion [1-3]. After successful reperfusion, reocclusion of the infarct related artery or its clinical correlate, reinfarction, or both, occur in 5 to 10% of the patients at hospital discharge [2,4], and in up to 30% of the patients at 3 months [5]. In unstable angina, progression to myocardial infarction still occurs in 5 to 7% of the patients [6, 7]. In coronary angioplasty, abrupt closure or myocardial infarction complicates the procedure in 5 to 10% of the patients. An additional 5 to 10% of the patients require urgent repeat revascularization [8-12]. In all of these syndromes, these thrombotic events are associated with increased mortality and thus are important targets for more effective antithrombotic therapy.

Antithrombin therapy Newer antithrombins that overcome the limitations of conventional antithrombotic drugs and reduce the complication rate in clinical care are being studied for use in acute coronary syndromes and interventions. These agents have the potential to be more effective, more specific and safer inhibitors of the various steps of the coagulation cascade. In this thesis we review the role of thrombin, thrombusformation and antithrombins – argatroban and desirudin in particular – in interventional cardiology.
Argatroban, an arginine derivative, is a potent, synthetic, small molecule (527 dalton) direct competitive thrombin inhibitor which is specific for the catalytic site of thrombin. The molecule is designed in 1971 to match this binding site precisely. It is in clinical use in Japan (licensed as NOVASTAN®) and is in worldwide, advanced clinical development as an antithrombotic agent for the treatment of heparin induced thrombocytopenia and heparin induced thrombocytopenia thrombosis syndrome, as well as for adjunctive treatment to thrombolytic therapy in acute myocardial infarction, in unstable angina pectoris and use as an anticoagulant in coronary interventional procedures (chapter 7 of this thesis).

Hirudin is a protein originally isolated from the medicinal leech (Hirudo Medicinalis). The anticoagulant property of leech saliva was discovered in 1884 by John B. Haycraft. It was needed by the leech to prevent the victim's blood from clotting. Initially, the compound was extracted from homogenized leech heads, and in 1913 it was processed for anticoagulant purposes during haemodialysis [13] and used first in humans in the 1920s [14]. A serious drawback was the limited availability of leeches, since breeding trials failed and the medicinal leech was placed on the list of endangered species. Interest in hirudin and its related compounds (hirulog and hirugen) has increased over the past decade as recombinant DNA technology has now become available to manufacture these drugs in pharmacological quantities.
In chapters 9 and 10 of this thesis we describe the results of our studies with desulphato-hirudin or desirudin (marketed as REVASC™).
In this thesis I hope to have clarified the current position and role of thrombin and antithrombin-therapy in interventional cardiology.

Jean-Paul R Herrman, Rotterdam
November 30, 1996

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Chapter 1

Pharmacological approaches to the prevention of restenosis following angioplasty

The search for The Holy Grail ?

Contents

Jean-Paul R. Herrman, Walter R.M. Hermans, Jeroen Vos, Patrick W. Serruys

Drugs 1993;46(1):18-52 (Part I) and (2):249-262 (Part II)

Summary Luminal renarrowing after balloon angioplasty still hampers the long term vessel patency in a substantial percentage of patients. Morphologically, the restenotic lesion comprises hyperplasia of intimal tissue, which is mainly characterised by proliferation of smooth muscle cells of the synthetic type with abundant extracellular matrix production, chiefly composed of proteoglycans. Unravelling the underlying pathophysiological process enables more specific intervention in basic interactions and cell responses. Critical events in the development of restenotic tissue are platelet aggregation and thrombus formation, while the release of several mediators promotes proliferation and migration of various cell types. All of these steps give access for a diversity of pharmacological interventions. With this in mind, antithrombotic, antiplatelet, antiproliferative, anti-inflammatory, calcium channel blocking and lipid-lowering drugs have been investigated in the prevention of restenosis. Other newer approaches likely to receive more attention in the future include antibodies to growth factors, gene transfer therapy and antisense oligonucleotides. Whether there is a feasible monotherapy, whether we have to focus on a drug combination or whether we are only searching for 'the holy grail' remain to be answered. (Drugs 1993;46(1):18-52 (Part I) and (2):249-262 (Part II).)

Percutaneous transluminal coronary angioplasty (PTCA) is an accepted treatment for providing relief of angina pectoris in patients with single- and multivessel disease. Increased experience and advances in technology have resulted in a higher primary success rate (90% to 95%) and a lower complication rate (4% to 5%). Despite the therapeutic success of coronary angioplasty, the exact mechanisms of dilatation remain speculative and involve multiple processes, including endothelial denudation with rapid accumulation of platelet and fibrin; cracking, splitting or disruption of the intima and atherosclerotic plaque; dehiscence of the intima and plaque from the underlying media; and stretching or tearing of the media, with persistent aneurysmal dilatation of the media and adventitia (McBride et al. 1988; Waller 1989).

The major limitation of PTCA is the high incidence of restenosis which limits the long term benefit of the procedure. Restenosis is the angiographic renarrowing at the site of PTCA, frequently accompanied by recurrence of symptoms of angina. The incidence of restenosis varies between 17% and 40%, the wide variation being a consequence of whether there has been complete angiographic follow up, as well as the way in which restenosis is defined (Holmes et al. 1984; Kaltenbach et al. 1985; Nobuyoshi et al. 1988; Serruys et al. 1988). Over the past ten years we have been unable to influence significantly the rate of this late complication. Although many of the risk factors for restenosis have been identified (Table I), most of these are difficult to influence and we are unable to

Table I
Variables associated with higher restenosis rates in patients with follow-up angiography.

Author	Year	Patients	Clinical	Hemodynamic or Procedure-related	Lesion-related
Holmes et al.	1984	557	Male sex Can. class III- IV No previous MI Diabetes Mellitus	Pre-PTCA TSG ≥ 40 mmHg Post-PTCA TSG ≥ 20 mmHg Angina onset < 2 months	Pre-PTCA DS > 70% PTCA on CABG
Kaltenbach et al.	1985	333	Medication ?		Repeat PTCA PTCA in graft
Scholl et al.	1981	45	Variant angina		Eccentric or calcified lesion
Dangoisse et al.	1982	31	Variant angina > 9 inflations		
David et al.	1984	191	Variant angina		Pre-PTCA DS > 90% Post-PTCA DS > 50% Concentric stenosis Diffuse disease Absence of dissection
Dorros et al.	1984	46			Prox graft
Marantz et al.	1984	73		Post-PTCA TSG > 18 mmHg > 7 atmospheres	Irregular lesion pre-PTCA Large change DS
Margolis et al.	1984	216	Diabetes Mellitus (insulin)		
Schmitz et al.	1984	86		Larger balloon size less restenosis	
Levine et al.	1985	100		Inflation pressure < 8 ATM	Post-PTCA DS > 30% Relative change less than 55%
Mata et al.	1985	60		Balloon artery ratio ≤ 0.9	LAD or LCX >RCA Calcified stenosis Post-PTCA DS > 40%
Probst et al.	1985	94		Collaterals pre-PTCA Occlusion pressure > 45 mmHg	
Serruys et al.	1985	28*			PTCA for total occlusion
Bertrand et al.	1986	229	'Dynamic' coronary stenosis (Spontaneous or provoked spasm)		
Clark et al.	1986	124*		More inflations Higher Inflation Pressure	
Hollman et al.	1986	536	Diabetes Mellitus		Multivessel PTCA Pre-PTCA DS ≥ 90% Post-PTCA DS > 40% Absence of intimal tear
Leimgruber et al.	1986	998	Old age Unstable angina Angina onset <2 mths	Post-PTCA TSG 15 mmHg Absence of dissection	LAD> RCA > LCX Post-PTCA DS > 30%
Powelson et al.	1986	50			Presence of intimal disruption [1]
Roubin et al.	1986	411**			Multi-lesion PTCA in one vessel
Uebis et al.	1986	100			Length of pre-PTCA stenosis >2mm
Hirshfeld Jr. et al.	1987	209	Short duration heparin therapy		LAD > LCX or RCA Minimal luminal diameter < 0.64 mm Normal diameter < 3.0 mm
Guiteras et al.	1987	100	Hypertension		Residual stenosis Eccentricity of lesion

Table I
continued

Author	Year	Patients	Clinical	Hemodynamic or Procedure-related	Lesion-related
Myler et al.	1987	164**	Diabetes Mellitus Hypercholesterolemia New onset angina Current smoking	Inflation pressure > 10 atm	Pre-PTCA DS > 95%
Rapold et al.	1987	178	Variant angina		Post-PTCA > 45% Multivessel Disease
Simonton et al.	1987	123	Diabetes Mellitus		Slow distal flow Difficulty crossing lesion History prior MI
Urban et al.	1987	91	C.W.P ≥ 30 mmHg		
Vandormael et al.	1987	129**	Male Gender Proximal LAD disease Diabetes Mellitus		Increased length stenosis (> = 10mm)
De Feyter et al.	1988	158***	Presence of collaterals Multivessel disease LAD disease Transient ST-depression		
Galan et al.	1988	160	Continuing Smoker		
Lambert et al.	1988	119**	Diabetes Mellitus		High grade stenosis Pre-PTCA Large residual stenosis
Weinstein et al.	1988	54			Dilatation of both bifurcation lesions
Bertrand et al.	1989	437			Presence of ergonovine induced spasm before and after PTCA
Ellis et al.	1989	308			Stenosis at bend point of coronary artery
Uebis et al.	1989	272		> = 3 inflations	
Renkin et al.	1990	111	Recurrence of angina		Post-PTCA luminal diameter
Rupprecht et al.	1990	473	Unstable Angina	Prolonged single inflation	Large residual stenosis post-PTCA High grade stenosis pre-PTCA

1= Early restenosis [≤ 2 days];
CABG= Coronary artery bypass graft;
DS= diameter stenosis;
TSG= Transstenotic gradient;
C.W.P= Coronary wedge pressure;

* = patients with total occlusion;
** = patients with multi-lesions;
*** = patients with unstable angina.

predict reliably which patients or vessel segments will develop restenosis. Until now, there has not been a pharmacological solution to restenosis. The use of one of the new interventional devices, such as the atherectomy catheter (Simpson et al. 1991), the excimer laser (Margolis et al. 1991), stent (Serruys et al. 1991a) or the rotablator (Buchbinder et al. 1991), has also not succeeded in preventing restenosis. The reason that a clinically significant restenosis occurs in only a minority of the dilated vessels remains an enigma. It seems that the solution to the problem of restenosis depends on an understanding of the controlled healing process, which occurs in 60-80% of the vessels dilated.

1. Possible Mechanism of Restenosis After PTCA

Beside the pathology of the dilated vessels of patients who died shortly after PTCA, or at a later stage (Essed et al. 1983, Austin et al. 1985, Nobuyoshi et al. 1991), it has become possible to remove and examine primary and restenotic lesions with the use of the transluminal atherectomy device (Johnson et al. 1990, Safian et al. 1990, Garett et al. 1990). Primary stenotic lesions consist in the majority of cases of atherosclerotic plaque composed of dense fibrous tissue and variable amounts of fatty atheromatous debris. However, in a small group only intimal hyperplasia was observed, histological identical to restenotic lesions. Restenotic lesions showed in most cases intimal hyperplasia (characterised by proliferation of smooth muscle cells of the synthetic type with abundant extracellular matrix chiefly composed of proteoglycans), and in a minority only atherosclerotic plaque was observed. Smooth muscle cell proliferation seems to play a pivotal role in the restenosis process.

Recently two models have been proposed to explain the very complex process of restenosis. Forrester et al. (1991) hypothesised that restenosis is a manifestation of the general wound healing process expressed specifically in vascular tissue following injury. The process that results in restenosis is indeed initiated at the time the disruptive action of the inflated balloon on the endothelium and/or intima and/or media takes place during PTCA. As intact endothelium prevents platelet aggregation, a superficial endothelial injury leads to local platelet and leucocyte adhesion, but most of the platelets do not undergo a release action. However, in case of a deep endothelial injury (as with a successful angioplasty) the haemostatic system is activated. Blood is exposed to collagen and other substances of the subintima which are potent stimuli for platelet aggregation mediated by the release of adenosine diphosphate (ADP), serotonin, thromboxane A_2 (TXA_2), and the adhesive molecules fibrinogen, fibronectin, thrombospondin and von Willebrand Factor (figure 1a). These substances activate neighboring platelets via different metabolic pathways, and promote intramural thrombus formation which could cause restenosis (Stein et al. 1989, Coller 1990). Thus the inflammatory phase begins with coagulation of blood and fibronectin, while platelets aggregate at the wound surface, releasing promoters for local vasoconstriction, coagulation and mitosis (figure 1b).

Removal of heparin from the surface of endothelial and smooth muscle cells by endoglycosidase creates receptivity for the action of growth factors. These are produced in an abundant variety, interacting in a complex way. Several growth

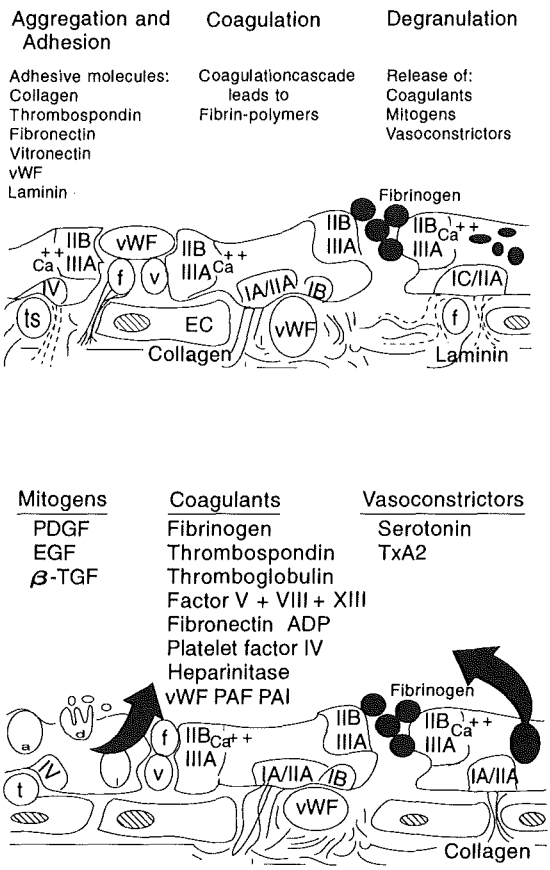

Figure 1 A and 1B

A. *Schematic presentation of platelet activation, caused by the exposure of subendothelial collagen and laminin to circulating blood. Platelet adhesion is mediated by ADP, serotonin, thromboxane and the adhesive molecules which bind to glycoprotein receptors on platelets.*

B. *This adhesion leads to activation and aggregation with release of coagulants, mitogens and vasoconstrictors.*

EC = endthelial cell;
Ca++ = Calcium ions;
IA (B or C)/IIA (B)/IIIA/(B) = glycoproteine receptor IA (B or C)/ IIA (B)/IIIA/(B);
Th = thrombospondin;
F = fibronectin;
V = vitronectin;
vWF = von Willebrand Factor;
PDGF-BB = platelet derived growth factor type BB;
EGF = epidermal growth factor;
bFGF = basic fibroblastic growth factor;
β-TGF = β-transforming growth factor.

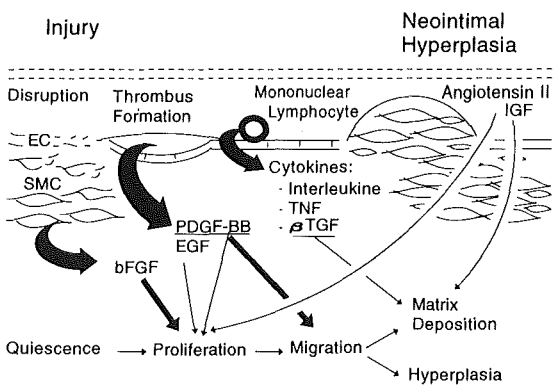

Figure 2

Schematic presentation of the stimulation of smooth muscle cells (SMC) by several growth factors, causing them to proliferate and migrate from the medial layer into the intima of the vessel wall. This response can be associated with abundant connective tissue formation.

EC = endthelial cell;
IGF = somatomedin (insulin like growth factor);
PDGF-BB = platelet derived growth factor type BB;
EGF = epidermal growth factor;
bFGF = basic fibroblastic growth factor;
β-TGF = β-transforming growth factor;
TNF = tumor necrosis factor.

factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor beta (TGF-ß) are released concomitantly from thrombocytes, smooth muscle cells, endothelium and macrophages. Also somatomedin (insulin like growth factor; IGF-I) and angiotensin have been identified as mitogenic contributors. They stimulate smooth muscle cells and fibroblasts to proliferate and migrate from the medial layer into the intima of the vessel wall. In some patients this response is exaggerated and is associated with overproduction connective tissue. This results in hyperplasia of the intima with a reduction in luminal diameter, and causes restenosis (Clowes & Schwartz 1985, Ross 1986, Liu et al. 1989), (figure 2). Thus the granulation phase is marked by endothelial cell migration and proliferation from the wound margin to cover the wound surface. Smooth muscle cells begin to migrate and proliferate from adjacent tissue to the injured area, determined by the action of growth factors and their opposites.

Contractile characteristics of smooth muscle cells disappear in favour of synthetic elements such as increased amounts of synthetic organelles, loss of capacity to contract and increased capacity to divide. Within about 7 days, the phase of extracellular matrix deposition and remodelling takes over, and large levels of chondroitin-sulfate and dermatan sulfate are produced. Both stimulate cell migration and proliferation, and therefore are central in the healing process. Within several months, replacement of the proteoglycanes by collagen and elastin completes the wound healing process.

Schwartz et al. (1991a) proposed an alternative model, based on observations in a porcine coronary injury model. In response to arterial injury, platelets, fibrin and red blood cells accumulate at the injured site, the so-called thrombotic phase. In the subsequent recruitment stage, this thrombus endothelialises and mononuclear cells infiltrate on the lumen side of the vessel. In the proliferative phase, cells (which stain positive for α-actin) form a thin cap just beneath the endothelial surface. These smooth muscle cells or myofibroblasts resorb the remaining thrombotic material towards the media. Since the smooth muscle cells first appear at the luminal side of the endothelialised thrombus, their origin is apparently not the media. Schwartz et al. (1991a) conclude from these observations that the magnitude of the luminal narrowing process may derive more from the volume of local mural thrombus at the site of arterial injury than from uncontrolled cellular proliferation.

Each of these steps could be sites of pharmacological intervention that may prevent the restenosis process (Forrester et al. 1991). The drugs which could reduce or prevent restenosis are listed in table II. In this review we will concentrate on the drugs that have been tested to prevent restenosis in animal models and in post-angioplasty patients. These are summarised in table III.

2. Animal Models

Lack of a practical animal restenosis model has limited the ability to investigate potential therapies. It is difficult to create arterial stenoses in animals that resemble exactly human coronary artery disease for the following reasons: a) the dimensions of the arteries in the animal model should be similar to these in

human beings; b) the development of lesions should occur in an accelerated fashion; c) lesions must produce high-grade, including total, angiographically detectable luminal diameter narrowing; and d) the histological composition of lesions should be similar to the complex lesions that are typical of human atherosclerosis (Gal et al. 1990).

At least 4 different species (pigs, rats, dogs and rabbits) have been used to test new drugs in their ability to prevent restenosis (LeVeen et al. 1982). There is no consensus as to the way that the stenosis should be created (i.e. inflated balloon, infused air, electrical stimulation, or oversized stent implantation) or whether an atherogenic diet should be added, as there is no animal with identical atherosclerotic disease to humans. The vessel studie (iliac, femoral, carotid or coronary arteries, aorta) is also different. Furthermore, the way in which restenosis is assessed differs between the different models. Some use the degree of platelet deposition at the site of arterial injury, while others use the percentage of intimal mitosis observed in the damaged arteries (Steele et al. 1985; Wilentz et al. 1987). Angiography is frequently used to assess restenosis, although some use visual evaluation and others use a computerised-assisted technique to assess restenosis. In summary, no consensus has been reached as to the definition of restenosis that should be used when a new drug is tested in animals.

Recently a model of human restenosis was developed in pigs fed a standard non-atherogenic diet (Schwartz et al. 1991b). Metallic foreign bodies were implanted percutaneously in porcine coronary arteries, with oversized PTCA balloons inflated to high pressure. Results of histological examination of lesions showed a marked proliferation of medial smooth muscle cells. The histopathological features of the proliferative response were identical to those observed in human cases of restenosis following angioplasty. This animal model may therefore be useful in understanding the development of the restenotic lesions and in testing restenosis-preventing drugs since this model more closely resembles the response in human restenosis.

3. Drugs Investigated for Restenosis Prevention

3.1 Antithrombotic Agents Exposure of collagen of the media, as well as material of the atheromatous plaque,activates the intrinsic and extrinsic coagulation system. Thrombin present first in minute amounts promotes its own generation by activating factors V, VIII and XIII by a positive feedback loop, and initiates an autocatalytic reaction leading to rapid production of large quantities of the prothrombin complex via the amplification mechanism. Thrombin stimulates platelets to release and promotes fibrinogen conversion. Antithrombotic and especially anti-thrombin drugs are therefore attractive agents in restenosis prevention.

3.1.1 Coumadin Coumadin has major effects on the coagulation proteins' factor II, VII, IX, and X, protein C and S. This vitamin-K-inhibitor affects the carboxylation system necessary for conversion of glutamic acid residues into gamma-carboxyglutamic acid, and results in a decreased capacity to bind phospholipid for its activation (Wessler & Gitel 1986). Correction of vitamin K inhibition can be readily achieved by administration ofphytomenadione (vitamin K-1) or transfu-

Table II
Summary of potential targets and drugs in preventing restenosis.

Thrombosis
Coumadin, heparin, low molecular weight heparin, hirudin, PPACK, r-ATS, TAP synthetic peptide inhibitor of thrombin
Aspririn, dipyridamole, thromboxane A2 synthetase inhibitor (nafragel), thromboxane A2 receptor blocker, synthetase inhibitor combined with endoperoxide receptor blocker, prostacyclin and analogues
Ticlopidine, serotonin antagonist, GPIIB/IIIA receptor blocker, Arg-Gly-Asp peptides, Lys-Gly-Asp peptides, monoclonal antibodies against von Willebrand Factor, omega-3 fatty acids
Cell proliferation
Angiotensin converting enzyme inhibitor, colchine, platelet derived growth factor antagonist, HGM Co-enzyme A reductase inhibitor, angiopeptin, serotonin antagonist, low molecular weight heparin, cytostatic agents and other antiproliferative drugs
Inhibitor of inflammation
Corticosteroids, non steroidal anti inflammatory drugs
Coronary vasospasm
Calcium antagonists
Lipid regulators
Omega-3 fatty acids, HGM co-enzyme A reductase inhibitor

Abbreviations: GP IIb/IIIa = glycoprotein IIb/IIIa; PPACK = d-phenyl-analyl-l-prolyl-l-arginyl-chloromethyl ketone; r-ATS = antistasin;TAP=tick anticoagulant peptide.

sion of blood, plasma, or plasma concentrates rich in vitamin K-dependent clotting factors.

There is evidence that coumadin is effective in preventing thrombosis in venous bypass grafts, and an anti-vitamin K regimen was tested in a trial in which 248 patients were randomly assigned to receive either aspirin 325 mg daily (n=126) or coumadin (122) at a dosage which resulted in a prothrombin time that was 2 to 2.5 times the normal value (Thornton et al. 1984). Restenosis was angiographically documented in 27% of the aspirin group, versus 36% of the patients treated with coumadin. A loss of >50% of the gain achieved at the time of PTCA or an increase in the stenosis of >30% (NHLBI IV), or the development of ischaemia during exercise testing if no angiogram was available, were used as restenosis criteria. The results favoured the aspirin strategy (figure 3), but the difference was only significant for a subgroup of patients with a long history of chest pain (> 6 months). In patients with poor compliance, restenosis rates were 32% in the group treated with coumadin versus 20% in the group given aspirin.

A more recent randomiszed trial involving 110 patients investigated the effect of a combination of coumadin with verapamil compared with verapamil alone (Urban et al. 1988). The incidence of restenosis (NHLBI IV) was 25% by lesion and 29% by patient in the group given coumadin, and 33 and 37%, respectively, in the control group. Although the incidence of angiographic restenosis tended to be lower with coumadin, none of the differences were significant (figure 3). Dill et al. (1992) failed to show any beneficial effect on the patency of chronically occluded coronary arteries, recanalised by PTCA, by using coumadin or aspirin. Ninety eight patients were randomised after a successful recanalisation to either aspirin 250 mg or coumadin (Quick's time $\leq$ 25%), in addition to intravenous heparin. In the aspirin treated group, 12 of 19 patients presented with restenosis at 3 months' follow up angiography, while 14 of the 24 coumadin treated patients had an angiographic recurrence of luminal narrowing. Reocclusion occurred in 37, respectively 42% (7/19 patients, 10/24 patients).

A randomised trial should be performed to evaluate the efficacy of coumadin in the prevention of restenosis that would ensure adequate medication compliance with reliable and safe monitoring of prothrombin time.

3.1.2 Heparin Heparin was discovered in the beginning of this century (McLean 1916). It is a heterogeneous mixture of glycosaminoglycans with a molecular weight ranging from 2000 to 40,000 D, composed of repeating glucosamine and glucuronic acid sugar residues (Casu 1985). It inhibits smooth muscle cell growth in vitro, as well as angiogenesis and proliferation of Schwann cells and epithelial cells. In animal studies, heparin has been shown to inhibit proliferation and migration of vascular smooth muscle cells (Clowes & Clowes 1986; Clowes & Karnovsky 1977). Growth inhibition with heparin is proportional to heparin fragment size, with a maximal potency observed in the decamers or larger segments. The earliest mechanism for this effect proposed that heparin scavenges cationic growth factors, such as fibroblastic growth factor (FGF) and PDGF. Furthermore, binding and internalisation of heparin by smooth muscle cells makes them less responsive to growth stimuli after vessel injury. Sulphation and

acetylation of the sugar moieties in this agent increases antiproliferative activity.

The anti-thrombotic capacity of heparin is achieved through reversible binding with the natural circulating anticoagulant antithrombin III. This binding is responsible for a 1000-fold acceleration of antithrombin III-heparin binding to factor IIa. This complex also involves activated coagulation proteins (factors X, IX, XI and XII) (Bettmann 1987; Ockelford 1986). A recent publication (Araki et al. 1992) reports the finding that heparin reduces the thrombogenecity of a damaged vessel wall in vivo. In mesenteric arteries of rats, they observed that the adherence of heparin was limited exclusively to the injured parts of the vessel, and inhibited clot formation by local antithrombin activity. They concluded that the antithrombotic action of heparin might be due to inactivation of the thrombogenic site.

Because of the central position of factor Xa in the coagulation cascade, the conversions downstream, including thrombin generation, are also inhibited by heparin.

Since early discontinuation of heparin after angioplasty is associated with acute occlusion of the dilated arterial segment, anticoagulation seems to be important in the early stages after PTCA. However, the optimal duration of heparin therapy is still unknown (Gabliani et al. 1988). A prospective trial involving 209 patients showed a possible beneficial effect of postangioplasty heparin therapy. Prolongation of heparin administration from 2 to > 30 hours reduced the incidence of stenosis from 60 to 40% (Hirshfeld et al. 1987). However, in a randomised study with 416 patients (469 stenoses) a decrease in restenosis following extended heparin treatment could not be confirmed (Ellis et al. 1989a). Restenosis was defined as a narrowing of >50% of the vessel at the time of follow-up angiography, which was performed in only 58.4% of the heparin recipients and 64.5% of those given placebo. No differences in restenosis rate were found in patients randomly assigned to placebo or 18 to 24 hours' heparin administration post-PTCA (all patients received aspirin for 6 months). More bleeding complications were observed in the group of patients who were treated with heparin (8.2% vs 3.8%). Acute total closure was observed more frequently in the group given placebo (2.4%) than in the group treated with heparin (1.8%) but the corresponding restenosis rate was 36.7% versus 41.2%.

Recently, Lehmann et al. (1991) randomised 23 patients to daily subcutaneous administration of heparin 10 000 units for an unspecified duration, or to usual (probably nonpharmacological care. 14 of 17 of the heparin treated patients (82%) experienced restenosis, compared with 2 of 6 (33%) in the placebo group. Restenosis was defined by quantitative angiographic analysis. Thus, long term heparin use after successful angioplasty paradoxically increases the likelihood of angiographic restenosis and adverse clinical outcome.

Perin et al. (1990) retrospectively compared baseline and post-heparin activated clotting times (ACT) between 76 patients with angiographic restenosis and 18 patients without restenosis. Patients with restenosis had significantly lower post-heparin ACT values than those without restenosis. There therefore appears to be a relationship between the procedural response to a bolus dose of heparin and the subsequent development of restenosis.

Another study (Walford et al. 1991) randomised 211 patients after successful PTCA to heparin 1000 U/hour for 12-24 hours or placebo. Paradoxically, early complications were significantly more common in the active treatment group, but no effect was documented on the restenosis rate and clinical endpoints at 19-months' follow up.

Thus, so far, conflicting data have been reported, and it appears reasonable to conclude, that heparin does not affect the restenosis rate dramatically.

Interestingly, cyclodextrin tetradecasulfate has been used during artery dilatation in rabbits (Herrmann et al. 1991). This synthetic heparin analogue resulted in a restenosis rate of 25% (2 out of 8) compared with 75% placebo (6 out of 8). The minimal luminal diameter was significantly increased in the treated group at 1-month follow up.

GM1-077 is a novel non-anticoagulant high molecular weight heparin derivative. This periodate oxidised heparin was investigated in rabbits undergoing balloon angioplasty in the femoral artery (Dubé et al. 1992; Timms et al. 1992). The decrease in lumen diameter from immediately after angioplasty to 28 days after continuous intravenous infusion of GM1-077 was investigated at two dose regimens, 0.1 or 0.33 mg/kg/hr, and compared to a control group (Timms et al. 1992). The decrease in lumen diameter in 14 animals given the higher dosage was significantly lower than in the 17 controls, with those given the lower dose somewhere in between.

Dubé et al. (1992) studied the effect of GM1-077 22 mg/kg/day subcutaneously on morphometric parameters, and detected a 1.8-fold greater inhibition of intimal thickening than with the control drug, subcutaneous heparin 7.6 mg/kg/day.

3.1.3 Low Molecular Weight Heparins Because low molecular weight heparins (LMWH) affect platelet aggregation and platelet-dependent thrombin generation to a lesser extent than conventional heparin, they have fewer adverse effects. They have weaker antifactor IIa activity, but greater antifactor Xa effects. Both are known to inhibit the proliferation of vascular smooth muscle cells (proportional to heparin fragment size and concentration), and thrombosis after endothelial injury in rats (Clowes et al. 1977; Majesky et al. 1987). The mechanism of this inhibition is not clear (Gordon et al. 1987).

LMWH have been used after transluminal angioplasty (TA) of rabbit iliac arteries (Pow et al. 1989). One group (n=9) received LMWH 10 mg/kg/day subcutaneously immediately prior to TA until follow-up angiography 1 month later, and another second group (n=12) received placebo. After 4 weeks, all animals in the placebo group and 3 of 9 in the group given LMWH had a loss of >50% of the gain in diameter after TA. Histologic findings showed reduced intimal hyperplasia and no formation of thrombus in the group tested with LMWH.

Another study in atherosclerotic rabbits by Currier et al.(1991) examined the effect of enoxaparin low dose (1 mg/kg/day) for 4 weeks or high dose (10 mg/kg/day) for 2 or 4 weeks. At follow-up angiography the mean luminal diameter was 0.82 ± 0.17mm for low dose enoxaparin, 1.04 ± 0.20mm for 2-week high dose ($p=0.03$ vs control) and 1.19 ± 0.09mm for 4-week high dose enoxaparin

(p=0.001 vs control). Restenosis, defined as a loss of 50% of the initial gain, was found in all control vessels, in 2 of 9 vessels given the 2-week high dose regimen, and in 3 of 9 vessels in the 4- week high dose group, demonstrating that LMWH can inhibit restenosis in animal models, and may be useful in humans.

Oda et al. (1992) investigated the long term effect (8 weeks) of enoxaparin administration on intimal hypertrophy in normolipidaemic and in hypercholesterolaemic rabbits after balloon injury in the abdominal aorta. The labelling index and the ratio of intimal to media thickness were used to detect drug effects. At the end of the study period, the inhibitory effect of the drug seen earlier, after intra-aortal intervention in the hyperlipidaemic rabbits, was lost, while after 56 days, a significant inhibitory effect on intimal hypertrophy could be detected in the normolipidaemic animals (intima/media ratio 1.45 in controls vs 0.65 after treatment; $p<0.01$).

Oberhoff et al. (1990) showed a significant reduction in the extent of intimal mitosis in the 7 days after balloon angioplasty in rabbits given LMWH, resulting in only a moderate increase of intimal thickness after 28 days compared with a control group. In two additional studies a significant effect of LMWH and unfractioned heparin on mitogenic activity of smooth muscle cells compared to placebo in carotid arteries of rabbits was confirmed (Oberhoff et al. 1991). They concluded that migration and proliferation of smooth muscle cells can be reduced by early treatment with LMWH after balloon angioplasty and further clinical investigation is advocated.

Similar data were reported by Unterberg et al. (1992), who implanted stents in the coronaries of hypercholesterolaemic pigs. Angiographic follow up after 4 weeks' administration of LMWH 200 U/kg showed 2 significant stenoses in the placebo group (33%), whereas all the vessels in the LMWH group were patent. Diameter reduction was 41 versus 22% ($p<0.01$).

Heras et al. (1992) investigated the inhibition of thrombus formation in pig arteries with deep injuries, using four dosages (100, 200, 400 and 500 U/kg) of the LMWH CY 216 in comparison with unfractionated heparin (100 U/kg) and placebo. Examination of the treated injured vessels after 1 hour showed platelet deposition of 22, 29, 9, 9, 11 and 42 $* 10^6/cm^2$, respectively, with these drugs. For fibrinogen deposition, the corresponding values were 19, 19, 21, 14, 12, and 35 molecules $*10^{12}/cm^2$. Thus, the predominantly anti-factor X activity does not clearly inhibit the deposition of thrombotic material after deep arterial injury.

In addition to routine post-PTCA medication, De Vries and associates (1991) treated 49 patients with tedelparin (dalteparin sodium) 5000U, starting on the day of PTCA and subsequently daily for 30 days. Restenosis, defined as >50% reduction in lumen diameter, was documented in 18 of the 46 patients (39%) who had follow up angiography at 4 to 6 months, and thus LMWH did not affect the restenosis rate.

A pilot investigation reported the restenosis rates in 22 patients after 3 months' treatment either aspirin 100 to 300 mg/day (n=11) or tedelparin 80 U/kg (Schmidt et al. 1990). Repeat coronary angiography at the end of this treatment period showed 6 stenoses in the aspirin recipients, and only 3 in the LMWH group. The small number of cases actually does not allow statistical analysis and conclusions, but larger scale studies are in preparation.

Recently a group in Boston completed the ERA (Enoxaparin Restenosis after Angioplasty) study comparing Enoxaparin 40 mg q.d. subcutaneously with placebo, for 28 days, starting within 2 hours after angioplasty, in the prevention of restenosis (Faxon et al. 1992). A total of 459 patients were enrolled and 86% completed the study protocol, including angiographic follow-up. Treatment comparison resulted in an insignificant difference between the two groups (52% with active treatment vs 51% with placebo). Futher, analysis of demographic data and the use of other definitions for angiographic restenosis did not reveal any difference between the two groups.

3.1.4 Hirudin A new anticoagulant drug, originally extracted from the leech Hirudo medicinalis and now produced by genetically manipulated micro-organisms, is hirudin. This 7 kD single-chain polypeptide composed of 65 amino acids, prevents fibrinogen clotting and thrombin-catalysed activation of factors V, VIII and XIII, protein C and thrombin-induced platelet activation by directly binding to thrombin at multiple sites, and also where thrombin is clot-bound (Weitz et al. 1990). Hirudin has been shown to be more effective in preventing thrombosis than heparin in a porcine model, by quantifying platelet and fibrinogen deposition (Dewanjee et al. 1984). This latter effect is probably because hirudin is a more potent and more specific thrombin antagonist (Heras et al. 1989, 1990), that does not require antithrombin III or heparin cofactor II for its activity, and is not neutralised by platelet factor IV or other heparin-neutralising factors (Fareed et al 1991).

Sarembock et al. (1991) evaluated the effect of hirudin on restenosis following balloon angioplasty in 29 rabbits. Given a 2-hour infusion, the hirudin-allocated animals showed significantly less restenosis 28 days after dilatation, assessed by angiography (luminal diameter reduction) and by quantitative histopathology (luminal cross-sectional area narrowing by plaque at necropsy).

Buchwald et al. (1993) were able to influence platelet and fibrinogen deposition after stentimplantation in minipigs; this was significant in a subgroup where medial tear was present at histologic examination. Hirudin (1 mg/kg bolus then 1 mg/kg/h intravenously) in combination of aspirin 250 mg, was compared with a combination of heparin (100 U/kg bolus then 50 U/kg/h), aspirin 250 mg, and dextran 500 ml. Examination 12 hours after stenting showed a siginificantly lower average platelet count in the hirudin group than in the heparin group (64.3 vs $19.7^{\star}\ 10^{6}$platelets/stent; $p<0.05$).

The effects of three dosages of recombinant hirudin versus a single dose of heparin and placebo on deep carotid injury caused by balloon dilatation in 50 pigs was studied by Heras et al. (1990). Platelet deposition in 5 groups given placebo, heparin 50 U/kg, or hirudin 0.3, 0.7, or 1.0 mg/kg was 54 ± 21, 33 ± 9, 22 ± 6, 8 ± 1 and 7 ± 1, respectively. The significance of these observations was confirmed by electron microscopy. In the high dose hirudin-treated groups, platelet deposition was reduced to only one single layer.

A pilot study in 79 hirudin-treated patients and 39 controls given heparin revealed similar safety and tolerability for both drugs (van den Bos et al. 1992). Trials designed to test the efficacy of hirudin on early and late complica-

tions of PTCA have just started recruiting patients in Europe and the United States.

Hirudin binding to thrombin is achieved by two specific binding sites. Hirugen mimics the peptide domain of hirudin, and binds to the fibrinogen binding and cleavage site of thrombin. PPACK (d-phenyl-analyl-l-prolyl-l-arginyl-chloromethyl ketone) binds the active site of thrombin.

With the use of a new infusion balloon catheter, the effect of local delivery of PPACK on platelet deposition was tested at the site of balloon angioplasty in an ex vivo whole artery perfusion model (Leung et al. 1990). Platelet deposition was significantly reduced with the use of this new antithrombin agent compared with the control model.

Hirulog is the name for a novel class of thrombin inhibitors that inhibits both the thrombin catalytic site and the fibrinogen binding exosite of thrombin (Maraganore et al. 1990). Hirulog has been administrated safely to healthy human volunteers (Dawson et al. 1991), and in patients undergoing PTCA (Bonan et al.1992a; Topol et al.1991). The Cleveland group recently reported the results of hirulog administration in atherosclerotic rabbits after angioplasty. The drug was implanted around dilated femoral segments in the adventitial layer, but did not influence neointimal proliferation (Guzman et al. 1993).

Another leech-derived drug is antistasin (r-ATS), a long-acting factor Xa-inhibitor, initially isolated from the salivary glands of the Mexican leech (Nutt et al. 1988), has been studied together with tick anticoagulant peptide (TAP), a short-acting specific inhibitor of factor Xa. These experimental drugs were tested in rabbits during balloon angioplasty, followed by 2-hours infusion (Ragosta et al. 1992). The reduction in luminal diameter, measured at 28 day follow-up angiography, was significantly less in the antistasin (-0.17 $\pm$0.11 mm) and TAP (- 0.26 $\pm$0.22 mm) groups compared with the control group given heparin (-0.66 $\pm$0.58 mm, $p \leq 0.02$).

3.1.5 Synthetic Peptide Inhibitor of Thrombin In 1988, two abstracts reported the efficacy of synthetic peptide inhibitor of thrombin. Schneider et al. (1988) showed that treatment with synthetic peptide inhibitor of thrombin morphologically abolished acute thrombus formation and significantly reduced deposition of ^{111}In-labelled platelets on carotid endarterectomy sites in baboons.

Another study showed that argatroban (argipidine) prevented occlusion in rabbits, while all control animals showed occlusion that was not prevented by heparin alone or in combination with alteplase (tissue plasminogen activator) after exposure to collagen in a femoral arterial thrombosis model (Jang et al. 1988). The same group recently described the effect of the synthetic and competitive thrombin inhibitor argatroban on trombus formation in the rabbit femoral artery model. When compared with heparin treatment, a 1-hour infusion of argatroban reduced residual thrombus severity significantly (Jang et al. 1992). In this respect it could have properties that diminish the long term restenosis.

The interest in the antithrombotic approach can be illustrated by the number and size of trials that are planned for the near future.

3.2 Antiplatelet Agents

Platelets play an important role in the development of restenosis after PTCA (Faxon et al. 1984), since postinjury myointimal hyperplasia in at least some animal models does not occur in the presence of profound thrombocytopenia. To prevent platelet deposition (which occurs within minutes of the procedure) and the associated release of smooth muscle cell mitogenic factors, anti-platelet therapy appears to be a logical approach.

3.2.1 Aspirin *Dose finding studies* Aspirin is a popular drug in restenosis prevention trials. In animal models it reduces platelet-thrombus deposition in a dosage of 1 mg/kg/day when given in addition to heparin (Perin et al. 1990). Aspirin has the ability to inhibit platelet TXA_2 synthetase and subsequent platelet activation, by irreversibly blocking the enzyme cyclo-oxygenase which is responsible for the conversion of arachidonic acid to intermediate prostaglandin G_2 (PGG_2). This latter compound normally leads to the formation of TXA_2 and thromboxane B_2. Thromboxane A_2 is a powerful platelet aggregator and vasoconstrictor. Low doses of aspirin result in decreased production of this substance in platelets. However, high dose aspirin results in cyclo-oxygenase inhibition in endothelial cells as well, which is usually responsible for the conversion of arachidonic acid to prostaglandin I_2 (Oates et al. 1988). Since the biologic actions of TXA_2 and PGI_2 are opposing, aspirin at high doses may be less effective in preventing restenosis. In addition, it only partially inhibits platelet aggregation induced by ADP, collagen or thrombin. Consequently, the effect of PDGF and other mitogens released from platelets may still affect proliferation of smooth muscle cells (Stein et al. 1989). Several studies have been carried out to evaluate the advantages of different doses of aspirin (Figure 3).

Intravenous given ASA in a high dosage (1000 mg), in addition to a bolus of 10.000 U of heparin suppressed thrombin generation beyond the reduction induced by 10.000U of heparin solely (plasma fragment F1.2 monitoring in 24 patients), and could therefore reduce the mitogenic respons in PTCA patients (Andreotti et al. 1992).

Dyckmans and colleagues (1988), randomly assigned 203 patients to either aspirin 1500 mg/day or 320 mg/day. In a preliminary report, 40% of these patients had been restudied 6 months after PTCA. Results of follow-up angiography showed restenosis (>50% diameter stenosis) in 13 of 44 patients in the group taking the lower dose (31%) compared with 9 of 42 patients in the group with the higher dose (21%).

In another randomised trial, the effectiveness of aspirin 80 or 1500 mg/day, starting on the day before PTCA, in the prevention of restenosis and acute complications after PTCA was compared in 495 patients (Mufson et al. 1988). Results of follow-up angiography were available in only 166 patients. In the group treated with the lower dosage of aspirin, 47% had restenosis (>50% diameter stenosis in 1 or more sites) compared with 51% in the group receiving the higher dosage. There were no differences in success or acute complication rates between the groups. Thus, restenosis was not influenced favourably by the use of a higher dose of aspirin.

A smaller trial compared the effects of aspirin 100 with 1000 mg/day, starting 1 day before PTCA for 6 months (Schanzenbacher et al. 1988). In addition, all patients received calcium antagonists and long-acting nitrates. Restenosis (clinically significant stenosis) requiring repeat-PTCA or coronary artery bypass graft (CABG) occurred in 7 of the 40 patients in the group taking aspirin 100 mg daily (18%) and in 8 of the 39 patients treated with 1000 mg aspirin (21%). The investigators concluded that restenosis is not favourably influenced by the use of high versus low dose aspirin.

Kadel et al. (1990) also were unable to detect significant differences regarding the rate of restenosis in their patients, randomised to 350 or 1400 mg of aspirin daily,although the results tended to favour the lower dosage (21 versus 31% restenosis).

Data from studies of similar design can be combined to obtain a more powerful analysis of the effects of treatment. Meta-analysis of the pooled restenosis data of the investigations of Dyckmans et al. (1988), Mufson et al. (1988), Schanzenbacher et al. (1988) and Kadel et al. (1990), using the Cochran-Mantel-Haenszel method, gives an overall relative risk for restenosis of 1.2 when a daily aspirin dosage of > 1000 mg is used, compared with a dosage of < 350 mg. The 95% confidence interval (CI) is insignificant.

Comparisons with Placebo In several studies, the effect induced by aspirin, with or without dipyridamole 225 mg, was compared with placebo (Figure 3).

Finci et al. (1988) designed a single-blind trial to compare aspirin 100 mg daily with placebo. However, it was prematurely discontinued after 40 patients were enrolled because of reports showing the benefit of aspirin (combined with dipyridamole) in preventing acute thrombosis in dilated vessels and the need for urgent bypass surgery (Barnathan et al. 1987). In this particlar study, results of follow-up angiography at 6 months (95% of the patients) surprisingly showed an incidence of restenosis (>50% diameter stenosis) that was two times higher in aspirin recipients compared with the group given placebo (33% versus 14%). Although this difference seems impressive, it was not statistically significant because of the small numbers.

Taylor and associates (1991) compared aspirin 100mg and placebo in 216 patients for 2 weeks, starting before PTCA. Follow-up angiography 6 months later showed restenosis in 45 of the 104 placebo-allocated and in 38 of the 108 aspirin treated patients (35% vs 43%, nonsignificant). Thus, no convincing results could be shown for the beneficial effect of aspirin.

In a well designed trial at the Montreal Heart Institute and Toronto General Hospital (Schwartz et al. 1988), 376 patients were randomly assigned to a daily combination of aspirin 990 mg and dipyridamole 225 mg, or to placebo, beginning the day before PTCA until follow-up angiography 4 to 7 months later. More acute complications were observed in the placebo group, including 13 periprocedural myocardial infarctions versus 3 in the actively treated group ($p < 0.05$). However, no differences were observed in the restenosis rate (increase in diameter stenosis from <50% after-PTCA to >50% at follow-up): 39% (127 patients) in the placebo recipients versus 38% (122 patients) in the active treatment group.

All patients received heparin until 12 hours after the procedure (500 U/hour) and diltiazem until follow-up angiography.

Chesebro et al. (1989) randomly assigned 207 patients (297 stenoses) to either aspirin 975 mg/day and dipyridamole 225 mg/day or to placebo from the day before PTCA until 6 months later. There was no difference in restenosis rate, defined in a linear model based on the minimum lumen diameter obtained by quantitative angiography. There were fewer acute complications (occlusion, myocardial infarction, repeat PTCA, CABG wihin 48 hour) in the aspirin and dipyridamole recipients (11 versus 20%) confirming the results of the Canadian trial.

White et al. reported in 1987 a restenosis study in which they compared a two drug regimen versus placebo. Active therapy consisted of either 750 mg ticlopidine, or 650 mg aspirin plus 225 mg dipyridamol, starting 4 to 5 days prior to PTCA. Repeat angiography in 75% of the study population showed a small but insignificant beneficial effect of aspirin/dipyridamol at visual assessment, although quantitative analysis in a small subset of patients (32%) demonstrated an opposite risk ratio.

Meta-analysis according to the Cochran-Mantel-Haenszel method of the pooled restenosis data from the four studies of Taylor et al.(1991), Finci et al. (1988), Schwartz et al 1988 and White et al. (1987) gives an overall relative risk of restenosis with aspirin treatment versus placebo of 0.932 (95% CI includes 1). It has to be mentioned that the dosage of aspirin used was quite divergent, making meta analysis less justifiable. These trials clearly showed that, although aspirin does not influence the incidence of restenosis, it definitely has a positive influence on acute complications during or immediately after angioplasty.

Recent work in atherosclerotic rabbits, however, showed different results. Aspirin 60 mg/day and coumadin (1.5 normal prothrombin time) were given as combination therapy starting 7 days before iliac transluminal angioplasty until final angiography 4 weeks later. Luminal diameter decreased from 1.7mm post angioplasty to 0.6 mm at follow-up in the control group, while in the treated rabbits the stenosis diameter only changed from 1.4 mm post intervention to 1.3 mm after 4 weeks. Restenosis (loss of >50% of initial post-transluminal angioplasty gain) was observed in 2 of 10 rabbits given aspirin plus coumadin versus all 12 control rabbits. This suggests strongly that early combination therapy reduced restenosis in this animal model, and that platelet activation is a key element in the restenosis process (Franklin et al. 1990).

Five days' pretreatment with aspirin 75 to 300 mg/day or an intravenous bolus of 1000mg during PTCA, in addition to a bolus of heparin 10,000U suppressed thrombin generation beyond the reduction induced by heparin alone in 24 patients (Andreotti et al. 1992). The results favoured those receiving aspirin 1000 mg.

3.2.2 **Dipyridamole** In a rabbit model, aspirin and dipyridamole decreased platelet-thrombus deposition and restenosis after transluminal angioplasty (Faxon et al. 1984a). The mechanism(s) by which dipyridamole has been suggested to inhibit platelet function include: 1) inhibition of the phosphodiesterase enzyme

in platelets, resulting in an increase in intraplatelet cyclic AMP and the consequent potentiation of the platelet-inhibiting actions of prostacyclin; 2) direct stimulation of the release of this eicosanoid by vascular endothelium; and 3) inhibition of cellular uptake and metabolism of adenosine, thereby increasing its levels at the platelet-vascular interface. Finally, in addition to such direct effects, dipyridamole may augment the platelet-inhibiting action of aspirin through pharmacokinetic interaction (Fitzgerald 1987). However, in clinical trials (Schwartz et al. 1988, Chesebro et al. 1989, White et al. 1987) no effect has been shown on restenosis rate after angioplasty (Figure 3).

3.2.3 **Thromboxane A_2** Since aspirin blocks cyclo oxygenase in both platelets and endothelial cells, it inhibits the formation of TXA_2, as well as prostacyclin. TXA_2 is a potent aggregating agent and vasoconstrictor, and it was deemed useful to antagonize these actions during and after PTCA, while at the same time leaving prostacyclin production of the vascular endothelium unaffected. Therefore, a specific approach has been attempted, such as the use of TXA_2 synthetase inhibition or TXA_2 receptor blockade (Figure 3).

Thromboxane A_2-synthetase inhibitor In rabbits, it was shown that a selective TX synthetase inhibitor was more effective than heparin or aspirin in inhibiting platelet deposition after balloon angioplasty (Sanborn et al. 1986). Another TXA_2 synthetase inhibitor nafragel (DP 1904), was tested in a small number of patients to prevent restenosis after PTCA (Yabe et al. 1989). It was given a minimum of 5 days before PTCA and was continued until follow-up angiography >3 months later. Restenosis was defined as > 50% loss of initial gain in luminal diameter. The results showed that 4 of 18 patients (22%) given with nafragel had restenosis versus 8 of 15 patients (53%) in the placebo group.

Thromboxane A_2-receptor blocker Vapiprost (GR32191), a new TXA_2 receptor blocker, has been shown to inhibit completely prostaglandin endoperoxide and TXA_2 induced platelet aggregation, serotonin secretion and beta-thromboglobulin secretion without any effect on the actions of prostacyclin (Hornby et al. 1989). Because of the inhibitory action of vapiprost on platelet aggregation, mural thrombus formation and platelet protein storage granule secretion, a trial was conducted to assess its effect on the prevention of restenosis after coronary angioplasty (Serruys et al. 1991b). This trial (CARPORT; Coronary Artery Restenosis Prevention On Repeated Thromboxane-Antagonism) randomly assigned 697 patients in 6 clinics throughout Europe to a TXA_2-receptor blocker, 80 mg intravenous before PTCA and 40 mg orally for 6 months, or 250 mg intravenous aspirin before PTCA and placebo for 6 months. No difference was observed between the mean difference in diameter of treated lesions between post-PTCA and follow-up angiogram: -0.31 ± 0.53 in the control group and -0.30 ± 0.54 in those receiving the TXA_2 receptor antagonist. Using a loss of 0.72 mm or more as restenosis criterion (Reiber et al. 1985) restenosis rates were 19% in the control group and 21% in the vapiprost group. There were also no difference in clinical events (death, CABG, repeat-PTCA, non-fatal myocardial

infarction) between the 2 groups. Thus, no benefit was shown of long term TXA_2 receptor blockade with vapiprost used as single pharmacological agent against restenosis.

In the US, vapiprost was tested in a similar multi-center restenosis trial (Feldman et al. 1992). 1192 patients were randomised to aspirin 325 mg/day before PTCA and placebo afterwards, or to vapiprost 80 mg/day followed by 40, or 80 mg daily for 6 months. Angiographic restenosis, defined as a change in minimal luminal diameter of more than twice the standard deviation, resulting in a cut-off point of 0.62 mm, was not affected by the drug (restenosis rate 39% with vapiprost versus 35% with aspirin plus placebo). The clinical event rate in both groups however was reduced by 21%, primarily because of a reduction in MI and repeat PTCA ($p = 0.02$).

Finci et al. (1989) tested the sulphonamide derivate sulotroban (BM13177) which blocks TXA_2-receptors, versus placebo in 107 patients. Fiftyseven patients completed the study protocol, including 6 months' angiography. The difference in restenosis rate was insignificant, namely 57% in the treated and 56% in the control group .

Sulotroban was also investigated by Bove et al. (1992). 755 patients were randomized to sulotroban 3200 mg/day, aspirin 325 mg/day, or placebo for 6 months. Stenosis diameter changes after PTCA were not influenced by selective TXA_2 antagonism (minimal luminal diameter at 6 months in placebo group 1.43mm, in the sulotroban group 1.43mm, and in the aspirin group 1.54mm).

3.2.4 Ridogrel Ridogrel (R68060) is a combined TXB_2 synthetase inhibitor and thromboxane-prostaglandin endoperoxide receptor blocker that eliminates almost all TXB_2 with a concomitant increase in 6-keto-PGF1a in serum.

It was tested recently in 32 patients in combination with heparin in a PTCA setting and appeared to be safe. Further studies seem to be warranted to assess the effectiveness in preventing early and late restenosis (Timmermans et al. 1991).

3.2.5 Sulfinpyrazone In contrast to aspirin, sulfinpyrazone is a competitive (reversible) inhibitor of platelet cyclo-oxygenase, but the exact mechanism of its antithrombotic activity is not clearly understood. Faxon et al. (1984a) showed a reduction in restenosis in rabbits. There is no clinical evidence to date to support a role for sulfinpyrazone in the prevention of restenosis after coronary angioplasty.

3.2.6 Dextran Dextran interferes with platelet activity by changing membrane function or by interacting with von Willebrand factor-factor VIII complex. Although it has been shown to be efficacious during PTCA there is no role established for dextran in restenosis prevention (Harker & Fuster 1986, Swanson et al. 1984).

3.2.7 Prostacyclin or Prostacyclin Analogues Vessel wall injury and platelet adhesion to endothelium might interfere with local production of prostacyclin,

and promote further platelet aggregation and thrombus formation. Prostacyclin is a potent naturally occurring vasodilator that protects platelets from being activated (Moncada & Vane 1976). Regular aspirin therapy eliminates, in the greater part, both TXA_2 and TXB_2 production, but also affectsendothelial production of prostacyclin. This reduces the usefulness of aspirin treatment, although it has advantageous effects. Therefore, administration of prostacyclin as additional therapy might play an important role in restenosis prevention (Figure 3) and has been tested in a prospective trial. Two hundred and seventy patients were randomly assigned to placebo (n=136) or prostacyclin 5 to 7 mg/min intravenously) (n=134) just before PTCA and up to 48 hours after PTCA (Kundtson et al. 1990). All patients received aspirin 325mg and dipyridamole 225mg beginning before angioplasty until follow-up angiography 6 months later. Short-term administration of prostacyclin did not significantly reduce the risk of restenosis compared with placebo (27 versus 32% rate). Restenosis was defined as >50% narrowing at follow-up angiography or > 50% loss of immediate gain after angioplasty. Acute vessel closure and ventricular tachyarrhythmias were more common in the control group than in patients who received prostacyclin.

Another study randomised 132 patients to either prostacyclin (4ng/kg/min) or placebo infusion for 36 hours after PTCA. Patients were followed for 6 months and were then restudied. No benefit was seen in prevention of restenosis (defined as a loss of 50% of the gain achieved at PTCA) (Gershlick et al. 1990).

Ciprostene is a chemically stable analogue of prostacyclin. To study the effect of ciprostene during PTCA, 311 patients were randomly assigned to ciprostene shortly before PTCA (40ng/kg/min) until 48 hours after PTCA (120 ng/kg/min), or to placebo (Raizner et al. 1988). Acute closure occurred in 3 patients in the placebo group and none in the ciprostene-treated group. The clinical endpoints of this trial included death, myocardial infarction, repeat-PTCA and CABG. In 23 of the 139 patients treated with ciprostene (17%), one or other of these clinical endpoints was observed, compared with 47 of the 137 placebo recipients (34%; p=0.0008). Angiograms of 86% of the patients who completed the protocol were retrospectively analysed (Raizner et al. 1993). Change in minimal lumen diameter was 0.32mm in the ciprostene group and 0.57mm in the control group (p=0.025), confirming the earlier experience.

The same protocol was recently followed by Darius et al. (1991), who randomised 32 patients to ciprostene or placebo. Twenty minutes of intra coronary infusion were followed by a 24 hour intravenous infusion, combined with a 24 hour heparin infusion, aspirin 100 mg and nifedipine 60 mg daily. In 24 patients, a 6 month follow-up angiogram was obtained. Coronary artery stenosis was reduced by PTCA from 83 to 31% in the ciprostene group, and from 81 $\pm$3 to 34 $\pm$3% in the placebo group. At 6 months follow-up angiography, the percent stenosis in the ciprostene-treated group was still significantly lower when compared with pre-PTCA status (55 versus 83% ; p=0.012),in contrast to those given placebo 63 versus 81%). Platelet aggregation studies revealed significant inhibition of PAF-induced aggregation after intra-coronary and after 4 hours of i.v. infusion. More clinical testing is necessary for the correct interpretation of these results.

Meta-analysis of the trials performed by Raizner et al.(1988), Knudtson et

al. (1990) and Gershlicket al. (1990)[Darius did not define a cut-off point for restenosis], gives an overall relative risk of 0.82, with the upper limit of the 95% CI of 1.008.

3.2.8 Prostaglandin E_1 Prostaglandin E_1 (PGE_1) differs from prostacyclin (PGI2) in the number of double bonds in the acyl side chain, and by the type of substituent group that is attached to the ring.

Since the deposition of platelets after angioplasty in porcine carotid arteries was reduced significantly more after infusion with PGE_1 even more than with prostacyclin or dipyridamole, a study was attempted to determine the effect of intra-coronary administration followed by intravenous PGE_1 on early restenosis (See et al. 1987). Eighty patients were randomly assigned to placebo or infusion of 20 to 40 ng/kg/min, 12 hours before PTCA. Clinical follow-up showed abrupt occlusion in 3 of 40 patients in the placebo group compared with none in the PGE_1-treated group. An additional repeat PTCA was necessary in 4 of 40 placebo recipients but in none of thase given PGE_1. No angiographic study has assessed the effect of PGE_1 on late restenosis.

3.2.9 Ticlopidine Ticlopidine is chemically unrelated to other antithrombotic agents. Its mechanism of action is not exactly known, but there is evidence that this potent platelet inhibitor alters platelet membrane activity, probably by blocking the interaction between platelet receptors with fibrinogen and von Willebrand factor (vWF) (Lee et al, 1981). The optimal effect occurs 3 days after the first administration and lasts for at least several days, and its effect on restenosis is documented in three abstracts (Figure 3).

In a multi-center trial in the United States (White et al. 1987), patients were randomly assigned to ticlopidine 750 mg/day, a combination of aspirin 650 mg/day and dipyridamole 225 mg/day, or to placebo. Restenosis was defined as a diameter obstruction of ≥70% at follow-up angiography 6 months later, by visual assessment. There was no difference in restenosis rate; in the 65 patients who received ticlopidine the restenosis rate between the 2 actively treated groups; in the 65 patients who received ticlopidine the restenosis rate was 29%, compared with 18% of 57 patients who received aspirin plus dipyridamole. The restenosis rate was 20% among the 54 patients taking placebo. There was no difference in acute complication rate. QCA in a small subset of patients (32%) yielded similar results.

In Japan, data collected retrospectively showed a lower restenosis rate when patients received a combination of ticlopidine 200mg/day, nicorandil 30mg/day and aspirin 300mg/day (Kitazume et al. 1988).

In the Ticlopidine Angioplasty Coronary Trial (TACT), there was no benefit of therapy with ticlopidine 500 mg/day versus placebo in 266 patients randomised for prevention of restenosis (loss of 50% of the gain achieved at PTCA) (Bertrand et al. 1990). However, acute closure was significantly reduced in the ticlopidine group compared with placebo. These patients did not receive concomitant heparin or aspirin. The French experience is confirmed by the results of a Spanish trial (Iniguez et al. 1991), which assigned a total of 179 patients to

ticlopidine 250 mg or placebo after a single lesion angioplasty. There was no difference in the restenosis rates between the two groups (28% with ticlopidine versus 24% with placebo). Possibly the time interval from first administration to angioplasty was too short for ticlopidine to become effective. Overall there seems no role for ticlopidine in restenosis prevention.

3.2.10 Serotonin Antagonists In the 1950s, the isolation was reported of a substance, extracted out of serum, that is responsible for a tonic reaction of vascular smooth muscle cells (Page 1958). Due to its properties it was called serotonin (5-hydroxytryptamine; 5-HT). Interaction with intact endothelial cells causes them to produce endothelial-derived relaxation factor (EDRF) which leads to the relaxation of the surrounding muscular layer. In the setting of damaged endothelium, however, serotonin gains direct access to the smooth muscle cells, causing them to contract. Serotonin seems to be an important mediator in cyclic flow reduction in stenotic and injured arteries. It is released from the dense granules in aggregating platelets. It is a weak agonist of platelet activation, but it enhances the activity of other platelet agonists like ADP, TxA_2, catecholamines and thrombin via a positive feedback loop. In vitro experiments show the stimulation of mitogenesis, migration and retraction of vascular smooth muscle cells following serotonin exposure (Rooman et al. 1990). In low concentrations it substantially enhances the mitogenic response of these cells to PDGF (Nemecek et al. 1986), as well as the proliferation of cultured fibroblasts and matrix synthesis (De Clerck & Janssen 1990).

Ketanserin is a potent 5-HT_2-receptor antagonist, with weak alpha1-receptor blocking properties. It inhibits serotonin-induced platelet activation and vasoconstriction, and has also been shown to inhibit DNA synthesis in vascular smooth muscle cells in vitro following serotonin stimulation. Klein et al. (1989) studied the effect of ketanserin on the incidence of early and late restenosis (Figure 3). Ketanserin 0.1 mg/min or placebo was given intravenously for 24 hours after PTCA to 21 patients. After 24 hours, 3 patients in the placebo group had an occlusion compared with none in the ketanserin group. Follow-up angiography 4 to 6 months later showed no difference in restenosis rates (29% versus 33%).

The large multi-centre interventional trial, PARK (Post- Angioplasty Restenosis Ketanserin), has recruited and randomised more than 700 patients to Ketanserin 40 mg twice daily or placebo, both in combination with aspirin. Analysis of post-angioplasty angiograms revealed a loss in MLD of 0.27 mm in the ketanserin group versus 0.24 mm in the placebo group (Serruys et al. 1992). When restenosis is defined as a loss in MLD of ≥ 0.72 mm, the restenosis rate was 15 versus 14%. Thus, no significant effect of ketanserin on angiographic parameters could be demonstrated (Figure 3).

The same conclusions were drawn from another double-blind randomised trial in 97 patients (Heik et al. 1992). Ketanserin administration started 1 day before angioplasty (10 mg iv), increased to 40 mg bid for 2 weeks, and continued at a dosage of 20 mg bid until follow-up. All patients received aspirin 100 mg per day. Changes in arterial dimensions, measured by QCA, were not significantly different between the groups.

Table III

The effect of drug therapy on restenosis per patients after successful coronary angioplasty with follow-up angiography and or clinical follow-up.

Author		Drug	Dose	Patients total	F-up
ERA/Faxon	1992	Enoxaparin placebo	40 mg q.d. s.c.	459	86%
Thornton et al.	1984	ASA Coumadin	325 mg/day 2-2.5 norm PTT	248	72%
Urban et al.	1988	Coumadin+Verapamil	> 2.5 nor PTT	110	77%
		Verapamil	Not Reported		
Hirshfeld et al.	1987	Heparin	Different duration	209	NR
Ellis et al.	1989	Heparin (18-24h) Dextrose	< 2.5 norm PTT	416	61%
Lehmann et al.	1991	Heparin (daily)	10,000 I.U./day	30	77%
Walford et al.	1991	Heparin	1.000 I.U./hour	211	88%
		Placebo			
De Vries et al.	1991	LMWH	5.000 I.U./day	49	94%
Schmidt et al.	1990	LMWH Fragmin	80 U/kg	22	100%
		ASA	100-300 g ASA/day		
Dyckmans et al.	1988	ASA	1500 mg/day	203	40%
		ASA	320 mg/day		
Mufson et al.	1988	ASA	1500 mg/day	453	37%
		ASA	80 mg/day		
Schanzenbacher et al.	1988	ASA	1000 mg/day	79	100%
		ASA	100 mg/day		
Kadel et al.	1990	ASA	1400 mg/day	188	92%
		ASA	350 mg/day		
Finci et al.	1988	ASA	100 mg/day	40	73%
		Placebo			
Taylor et al.	1991	ASA	100 mg/day	216	98%
		Placebo			
				303 lesions	
Schwartz et al.	1988	ASA+Dipyridamol	990-225mg/day	249	100%
		Placebo			
Chesebro et al.	1989	ASA+Dipyridamol	975-225mg/day	207	85%
		Placebo			(QCA)
White et al.	1987	ASA+Dipyridamol	650-225mg/day	236	75%
		Ticlopidine	750 mg/day		
		Placebo			
		ASA+Dipyridamol		*76*	*32%*
		Ticlopidine			
		Placebo			
Yabe et al.	1989	TXA2 synth inhibitor	600 mg/day	33	100%
		Placebo			
Serruys et al.	1991	TxA2 receptor blocker	40 mg/day	650	89%
		Placebo			
Feldman et al.	1992	GR32191	20 mg	1089	86%
		GR32191	40 mg		
		Placebo			
Finci et al.	1989	Sulotroban	4 x 800 mg	107	53%
		Placebo			

Time	Definition restenosis	Restenosis(%) drug vs placebo		Sign	Risk ratio	95% CI
6 months	loss > 50% gain	52%	51%	p=ns	1.0	0.76 to 1.07
6-9 mths	loss> 50% gain stress test – : +	27%	36%	p=ns	0.7	0.5 to 1.1
5 months	> 50% DS Fup	29%	37%	p=ns	0.8	0.4 to 1.4
4-12 mths	> 50% DS Fup (visual)	Longer heparin less restenosis				
3-9 mths	> 50% DS Fup (visual)	41%	37%	p=ns	1.1	0.8 to 1.5
NR	NR	82%	33%	p<0.05	2.5	0.8 to 7.8
19 months	Clinical	22%	24%	p=ns	0.9	0.6 to 1.6
4-6 mths	> 50% reduction in lumen diameter	39%	NR			
3 months	reduction of residual stenosis area > 30% reduction of stenosis diameter > 50%	27%	54%	p=ns	0.5	0.2 to 1.5
6 months	> 50% DS Fup	21%	31%	p=ns	0.7	0.4 to 1.5
3-8 mths	> 50% DS Fup (visual)	51%	47%	p=ns	1.1	0.8 to 1.5
6 months	Clinical (re-PTCA, CABG)	21%	17%	p=ns	1.2	0.5 to 2.9
4-6 mths	NR	31%	21%	p=ns	1.5	0.9 to 2.5
6 months	> 50% DS Fup (visual)	33%	14%	p=ns	2.3	0.5 to 10.1
6 months	> 50% reduction in lumen diameter and > 50% reduction of the gain	35%	43%	p=ns	0.8	0.6 to 1.1
		25%	38%	p=0.03	0.7	0.5 to 0.95
4-7 mths	> 50% DS Fup (QCA)	38%	39%	p=ns	1.0	0.7 to 1.3
5 months	MLD (Post-PTCA) - (Fup)	0.14 mm	0.18 mm P	p=ns		
6 months	> 70% DS Fup (visual)	18%	29%	p=ns	0.9	0.4 to 1.9
			20%	p=ns	1.4	0.8 to 2.8
	> 70% DS Fup (QCA)	*33%*	39%	*p=ns* p=ns	*1.3* 1.6	*0.5 to 3.7* 0.7 to 3.3
			25%			
> 3 mths	loss > 50% gain	22%	53%	p=ns	0.4	0.2 to 1.1
6 months	MLD (post-PTCA) -MLD (Fup)	0.31 ±0.53 TxA2 0.30 ±0.54 P		p=ns		
6 months	≥ 0.62 mm change in MLD	39%	35%	p=ns	1.2	0.9 to 1.6
6 months	loss of ≥ 50% of the initial gain	65%	61%	p=ns	1.1	0.7 to 1.6

Table III continued

Author		Drug	Dose	Patients total	F-up
M-Heart et al.	1992	Sulotroban	3200 mg/day	755	82%
		ASA	325 mg/day		
		Placebo			
Timmermans et al.	1991	Ridogrel	300 mg iv bolus	32	100%
			300 mg p.o. b.d.		
		plus Heparin	10.000 I.U. bolus		
			1.000 I.U./hour/24 hours		
Knudtson et al.	1990	Prostacyclin+ASA+D	5 ng/kg/min	270	93%
		ASA+Dipyridamol(D)	325 + 225 mg/day		
Gershlick et al.	1990	Prostacyclin	4 hg/kg/min	132	80%
		Placebo			
Raizner et al.	1988	Ciprostene	120 ng/kg/min	311	80%
		Placebo	max. 48 hours		
Darius et al.	1991	Ciprostene	120 mg/kg/min.	32	67%
		Placebo	for 48 hours		
Kitazume et al.	1988	ASA (Placebo)	300 mg/day	280	100%
		ASA+Ticlopidine(Tic)	+ 200 mg/day		
		ASA+Tic+Nicorandil	+ 30 mg/day		
Bertrand et al.	1990	Ticlopidine	500 mg/day	266	93%
		Placebo			
Klein	1989	Ketanserin	0.1 mg/min (24 hr)	43	100%
PARK	1992	Ketanserin + ASA	80 mg/250-500 mg	704	84 %
		ASA	250-500 mg		
Brozovich et al.	1991	ACE-inhibitor	different dosages	322	retrosp.
		non ACE-inhibitor			
Serruys et al.	1992	Cilazapril	40 mg/day	700	89%
		Placebo			
O'Keefe et al.	1992	Colchicine	1.2 mg/day	197	74%
		Placebo			
Grines et al.	1991	Colchicine	1 mg/day	253	79%
		Placebo	for 1 month		
Okamoto et al.	1992	Trapidil	600 mg/day	97	74%
		ASA + D	300 + 150 mg/day		
Maresta et al.	1992	Trapidil	300 mg/day	251	46%
		ASA	300 mg/day		
Rose et al.	1987	Steroid	48 mg/ day	66	88%
		Placebo			
Stone et al.	1989	Meth. prednisolone	125mg mp/day	102	53%
		prednisolone	240mg p/week		
Pepine et al.	1990	Steroid	1.0 g mp	722	71%
		Placebo			
Hirayama et al.	1992	Ebselen	200 mg p.o bid	79	100%
		Placebo			
Corcos et al.	1985	Diltiazem+ASA+D	270 mg/day	92	100%
		ASA+D	650-225 mg/day		
O'Keefe et al.	1991	Diltiazem+ASA+D	240-360 mg/day	201	60%
		ASA+D	325-225 mg/day		
Whitworth et al.	1986	Nifedipine+ASA	40 mg/day	241	82%
		ASA			

Time	Definition restenosis	Restenosis(%) drug vs placebo		Sign	Risk ratio	95% CI
6 months	MLD (post-PTCA)	Sulo: -0.45 mm	p=ns			
	-MLD (Fup)	ASA: -0.37 mm				
		Plac: -0.48 mm				
6 months	Clinical	10%		non-randomised		
6 mth	> 50% DS Fup	27%	32%	p=ns	0.9	0.6 to 1.3
	loss > 50% gain					
5-7mth	loss > 50% gain	31%	34%	p =ns	0.9	0.6 to 1.5
6 months	> 50% DS Fup	41%	53%	p=ns	0.8	0.6 to 1.0
	(visual)					
	Clinical	17%	34%	p<0.01	0.5	0.3 to 0.8
	(MI,re-PTCA,CABG,Death)					
6 months	change in % -D.S.	-28%DS	-18%DS	p<0.05		
	(pre Fup)					
6 months	> 50% DS Fup		38%			
		27%		p=ns	0.7	0.2 to 2.0
		16%		p=0.002	0.4	0.3 to 0.7
6 months	loss > 50% gain	50%	41%	p =ns	1.3	1.0 to 1.8
4-6 mths	NR (QCA)	33%	29%	p=ns	1.1	0.4 to 2.8
6 months	MLD (F-up – pre)	0.27 mm	0.24 mm	p=ns		
	MLD ≥0.72 mm	15 %	14 %	p=ns	1.1	0.8 to 1.6
6 months	Clinical/>50% Fup	3%	30%	p<0.05	10	0.01 to 0.99
6 months	MLD (post-PTCA)	-0.27 ±0.51 mm		p=ns	1.0	0.7 to 1.4
	- MLD (Fup)	-0.29 ±0.49 mm				
NR	return ≥70% DS	41%	45%	p=ns	0.9	0.6 to 1.4
	and loss of ≥ 50% gain					
6 months	Clinical and	48%	41%	p=ns	1.1	0.6 to 1.9
	thallium exercise test					
6 months	loss > 50% gain	19%	42%	p<0.05	0.5	0.2 to 1.0
NR	loss of gain	43%	48%	p=ns	0.8	0.5 to 1.4
3 months	> 50% DS Fup	33%	33%	p=ns	1.0	0.6 to 1.7
6 months	> 50% DS Fup	59%	56%	p=ns	1.1	0.7 to 1.7
4-8 mths	> 50% DS Fup	43%	43%	p=ns	1.0	0.8 to 1.2
	(caliper)					
3 months	loss ≥ 50% gain	19%	38%	p<0.05	0.5	0.25 to 0.97
5-10mths	> 70% DS Fup	15%	22%	p=ns	0.7	0.3 to 1.7
	(visual)					
12 mths	≥ 70% AS + loss	36%	32%	p=ns	1.1	0.7 to 1.9
	of ≥ 50% gain					
6 months	loss > 50% of gain	29%	33%	p=ns	0.9	0.5 to 1.4
	> 50% DS Fup					

Table III continued

Author		Drug	Dose	Patients total	F-up
Hoberg et al.	1990	Verapamil+ASA+D ASA + D	480 mg/day 660 + 330 mg/day	196	88%
Slack et al.	1987	Fish oil Placebo	2.4 g/day	162	85%
Reis et al.	1989	Fish oil Placebo	6.0 g/day	186	30%
Milner et al.	1989	Fish oil Placebo	4.5 g/day	194	23% 100%
Dehmer et al.	1988	Fish oil Placebo	3.2 g/day	82	100%
Grigg et al.	1989	Fish oil Placebo	3.0 g/day	108	94%
Franzen et al.	1990	Fish oil Olive oil	3.15 g	151	85%
Cheng et al.	1990	max EPA ASA	10 caps./day 300 mg started 2 weeks prior to PTCA	50	86%
Bairati et al.	1992	max EPA Olive oil	15 g	205	58%
Sahni et al.	1989	Lovastatin Placebo	20-40 mg/day	157	50%

AS= Area stenosis; ASA= Acetylsalicylic Acid; D= Dipyridamole; Ds= Diameter stenosis; F-up= Follow up (% of successful PTCA); mp= methylprednisolone; ms= months; nor PTT= normal prothrombin time; NR= Not Reported; ns= not significant; p= p-value; pt= patient; Sign= Significance; TXA2= Thromboxane A2 synthetase inhibitor; tic= ticlopidine

Time	Definition restenosis	Restenosis(%) drug vs placebo		Sign	Risk ratio	95% CI
5 months	loss of ≥ 50% gain					
	in unstable pat.	56%	62%	p=ns	0.9	0.6 to 1.3
	in stable pat.	38%	63%	p<0.05	0.6	0.4 to 1.0
6 months	Clinical	16%	33%	p<0.05	0.5	0.2 to 1.0
	Stress test – Ä +	67%	58%	p=ns	1.2	0.7 to 1.8
6 months	> 70% DS F'up	34%	23%	p=ns	1.5	0.9 to 2.5
6 months	> 50% DS F'up	18%	27%	p=ns		
	Clinical					
	Stress test – Ä +	19%	35%	p<0.01	0.6	0.4 to 1.0
6 months	> 50% DS F'up	19%	46%	p<0.007	0.4	0.2 to 0.8
	(visual)					
3-5 mths	loss > 50% of gain	34%	33%	p=ns	1.0	0.6 to 1.8
	(caliper)					
6 months	NR	37%	33%	p=ns	1.1	0.7 to 1.8
6 months	> 50% DS at F'up	20%	26%	p=ns	0.8	0.3 to 2.3
6 months	MLD follow-up	35.6%	53.3%	p=0.05	0.7	0.4 to 1.0
	- MLD post-P'TCA					
	≥ 0.50 mm					
2-10 mths	> 50% DS F'up	14%	47%	p<0.001	0.3	0.2 to 0.6

Risk ratio with 95% confidence intervals (CI). An risk ratio of < 1 means that a lower restenosis rate is seen among the patients treated with the new drug compared with those who received placebo. A statistical significant ($p < 0.05$) lower restenosis rate is seen in those studies where the 95% confidence intervals do not cross risk ratio of 1. Risk ratio of more than 1 indicates a higher restenosis rate among the patients treated with the new drug.

3.2.11 GlycoproteinIIb/IIIa receptor blockers The clinical importance of the glycoprotein IIb/IIIa (GP IIb/IIIa) receptor is indicated by the effect of its absence in patients with Glanzmann thrombastenia who suffer from mucocutaneous bleeding (George et al. 1984). The GP IIb/IIIa complex is one of the most abundant platelet cell surface protein (Phillips et al. 1988), which, when activated, binds to the adhesive protein vWF, fibronectin, vitronectin and fibrinogen, possibly by sharing a common binding site or peptide recognition sequence, and can be considered to be a final common pathway by which all antagonists act as initiators of platelet aggregation. The dimeric structure of fibrinogen makes binding to, and thus aggregation of, two separate platelets possible. GP IIb/IIIa receptor antagonists may be effective in research in restenosis prevention, because of their ability to decrease acute platelet mass adherent at the site of angioplasty.

The antithrombotic properties of monoclonal antibodies against the GPIIb/IIIa receptor in dogs and baboons showed a remarkable inhibition of platelet deposition onto thrombogenic surfaces (Coller et al. 1986; Hanson et al. 1988).

Chimeric 7E3 is a fragment of a genetically reconstructed murine immunoglobulin G (IgG) antibody, inwhich portions of the murine-derived antibody have been replaced by corresponding regions from human IgG. Subsequently, the protease papain cleaves the Fab fragment, resulting in a pure substance with a high affinity for GPIIb/IIIa. Receptors blocked by c7E3 are unable to bind the natural ligands fibrinogen and vWF, thereby preventing platelet aggregation. Platelet adhesion, however, and as subsequent release of mitogens from alpha-granules, is unaffected.

The activity of c7E3 was demonstrated by Kaplan et al. (1991a) who observed platelet deposition in an ex vivo whole artery system, following balloon angioplasty. Platelet deposition at the site of injury was reduced 70% by c7E3, when compared with heparin. One month after rabbit femoral artery was dilated with prior infusion of a GP IIb/IIIa antagonist, lumen diameter in the treated group was significantly wider than in the controls (Azrim et al. 1991).

C7E3 appears to be safe during PTCA, with inhibition of platelet function in a dose-dependent fashion, and may be useful in prevention of abrupt closure and possibly of restenosis (Coller 1990; Ellis et al. 1990).

Numerous cysteine-rich peptides, containing the tripeptide Arg-Gly-Asp (RGD), have been isolated from the venom of various vipers, and have shown potent inhibitory effects on platelet aggregation and fibrinogen binding (Gould et al 1990). This RGD sequence is found in many extracellular matrix and platelet adhesion proteins, such as the GPIIb/IIIa receptor, acting as a common binding sequence. Barbourin, a peptide from the southeastern pygmy rattlesnake, shows a very high specificity for only the GPIIb\IIIa receptor. This venom derived protein contains a Lys-Gly-Asp sequence (KGD) as the recognition site (Scarborough et al. 1991). A report from Hanson et al. (1991) reported the evaluation of two cyclic synthetic peptides (SB-1, and SB-6). Using these barbourin-based structures, platelet deposition was significantly reduced on Dacron implants in baboons.

Shebuski and coworkers (1990) used synthetic Echistatin in a dog model.

Intravenous infusion with Echistatin 10 and 20 μg/kg/min was a potent aggregation inhibitor, prolonging bleeding time 3-fold. In vivo experiments, evaluating the effects of RGD- and KGD- containing peptides on thrombus formation and subsequent neointimal formation after balloon induced vessel wall injury, are still awaited.

3.3 Anti-proliferative drugs

After the disruptive action of balloon dilatation, smooth muscle cells respond by proliferation. Cell characteristics shift from the contractile to the synthetic phenotype, which results in an extra cellular matrix deposition. Since one of the key features of restenosis is the uncontrolled proliferation of vascular smooth muscle cells, antiproliferative agents have been considered as an attractive concept.

3.3.1 Angiotensin converting enzyme inhibitors *Animal studies.* Several organs contain local angiotensin converting enzyme (ACE) systems (Dzau 1988). It appears that both the production of angiotensin II (A II) and its interaction with specific A II receptors (e.g. on medial smooth muscle cells) may take place in these tissues independent of the plasma renin angiotensin system (Dzau 1988). In the hypertensive rat model, the formation of a neointima (SMC proliferation) in aging rats is accelerated, and it has been demonstrated that treatment of chronic hypertensive rats with ACE inhibitors reduces the medial hypertrophy of muscular arteries (Owens 1987), and it has been postulated that the local renin-angiotensin system plays an important role in the remodelling process after arterial injury (Powell et al. 1989a). Furthermore, there is evidence to support the role of A-II as a co-mitogen responsible for intimal hyperplasia after PTCA (Deamen et al. 1991). Several research groups have investigated the effect of A-II and ACE-inhibition on the proliferation of vascular smooth muscle cells. Genes encoding platelet derived growth factor (PDGF-A), transforming growth factor-ß (TGF-ß), and thrombospondin could be induced by A-II to express m-RNA activity, and thus result in cell proliferation (Scott-Burden et al. 1990; Naftilan et al. 1989; Powell et al. 1989b, 1991), but could not be inhibited by an ACE inhibitor or metabolites.

Thus the antiproliferative effect of ACE-inhibition is mediated through A-II, and consequently may prevent the proliferative response. In rats, neointima formation was reduced by 80%, 14 days after balloon dilatation of the left carotid artery when an ACE inhibitor was given either 6 days before, 1 hour before or 2 days after angioplasty and continued until 14 days after the procedure (Powell et al. 1989b). This effect seems to be dose dependent and is synergistic with the effect of heparin. There was no effect with administration of a single dose or when it was discontinued 2 days after balloon dilatation. Further study has shown that captopril 100 mg/kg/day also reduced intimal hyperplasia to almost the same extent. Two other non-ACE inhibitor vasodilators, verapamil and hydrazaline, demonstrated a lesser effect (Müller et al. 1989). These results indicate that hemodynamic effects on vascular walls may influence the formation of intimal hyperplasia after balloon catheterization, and that ACE-inhibitors may reduce intimal hyperplasia through additional mechanisms related to inhibition of the angiotensin system.

However, Lam et al. (1990) found no effect on atherosclerotic changes after balloon angioplasty of the carotid artery in 3-month-old pigs, despite adequate plasma ACE activity inhibition. Similarly, Bilazarian et al. (1991) demonstrated in a hypercholesterolemic rabbit atherosclerotic model of balloon angioplasty that cilazapril started 1 week prior to angioplasty of the iliac artery until final angiography gave less reduction in diameter compared with the placebo group. Miyauchi et al. (1992) showed that this effect on restenosis was limited to vessels with intimal damage but without interruption of the internal elastic membrane. Cilazapril 10 mg daily, starting 7 days before balloon inflation and continued for 21 days could not reduce intimal hyperplasia when both the intima and the media were damaged.

Bilazarian (1992) studied angiotensin II antagonism in rabbits. To determine whether the effects were due to a reduction of A II or to another effect of ACE-inhibitors, this group gave losartan potassium 50 mg/day (a direct inhibitor of A II) or placebo starting 1 week before balloon angioplasty, continuing until 4 weeks later. Minimum lumen diameter was not reduced significantly, which suggests that reduction of angiotensin levels is not the primary mechanism leading to neointima formation inhibition.

Santoian et al. (1991) treated normolipidaemic swines with enalapril prior to balloon angioplasty until they were sacrificed at 14 or 28 days. They could not show significant effects on reduction of neointimal hyperplasia. Huber et al. (1991) administered two different ACE-inhibitors in 20 animals, 6 days prior and 28 days after injury in porcine coronary arteries. Mean neointimal thickness was not significantly different versus controls.

Human studies Several studies have assessed the effect of ACE-inhibition on renarrowing of the coronary vessel after treatment with balloon angioplasty in humans.

Recently Freed et al. (1993) reported the results of a nonrandomized pilot study using multiple drugs, namely enalapril 2.5 mg bid, lovastatin 20-40 mg bid., and colchicine 0.6 mg bid. This drug cocktail was administered from 1 week prior to intervention to folluw-up angiography. Despite the multiple targets of drug action, restenosis occurred in 33% of 50 patients.

Brozovich et al. (1991) retrospectively analyzed records of 322 patients with a successful angioplasty and separated them into 2 groups according to presence (N=36) or absence (286) of a drug regimen that included an ACE inhibitor. Restenosis (return of symptoms of angina with a significant stenosis on the angiogram) occurred in 30% of the patients treated without ACE-inhibitors versus 3% in the group who received this class of drug. Thus, it appears that inhibition of ACE may significantly reduce the incidence of restenosis after successful PTCA.

Currently, a large multicentre randomised trial in Europe (MERCATOR = Multi-center European Research Trial with Cilazapril after Angioplasty to prevent Transluminal coronary Obstruction and Restenosis) has just been completed to determine the effect of cilazapril on the incidence of restenosis (MERCATOR study group 1992). More than 700 patients were randomised to cilazapril

or placebo starting 4 to 6 hours after PTCA, in addition to standard therapy of aspirin 200 mg. After 6 months (or earlier if indicated by symptoms), a follow-up angiogram was performed. The loss in minimal luminal diameter at follow up was 0.29 ± 0.49 mm in the control group and 0.27 ± 0.51 mm in cilazapril treated patients. Using a dichotomous, frequently used definition of restenosis (loss of > 50% of the gain of PTCA or > 30% increase in diameter stenosis), the restenosis rate was 39% in the control group and 37% in cilazapril recipients. Treatment did not reduce the incidence of clinical events. A similarly designed trial (MARCATOR), investigating cilazapril 1, 5, or 10 mg/day versus placebo has recruited more than 1400 patients in the United States and Canada. The results of this trial do not indicate a role for cilazapril in restenosis prevention (Faxon et al. 1992a). Final end point data have yet to be published, as well as the results of an ongoing study in Belgium that was discontinued after randomisation of 508 patients to fosinopril 40 mg/day or placebo because of a disappointing interim analysis (Desmet et al. 1992). Thus, there seems no role for ACE inhibition in restenosis prevention.

3.3.2 Colchicine Colchicine is an alkaloid derived from the plant Colchicium autumnale, which causes arrest in the metaphase of cell division by binding to tubulin and interfering with microtubule-related functions, including synthesis and secretion of polymorphonuclear cell and monocyte chemotactic factors and collagen; increase in collagenase activity (Muller et al. 1991); inhibition of the proliferation and migration of smooth muscle cells; and inhibition of the release of chemotactants by leucocytes (Currier et al. 1989). Recently Bauriedel et al. (1991) showed a loss in regional pseudopodial activity in smooth muscle cells cultured from human plaque tissue after adding colchicine.

To study the effect of colchicine on restenosis in vivo, atherosclerotic rabbits with >50% diameter stenosis underwent iliac transluminal angioplasty (Currier et al. 1989). Colchicine was started 2 days before angioplasty (0.02 or 0.2 mg/kg per day) until follow-up angiography at 4 weeks. High dose colchicine significantly decreased the diameter of stenosis at follow up, although no effect was seen with the lower dose. It has to be mentioned that 0,2 mg/kg per day in humans would almost certainly will give severe adverse effects.

O'Keefe et al. (1992) randomised 197 patients (2:1) to colchicine 1.2 mg/day versus placebo within 24 hours of PTCA, until 6 months follow-up angiography. Because of side effects (diarrhoea and dyspepsia), there was a 6.9% withdrawal rate of colchicine-treated patients. The restenosis lesion rate (return to > 70% stenosis and loss of 50% of initial gain) was 22% in both groups.

In a double-blind placebo controlled study, 253 patients were randomised to colchicine 1 mg daily or placebo, initiated prior to balloon angioplasty and continued for 1 month (Grines et al. 1991). Clinical evaluation and exercise thallium tests in 79% of the studied population demonstrated no impact or clinical evidence of early or late restenosis. Thus colchicine seems to be ineffective for the prevention of restenosis after PTCA (fig 3).

Wilensky et al. (1992) evaluated the antiproliferative concept with locally delivered colchicine. A solution of 0.1 or 10 μM/L was delivered using a porous

balloon drug delivery catheter in animals. Contalateral arteries served as controls in the treated animals. The arterial dimensions, determined by angiography and histological examination, revealed no significant differences. Thus colchicine seems to be ineffective for the prevention of restenosis after PTCA.

3.3.3 Platelet-Derived Growth Factor Antagonists *Animal studies* Proliferation of vascular smooth muscle cells during the development of atherosclerosis and after coronary angioplasty might be initiated by the release of growth factors. Smooth muscle cells from diseased human arteries are known to possess mitogenic activity and these cells express the gene for the PDGF-A chain selectively (Ferns et al. 1991). Besides an excessive proliferative response, the formation of connective tissue of great importance in luminal narrowing. In this way, it could play an important role in the development of restenosis (Libby et al. 1988; Ross 1989; Majesky et al. 1990).

The squalene epoxidase inhibitor terbinafine, usually used as an antifungal agent, has shown inhibitory PDGF an FGF activity in in vitro experiments. However, pretreatment with terbinafine 200 mg/kg/day for two days prior to balloon angioplasty followed by 100 mg/kg/day for 28 days in 16 hypercholesterolaemic rabbits resulted in an only slight decrease in luminal narrowing compared with 15 controls on quantitatively analysed angiograms (Haber et al. 1991). Reduction in luminal diameter was 0.62 and 0.71 mm, respectively.

Trapidil (= triazolopyrimidine) has been shown to inhibit cellular proliferation induced by PDGF in cell culture, and intimal thickening in damaged carotid arteries. In a model of atherosclerosis, rabbits were assigned to placebo (n=8) or trapidil 60 mg/kg/day (n=9). The medication was started 2 days before balloon dilatation of the external iliac artery and continued for 4 weeks. Follow-up angiography showed a greater luminal reduction in the control group than in the trapidil group compared with baseline values ($p < 0.001$), and there was significantly less histologic al evidence of intimal hyperplasia associated with trapidil administration (Liu et al. 1990).

Human studies Okamato et al. (1992) randomised 97 patients to trapidil 600 mg/day, beginning 1 week before PTCA and continuing for 6 months, or to aspirin 300 mg/day and dipyridamole 150 mg/day. Restenosis (loss of ≥ 50% of initial gain) occurred in 7 of 36 trapidil recipients and in 15 of 36 control patients (42%) who had follow-up coronary angiography. Thus, trapidil has been shown to be effective in preventing restenosis after PTCA in this small group of patients (Fig. 3).

Nishikawa et al. (1992) randomised 160 patients to either trapidil 200 mg tid, or to dipyridamole 50 mg tid, both in combination with aspirin, starting 2 days prior to intracoronary intervention and continued for a 4 month period. Quantitatively assessed restenosis (> 50% loss of the initial gain) was present in 13 of 65 in the trapidil treated group (20%), and in 27 of the 72 controls (38%). Coronary dimensions evolved from 22 vs 21% stenosis directly after balloon angioplasty, to a final stenosis severity of 39 % in the trapidil group and 49% in the dipyridamole group.

Maresta and coworkers (1992) intend to enrol 360 patients in a double-blind randomised study comparing trapidil 100 mg tid with aspirin 100 mg tid. Preliminary results based on 260 patients, of whom 159 are restudied showed a slightly better outcome in the trapidil-treated group (loss of the gain of 33% vs 41% . Final analysis of this studie is awaited, but these studies indicate a possible role for trapidil in restenosis prevention.

Experimental work with monoclonal antibodies against PDGF is currently going on and described in the chapter 'future directions' (section 4).

3.3.4 Angiopeptin It has been known for some time that hypophysectomy inhibits neointimal plaque formation in response to endothelial injury (Tiel et al. 1978; Fingerle et al. 1992). The last group recently presented evidence that smooth muscle cells do not alter after hypophysectomy, but that proliferative capacities require pituitary hormones in order to respond after injury. This suggest that an endocrine factor may be involved in neointima formation.

Somatomedin (insulin-like growth factor; IGF-1) has been shown to be involved in the repair of the intima in injured arteries. Further, it is (like PDGF) a potent mitogen for porcine aortic smooth muscle cells, and when added together to quiescent cultures their effects are synergistic (Hansson et al. 1987; Clemmons et al. 1985). Recently the effect of a newly synthesised class of pituitary growth hormone inhibiting agents on vascular smooth muscle cell hyperplasia after endothelial cell injury in-vivo has been investigated. These compounds are peptide analogues of somatostatin, and have high affinity for somatostatin receptors on pituitary cells. They inhibit the release of pituitary growth hormone and somatomedin. One of these agents, angiopeptin, was shown to inhibit vascular smooth muscle cell proliferation in response to a variety of vascular injuries. This seems to be a result of a local effect directly on autocrine or paracrine mechanisms in cell replication (Foegh et al. 1989). This new group of agents is currently undergoing investigation as inhibitors of several variants of 'accelerated atherosclerosis' (postangioplasty, cardiac transplantation and coronary bypass surgery).

The somatostatin analogue octreotide reduced smooth muscle cell proliferation, stimulated by PDGF and somatomedin, in terms of thymidine uptake by 58% at a concentration of 100nM/L (Wargovich and Grant 1992). No significant effects on chemotaxis were seen in these cells.

Animal studies Hong et al. (1991) administered saline or angiopeptin in 3 doses (1, 10, or 100 μg/ml) during angioplasty in rabbit abdominal aortas. Electron microscopy after 3 weeks showed in all the angiopeptin allocated groups that the intima was lined by endothelial cells, while the control group showed mainly modified SMC and intimal hyperplasia. Using isotope-labelled angiopeptin, there was evidence that inhibition of SMC-proliferation occurs at the nuclear level.

A significant decrease of smooth muscle cell proliferation has also recently been reported a porcine modelin (Santoian et al. 1992). After angioplasty with oversized balloons and 14 days' subcutaneous administration of angiopeptine, the treated group showed a significant improvement in intimal and luminal area

(p=0.008) compared with untreated controls. After two more weeks without angiopeptin treatment, this beneficial effect was no longer seen. Santoian et al. (1993) demonstrated a greater inhibition of hyperplasia with systemic versus local angiopeptin administration.

Conte et al. (1990) showed that angiopeptin 20 μg/kg/day given one day before injury of the iliac artery and aorta and then continued for 2 or 6 significantly reduced intimal thickness in rabbit iliac arteries, when examined after 3 weeks.

Howell et al. (1991) showed a significant effect of angiopeptin 10 μg/kg given subcutaneously twice daily on intimal hyperplasia after 23 days, when the initial dose was given prior or during angioplasty. Angiopeptin treatment was ineffective when started 8 or more hours following angioplasty.

Both of these studies indicate an early mechanism of growth inhibition by angiopeptin.

Human studies An important restenosis prevention study in the US involving 1240 patients tested angiopeptine in four treatment groups. The results of a European trial, recruiting a total number of 450 patients to receive either routine angioplasty with aspirin and heparin therapy, or with an infusion of angiopeptin 6mg daily, starting 24 hours prior to PTCA and continued for 72 hours, will be published soon. These studies should answer the promising results achieved with angiopeptin in animals are applicable to the prevention of restenosis in humans.

3.3.5 Cytostatic agents In the proliferative concept, the use of cytostatics seems very useful. The principle concern with these agents is the potential for serious adverse effects, because of the capability of these drugs to damaging other rapidly dividing cells, e.g. those in the gastrointestinal tract, bone marrow and reproductive system.

Interest in the antimitotic approach has been stimulated by a study of Voisard et al. (1991) who collected human VSMC from primary stenosing lesions and from restenotic lesions, and observed a significantly increased growth rate in the latter group. Etoposide added to the cultures was able to inhibit VSMC proliferation by more than 50% . This agent has not yet been tested in vivo.

Recently the effects of locally administered doxorubicin on restenosis were reported by Currier et al. (1992) in hypercholesterolemic rabbits. Remarkably, local doxorubicin infusion showed significant inhibition of luminal renarrowing (reduction in lumen diameter from 1.56 to 1.00 mm) compared with balloon angioplasty combined with saline infusion (1.51 to 0.55 mm), but not when compared with balloon angioplasty alone (1.40 to 0.89) These changes suggest that the damage to vascular tissue caused by local drug delivery is greater than the antiproliferative effects of doxorubicin.

Barath et al. (1989) hypothesised that cytostatic agents may prevent restenosis by selective toxicity against active and proliferating smooth muscle cells without damaging normal smooth muscle cells. In their study, rabbits were divided into 4 groups: the first group was a control group, the second group had only a balloon dilatation of the aorta, the third group received the cytostatic agents

vincristine 0.075 mg/kg and dactinomycin 0.015 mg/kg, and the fourth group underwent balloon dilatation and received cytostatic agents. Three days later, electron microscopic findings showed that these cytostatic agents prevented smooth muscle cell proliferation without damaging the normal smooth muscle cells.

McKenney et al. (1991) examined the effects of administering cyclosporine 15 mg/kg, starting 3 days prior to angioplasty for 4 weeks, in 10 hypercholesterolemic rabbits. Comparison of angiographic and histological data with 10 controls showed no beneficial effect; on the contrary, an increase in media thickness occurred in the treated group, because of increased accumulation of foam cells. Similarly local delivery of methotrexate via an infusion balloon catheter into carotid arteries following balloon angioplasty in 10 Yucatan minipigs resulted in no difference in intimal proliferation compared with placebo (Muller et al. 1990). Murphy et al. (1990) used methotrexate 1.25 mg/day for 5 days each week or 20 mg intramuscular, or azathioprine 25 mg/day in stenotic porcine coronary arteries. They found no difference in stenosis size (measured by histology) in the different groups.

In another in vivo study, Cox et al. (1991) placed polymer-coated balloon-expandable stents into the circumflex artery of 40 pigs. Four groups were studied: the first received an uncoated tantalum stent, the second a heparin-coated stent, the third a methotrexate-coated stent, and the fourth group received a stent coated with both heparin and methotrexate. Analysis, 28 days after implantation revealed no significant differences in percentage restenosis or intimal area between the four groups.

The results reported so far suggest that there seems to be no role for cytostatic agents in the prevention of restenosis in human coronary vessels.

3.3.6 Photodynamic Therapy Deckelbaum et al. (1992) evaluated the effect of methoxsalen (8-methoxypsoralen) 1μg/ml in combination with different doses of visible light (420 nm) on the proliferation of cultured bovine smooth muscle cells. Low dose light caused a high percentage of proliferation inhibition, whereas high doses were cytotoxic.

March et al. (1992) tested ultraviolet radiation exposure in combination with the photoactive agent methoxsalen. They determined strong synergism in inhibiting DNA synthesis in cultured bovine aortic smooth muscle cells. Progression through the cell cycle could be blocked at any phase by inverse variations of delivered dose and energy.

A Japanese group (Asahara et al. 1993) used the combination of mercury-xenon flash lamp light and a haematoporphyrin derivative 24 hours before irradiation in rabbit aortas. Photodynamic therapy applied at different intervals after angioplasty demonstrated the most effective intima suppression at 1 week.

The new concept of photodynamic therapy seems promising, but needs more in vitro testing and in vivo validation.

3.3.7 New antiproliferative drugs Using a Wolinsky porous infusion catheter, Wilensky et al. (1991a) injected a solution of a thiol protease inhibitor (TPI) into

atherosclerotic rabbit femoral arteries immediately following angioplasty to evaluate the effect on restenosis. They found a smaller reduction in minimal luminal diameter after 2 weeks in the TPI-treated group than in the control group. They hypothesised that the process of vascular remodelling after angioplasty is significantly modified by TPI.

Betz et al. (1990) used a calcium-calmodulin antagonist and found it to be effective in inhibiting smooth muscle cell proliferation after application of atherogenic stimuli in a dose-dependent manner. These newer drugs could thus be used for inhibiting stenosis of intimal proliferation.

Lafont et al. (1992) reported the antiproliferative action of the well known anti oxidant α-tocopherol (vitamin E). Rabbits, pre-treated with α-tocopherol and a cholesterol-rich diet, underwent balloon angioplasty 28 days after induction of a stenotic lesion by air dessication. Angiographic follow up after 21 days showed a significantly greater minimum diameter in the treated group (1.70 vs 1.07 mm, $p < 0.01$). Histological evaluation of the narrowed segments showed significantly less intima and media area than in the control group (0.69 vs 1.16 mm^2,) and thus confirmed the angiographic results of this approach.

3.4 Anti-inflammatory agents

Corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) have proven immunosuppressive potency. Since restenosis can be explained as the result of a general wound healing process in injured vascular tissue (Forrester et al. 1991), inhibition of the inflammatory phase is an interesting goal. It is evident that accumulation of immuno-active cells and release of soluble mediators that stimulate migration, growth and activation of VSMC and fibroblasts, leads to a complex immunological and inflammatory response.

3.4.1 Corticosteroids Corticosteroids affect the quantity and the quality of circulating and accumulating lymphocytes and monocytes, while also inhibiting PAF-formation (MacDonald et al. 1987). Responsiveness of monocytes to lymphokines and the lymphocyte proliferation are suppressed, probably due to decreased release of PDGF and macrophage-DGF (Berk et al. 1991). Furthermore SMC chemotaxis and proliferation, as well as collagen synthesis by fibroblasts is inhibited by this class of drugs. The reduction in proliferation is the consequence of decreased RNA and protein synthesis inhibition (Gordon et al. 1987, Berk et al. 1991).

The results of local dexamethasone delivery in the carotid artery of rats, using silicone drug-eluting polymers as carriers, have been described (Villa et al. 1993). In 8 control animals, placebo polymer implants did not induce neointima formation. However, in treated and de-endothelialised segments, the neointimal area was significantly affected at 3 weeks and, due to systemic steroid activity, this effect was also seen in segments that did not contain the polymers in the adventitia.

To test these drug clinically, Rose et al. (1987) randomised 66 patients to placebo or methylprednisolone from 48 hours before to 5 days after PTCA. Angiographic restenosis (> 50% stenosis at 3 months' follow-up) revealed no dif-

ference between the two groups, 33% in each. The same results were achieved when corticosteroids were given to 102 patients with restenosis after PTCA (Stone et al. 1989). In addition to aspirin, dipyridamole and a calcium antagonist, patients received methylprednisolone 125 mg intramuscular 1 day before repeat-PTCA and prednisone 240 mg for 1 week. Only the 54 patients undergoing follow-up angiography were analysed. Restenosis (> 50% diameter narrowing) was found in 59% of the patients in the group treated with steroids compared with 56% of the patients receiving standard treatment.

Similarly, in a multicentre trial in the United States (Pepine et al. 1990), 850 patients were randomly assigned to methylprednisolone or placebo 2 to 24 hours before PTCA. In 71% of the patients, follow-up angiography was performed. The incidence of restenosis (> 50% diameter stenosis) was 43% both groups.

These trials demonstrate that administration of corticosteroids has no influence on the incidence of restenosis (Figure 3).

3.4.2 Nonsteroidal Anti-Inflammatory Drugs Ibuprofen is known to decrease platelet-thrombus deposition in arterial grafts. In a study of normal porcine common carotid arteries pretreated with heparin, balloon angioplasty was performed and followed by a bolus (12.5 mg/kg) and infusion (75-100 μg/kg/min) of ibuprofen or placebo. Quantitative ^{111}In labeled autologous platelet deposition at the site of angioplasty was significantly reduced by ibuprofen (Lam et al. 1987). Whether this will affect the risk of late restenosis is unknown.

Ebselen is a newly synthesised selenium containing compound with strong anti-inflammatory characteristics. Recently a Japanese group reported a randomised study that included a 3-month follow-up angiography (Hirayama et al. 1992). Twenty-nine patients received Ebselen 200 mg p.o. b.i.d., and the control group comprised 50 patients given placebo. Lesion restenosis rate (loss of ≥ 50% of the initial gain) was 48.2% in the placebo recipients and 28.6% in the Ebselen-treated group ($p < 0.05$) (Figure 3). These results suggest strong preventive effects on restenosis. Further details of this studie, as well as a larger scale trial with Ebselen are eagerly awaited.

3.5 Calcium antagonists

Coronary spasm is frequently seen during and shortly after PTCA and may have a role in the pathogenesis of restenosis (David et al. 1982). Platelet deposition at damaged endothelium induces coronary spasm and the formation and subsequent organisation of thrombus (Steele et al. 1985, Wilentz et al. 1987). Calcium antagonists may thus reduce the incidence of restenosis by inhibiting vasospasm.

In an animal model (Faxon et al. 1984) and in 3 randomised trials in humans (Corcos et al. 1985; O'Keefe et al. 1991; Whitworth et al. 1986), calcium antagonists have not been shown to influence the incidence of restenosis. However, verapamil was effective in patients with stable angina pectoris (Hoberg et al. 1990). Moreover, nifidepine and nicardipine are effective in trials investigating atherosclerosis progression and regression, so there may still be a role for calcium antagonists in preventing restenosis or the development of new atherosclerotic lesions (Lichtlen et al. 1990; Waters et al. 1990) (Figure 3).

3.5.1 Diltiazem In a study from the Montreal Heart Institute (Corcos et al. 1985), 92 patients received diltiazem 270 mg/day for 3 months and underwent a recatherisation 5 to 10 months after balloon angioplasty, or earlier if symptoms returned. All patients also received daily aspirin 650 mg and dipyridamole 225 mg for 6 months. Patients treated with diltiazem had a restenosis rate of 15% versus 22% in the patients not receiving the drug (restenosis defined as stenosis of ≥70% at follow-up angiography). The average decrease in diameter during follow-up was 4% in the diltiazem group and 7% in the control group. It was concluded that diltiazem had no effect on restenosis and that coronary spasm is not a major mechanism of restenosis.

In a further trial, a total of 201 patients were randomised to receive high dose diltiazem (started 1 day before PTCA, mean dosage 329 mg/day) or to placebo (O'Keefe et al. 1991). Repeat angiography at 1 year was obtained in 60% of the patients. Restenosis was assessed by quantitative angiographic techniques and was defined as return to ≥ 70% or luminal area stenosis and loss ≥ 50% of the initial gain with angioplasty. No difference in procedural complications (6 of 102 diltiazem vs. 8 of the 99 placebo recipients) or restenosis rate (36% versus 30%) were observed.

An abstract from Unverdorben et al. (1992) reported the beneficial effects of Diltiazem 180 mg, compared with placebo in 170 patients. Recatheterisation at 3 to 4 months showed a significant effect on average luminal narrowing (38.6 vs 50.3%, $p< 0.01$), while the restenosis rate (≥ 50%-criterium) also appeared to be significantly lower (18/84 vs 33/86 patients $p < 0.03$). These effects were more pronounced in subgroups such as diabetic patients, those with hypercholesterolemia or classified in CCS class II, and in calcified plaques.

3.5.2 Nifedipine In a 6 month follow up trial 241 patients were randomly assigned to receive either nifedipine 40 mg/day or placebo (Whitworth et al. 1986). All patients also received aspirin 325 mg/day. Restenosis was defined as a loss of more than 50% of the gain achieved at the time of the PTCA. In patients who were compliant with medication and underwent follow-up angiography (84 patients in both groups) there was no difference in restenosis rates: 29% in the nifedipine group and 33% in the placebo group.

3.5.3 Verapamil Recently Hoberg et al. (1990) conducted a restenosis prevention trial in which 196 patients were randomised to verapamil 480 mg/day or placebo in combination with aspirin 660 mg/day and dipyridamole 150 mg/day. Follow up angiography at 6 months was performed to detect recurrence of the lesion (≥ 50% loss of the initial gain). In a subgroup of 88 patients with unstable angina there was no beneficial effect of verapamil treatment (restenosis rate 56% versus 62% with placebo). However, in a subgroup with stable angina, there was a significant reduction in restenosis (38% vs 63%), demonstrating a relative risk of 0.6 ($p = 0.038$). This indicates that a high dose of verapamil prevents restenosis after successful PTCA in patients with stable angina. No difference was found in the group with unstable angina before PTCA.

Data pooling of these four clinical trials using calcium antagonists reveals no

effect of this type of drug on recurrence of stenotic lesions (Figure 3).

3.6 Lipid Lowering Drugs

3.6.1 Fish Oils Epidemiological trials have shown that a diet rich in omega-3 polyunsaturated fatty acids (present in high concentrations in most salt water fish) may account for the low incidence of coronary disease seen in Eskimos. Animal research has shown that these polyunsaturated fatty acids inhibit atherosclerosis in general. This can be partly explained through a reduction in triglycerides and VLDL cholesterol. Reduced VLDL levels is the consequence of suppression of hepatic synthesis and increased clearance by the liver or peripheral tissues. Another effect is on eicosanoid metabolism and function by inhibition of platelet production of TXA_2 while production of endothelial PGI_2 is only slightly reduced, and is partially compensated by the synthesis of PGI_3 (Leaf 1990). These effects enhance vasodilatory and antiaggregatory effects.

Omega-3 fatty acids also inhibit the production of leukotriene B_4 by a mechanism of competition with arachidonic acid that results in the production of the less potent leukotriene B_5 (Lee et al. 1985). Other cardiovascular and hemodynamic effects of fish oil include changes in membrane qualities, resulting in platelet aggregation inhibition (Croset & Lagarde 1986), and reduction in blood viscosity (Terano et al. 1983). Furthermore, vasodilatation is stimulated by increased endothelial-dependant relaxation and reduced sensitivity to vasospastic stimuli. Finally, reduction in blood pressure and increased fibrinolytic activity (Barcelli et al. 1985) have to be mentioned in the ever growing list of fish oil qualities.

Slack et al. (1987) showed that adding 2.4 g fish oil (rich in eicosapentaenoic acid (EPA)) each day to the usual post-PTCA regimen of calcium channel blocker, nitrates, aspirin and dipyridamole could reduce the incidence of clinical restenosis in patients with single vessel disease (16 vs 33% in the placebo group). In 49 patients with multi-vessel disease, no influence could be shown.

However, Reis et al. (1989) demonstrated in a double-blind trial that supplementing the normal diet with 6.0g of fish oil daily, starting just before PTCA until 6 months later, had no influence on restenosis rate in 186 patients in whom PTCA was successful. Angiographic restenosis (70% diameter stenosis at a site previously dilated to <50%) was present in 34% of the group taking fish oil and 23% of the control group. However, patients without symptoms and who performed a negative exercise test were classified as not having restenosis, so only 68 patients (37%) underwent repeat angiography; almost all patients had a recurrence of chest pain, which contributed to a selection bias at follow-up angiography.

Milner et al. (1989) found that the addition of 4.5 g/day fish oil to the normal diet of 194 patients had a positive influence on clinical restenosis, with 19% (16 of the 84 patients) in the fish oil group versus 35% (35 of the 99 patients) in the placebo group having a recurrence of chest pain. However, in the first week, 11 of the 95 patients stopped taking the medication because of side effects.

In another study, 82 patients were randomly assigned, in a non-blinded manner, to aspirin plus dipyridamole with or without EPA 3200 mg/day, the equivalent of 18 capsules (Dehmer et al. 1988). Treatment was started 7 days

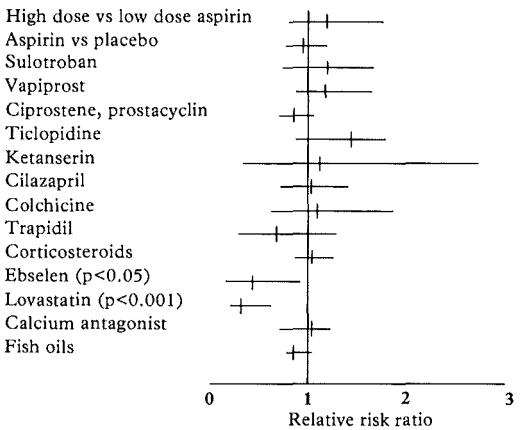

Figure 3

The relative risks of drugs investigated versus a control group in the prevention of restenosis in humans, with 95 % confidence intervals (CI). A risk ratio < 1 indicates that a lower restenosis rate is seen among the patients tested with the drug compared with those who received placebo. A statistical significant (p< 0.05) lower restenosis rate is seen in those studies where the 95 % CI do not cross the risk ratio of 1. A risk ratio of > 1 indicates a higher restenosis rate among the patients treated with the drug.

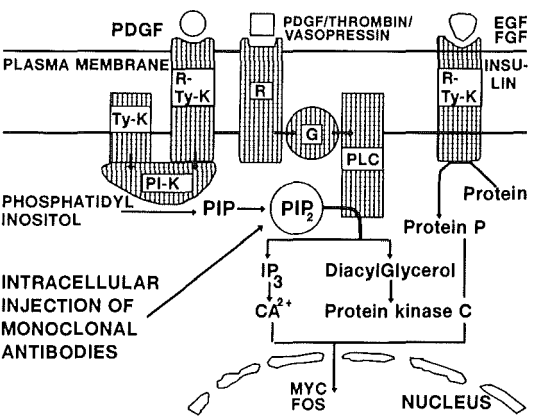

Figure 4

Schematic presentation of the phosphorylation and hydrolysis of phosphatidyl inositol (PI) and the conversion to inositol triphosphate (IP3) and diacyl glycerol. Several mitogens rapidly induce this process by stimulating phospholipasae C (PLC). Regulation of cytosolic calcium ion (Ca++) concentration is central to cell growth control (text). Stimuli to the nucleus result in MYC and FOS expression, oncogenes that initiate proliferation and phenotype changes. The dotted arrow indicates the target of the monoclonal antibodies against phosphatidyl inositol biphosphate (PIP2), which Takenawa et al. (1989) used to prevent cell proliferation.

Abbreviations:

Ty = tyrosine;

K=kinase;

R=receptor;

G=guanine;

EGF = epidermal growth factor;

FGF = fibroblastic growth factor;

PDGF = platelet-derived growth factor.

before PTCA and was discontinued 6 months later. In all 82 patients, a second angiogram (on average 3 to 4 months after PTCA) was performed. Restenosis (≥ 50% narrowing of the dilatation site at follow-up angiography) was seen in 46% in the placebo group and 19% of those receiving EPA. This trial suggests, that omega-3 fatty acids may effectively reduce restenosis in high risk patients, provided that they comply with the dosage regimen and that treatment is started 7 days before PTCA.

In contrast, no difference in angiographically defined restenosis rates was observed in another 108 patients randomly assigned to 10 capsules of fish oil (34% rate) or placebo (33%) (Grigg et all. 1989). Medication was started the day before angioplasty and continued until 4 months after angioplasty. All patients also received aspirin and verapamil.

Comparisons between fish oils and other treatments have been performed. Franzen et al. (1990) randomised 200 patients to assess the effect of 3,15 g omega-3-fatty acids versus olive oil. Follow-up angiography after 4 months showed 37% restenosis in the fish-oil group (25/67) and 33% restenosis in the olive oil group (21/62). Bairati et al. (1992) randomly assigned 205 patients to a daily dose of 'Maxepa' 15 g (each capsule containing EPA 0.18 g and docosahexaenoic acid 0.12g) or olive-oil from 3 weeks before PTCA until an angiogram 6 months later, while also performing ^{201}Thallium-scintigraphy at 3 months. This procedure showed a significant benefit ($p=0.02$) in the group given fish oil. At 6-month follow-up angiography, restenosis occured less often in the fish-oil group (22 vs 40% $p=0.03$). This group therefore documented the protective effect of fish-oil on the recurrence of coronary stenosis six months after PTCA.

Fifty other patients were randomised to 10 Maxepa-capsules or aspirin 300 mg daily for 2 weeks prior and 6 months after elective PTCA (Cheng et al. 1990). Angiography at 6 months revealed a restenosis rate of 20% in those taking fish-oil (4/20), and 26% in the aspirin group (6/23).

Very recently, Kaul et al. (1992) presented the results from a randomised trialin which 107 patients received aspirin and calcium antagonists alone or along with EPA and docosahexaenoic acid, starting 1 to 7 days before intervention. Restenosis occurred in 38% of the patients receiving fish oils, and in 29% of the controls. The length of pretreatment period did not influence the restenosis percentage.

It is thus clear that a consensus regarding the use of fish-oils to prevent restenosis can not be reached (Figure 3). These conclusions are in part related to differences in the design of the individual studies. Although all studies were randomised, only 4 were conducted double-blind. Different dosages and formulations were used and patient compliance and follow up rates varied in the studies. There were also differences in the timing of initiation of therapy and variable methods (coronary angiography, stress test, symptoms) used for the detection of restenosis.

Nevertheless, when meta-analysis is performed on the pooled data, there is a more convincing and powerfull relative risk as shown in figure 3. The upper value of the 95% confidence interval is 1.01, thus approaching the level of significance.

Two large scale trials, the EMPAR- and the FORT-trials, are currently

recruiting patients and we can probably draw final conclusions after the results of these studies have been published (Figure 3).

3.6.2 Cholesterol Lowering Drugs Hypercholesterolaemia is a well known risk factor for ischaemic heart disease. Lipid modification is an important goal in secondary prevention in halting the progression of atherosclerosis in general, and possibly after angioplasty. The total cholesterol/HDL-cholesterol ratio post-PTCA seems to be related to the risk for restenosis (Reis et al. 1990).

Recently it was shown that lovastatin reduces intimal hyperplasia after balloon angioplasty in rabbits with hypercholesterolaemia (Gellman et al. 1991), which is confirmed by observations in normolipidaemic rats (Ferns et al. 1991). Plaque size of the dilated carotid artery decreased significantly in lovastatin treated animals, reducing the formation of neointimal plaques. Constantinescu et al. (1992) demonstrated that the effect might be the result of direct inhibition of smooth muscle cell proliferation. Lovastatin added to cultured SMC's and EC's that were stimulated with either PDGF or EGF, showed a dose dependent inhibition in cell proliferation ($p < 0.01$).

However, two trials with postangioplasty patients in which the effect of lipid lowering on the incidence of restenosis after PTCA were tested, yielded conflicting results. In the first trial (Sahni et al. 1989), 157 patients were randomly assigned to lovastatin or to placebo following successful PTCA, for an unstated period of time. Only 50% of the patients underwent follow-up angiography at an average of 4 months after PTCA (50 patients in the lovastatin group and 29 patients in the control group). Restenosis (narrowing of ≥50% at follow-up angiography) was seen in 14% of the sites in the lovastatin group and 47% in the placebo group, reaching the level of significance in this select group.

In a second nonrandomised trial (Hollman et al. 1989), an aggressive treatment was used in 55 consecutive patients to lower serum cholesterol, including dietary modification, colestipol and lovastatin, starting on the day of PTCA. After 2 weeks, cholesterol levels were reduced by 50%. A restenosis rate of 34% in 44 of 55 patients was obtained. There was no difference in cholesterol levels between patients with and without restenosis.

4. Future directions

Molecular biology has provided us with detailed information about an important family of 'peptide cell regulator factors' (PRF) (Ross 1989; Majesky et al. 1990; Michell 1989; Schneider & Parker 1990; Waterfield 1989; Green 1989). Although it seems to be a long way from the bedside of the patient, this new family could lead to drugs that prevent restenosis after PTCA. In recent years it has become clear that cell proliferation and differentiation are controlled by many peptides and other agents through their interactions with cell surface receptors that send signals to the cell interior.

4.1 Antibodies to Growth Factors and Inositol Diphosphate PDGF has been shown to stimulate SMC-migration in an in vivo system. Intravenous infusion of PDGF for one week resulted in a 15- fold increase in intimal lesion area following

injury with a filament loop catheter. In addition, analysis of autoradiograms after suppletion of (^{3}H)-thymidine demonstrated a 4-fold increase in dividing cells and a 20-fold increase in non-dividing cells, suggesting the increase in cell number is due mainly to migration (Jawien et al. 1991).

Ferns et al. (1991) used goat polyclonal antibodies to PDGF to examine the formation of intimal lesions in de-endothelialised dilated rat carotid arteries, and administered anti-PDGF before and 9 days after the procedure. This resulted in a 40.9% reduction in the area of the neointima ($P< 0.01$). A (^{3}H)-Thymidine test showed no significant difference in labeling indices, and so suggests that the decrease in initial area is primarily due to reduction of chemotactic migration. The lack of effect on mitogenesis is probably the effect of endogenously produced PDGF-AA, a homodimer peptide chain that in in vitro studies needs larger amounts of anti-PDGF than PDGF-AB or -BB to block mitogenic activity (Raines et al. 1989).

Transforming growth factor-ß (TGF-ß) can either stimulate or retard cell growth, depending on concentration, cell age and density. Majesky et al (1991) showed in a rat carotid artery model increased TGF-ß synthesis after balloon injury, which stimulated SMC proliferation. Of particular interest is the report by Nikol et al. (1992) who observed the in vitro expression of TGF-ß in primary atherosclerotic and restenotic lesions. Messenger-RNA in restenotic material, obtained by directional coronary atherectomy, was detected at a significant higher level than in atherosclerotic cell cultures. This may indicate a role for TGF-ß in an exaggerated repair response following vessel wall injury.

Shah et al. (1992) injected antibodies to TGF-ß in disrupted dermal tissue in rats and investigated the wound healing process. Scar tissue of the treated animals had a lower rate of angiogenesis and infiltration of macrophages, which can release their TGF-ß stores. Furthermore, the collagen and fibronectin fibers of this tissue were found to have a smaller volume and normal orientation, and leaving scar tissue strength unaltered by TGF-ß down-regulation. Bonan et al. (1992b) described the in situ delivery of anti-TGF-ß antibodies in the coronary arteries of 5 minipigs. The extent of vessel wall injury, however, as well as was the restenosis-injury index (ratio of neointimal area to the total wall area over extent of injury) was the same in treated and untreated animals. This study suggests an ineffective role for ß-TGF antagonism.

Epstein et al. (1991) linked the nonspecific but effective Pseudomonas exotoxin A, lacking its cell recognition domain (PE40), to TGF-α. This growth factor is recognized by the EGF receptor, present in abundance on rapidly proliferating SMC's. Due to this new recognition site, PE40 is able to attach to cells expressing this EGF receptor, be internalised, and inhibit protein synthesis. The PE40-TGF-α complex has an extreme affinity to cells expressing the EGF receptor. In the same way, Cascells et al. (1990) used a saporin-FGF conjugate to inhibit DNA synthesis and intimal thickening in injured vessels.

Basic FGF is another mitogen, synthesised in both endothelial and SMC's, and is thought to be stored in the subendothelial matrix (Vlodavsky et al. 1987). The replicative capacities were evidently shown in endothelium after denudation with a filament loop in carotid arteries of rats. In damaged vessels, labelled thym-

idine index increased from 11.5% in controls to 54.8% in treated animals. Arteries with an intact endothelium did not respond to this mitogen.
Lindner and Reidy (1991) investigated the effect of a rabbit antibody to b-FGF on proliferation in cultured cells, after exposure to bFGF, PDGF-BB, EGF and calf-serum. The anti b-FGF antibody appeared to be highly specific in vitro. Administration in rats prior to and following balloon inflation reduced the SMC response significantly. One single injection of anti b-FGF prior to balloon angioplasty caused a similar reduction in (3H-)thymidine incorporation as when the angioplasty was followed by 5 additional injections, every 8 hours.

All the above results suggest a significant role of growth factors in the response of SMC's to vessel wall injury.

Among the important signals that have been implicated in these processes are the phosphorylation of tyrosine residues on proteins (Figure 4) and changes in the intracellular concentrations of the messenger molecules c-AMP, diacylglycerol, Inositol-1,4,5-triphosphate (IP3) and Ca^{++}, which directly or indirectly exert most of their regulation on the phosphorylation and dephosphorylation of serine and threonine residues of particular proteins (Michell 1989). Recent studies have demonstrated that mitogens such as PDGF and thrombin rapidly induce the hydrolysis of phospatidylinositol biphosphate (PIP2) by phospholipase C (Michell 1989; Takenawa & Fukami 1989).

PIP2 hydrolysis produces two compounds. The first of these is inositol triphosphate, a water-soluble molecule whose formation triggers the mobilisation of Ca^{++} in the cytoplasm. Within cells there is a membrane compartment, probably a part of or closely related to the endoplasmic reticulum, into which Ca^{++} is continuously pumped by an ATP-driven pump, so maintaining the cytoplasmic concentration at approximately 0.1 umol/L. When receptors trigger the formation of IP3, it binds to IP3 receptors on the membrane enclosing this reservoir, thus opening channels through which Ca^{++} is released to raise the cytoplasmic Ca^{++} concentration to somewhere in the range of 0.2-0.1 umol/L within seconds. It has long been known that regulation of cytosolic Ca^{++} levels is central to control of cell growth, and that this regulation may go awry in malignantly transformed cells. The second product of PIP2 hydrolysis is 1,2 diacylglycerol; this compound activates one or more of the protein kinase C (Figure 4).

These signalling pathways have been considered to play important roles in cellular responses. Unfortunately, they are not the sole signal transduction system. At least four structural classes of growth factor receptors have been identified, all of which phosphorylate on tyrosine residues. It is not clear to what extent these different tyrosine kinases share the same protein substrate. In some cases the ligand-binding and tyrosine kinase domains of a receptor protein are separate portions of a single polypeptide chain that spares the plasma membrane, whereas in others these sites are on separate subunits of a multisubunit membrane-spanning receptor protein. In this system there is clear evidence that receptors transmit their information to phospholipase C via a coupling protein G (guanine-nucleotide-dependent) (Figure 4).

A Japanese group (Takenawa & Fukami 1989) has developed a monoclonal antibody against the PIP2. Microintracellular injection of the antibody into the

transformed cells causes reversible and dose-dependent decrease in DNA synthesis and in the rate of cell proliferation, and reverts the cell morphology to that of untransformed cells, the normal phenotype. As predicted from the proposed scheme for growth factor transduction, microinjection and overproduction of phospholipase C or protein kinase C also can substitute for exogenous mitogens, whereas antibody to 1.4 inositol diphosphate or protein kinase C prevents proliferation (Figure 4). Thus, development and local release of new agents which inhibit inositol-phospholipid metabolism may be useful for treatment of human restenosis.

4.2 Gene Transfer First steps are being taken to explore the field of genetics. Methods for the incorporation of foreign DNA into the endothelial and VSMC have been developed recently and could be useful in protecting vessels from vascular diseases, including atherosclerosis and restenosis. The most elegant way to deal with this sophisticated technique is to augment genetic sequences, leaving the defective host genes unaltered.

Transfection can be achieved by physical means such as microinjection (Anderson et al. 1980), or electroporation (Neumann et al. 1982), or by a chemical approach, using liposomes as carriers (Felgner et al. 1987), or as shown by Leclerc et al. (1992), by bombardment of vascular muscle cells, using DNA-coated micro projectiles as carrier for transfer. A commonly used medium is the replication-defective retroviral vector, although amphotropic adeno- and DNA-viruses can be used also. The advantage of defective retroviruses is that these produce efficient infection followed by integration, and hence stable gene expression. In 1989, Nabel and colleagues succeeded in in vitro implantation of genetically modified endothelial cells expressing ß-galactosidase using a double balloon system to introduce the cells. Histochemical staining of this enzyme was observed in the intimal layer several weeks later and thus proved gene-expression.

Introduction of new genetic material into the wall of coronary arteries can give rise to the detection of enzyme activity. Transfection of luciferase DNA could be effected by bombardment of vascular muscle cells in vitro, using DNA-coated microprojectiles (1 μm diameter) and Biolistic PDS-1000 as delivery system (Leclerc 1992). Transfection of luciferase -an enzyme not expressed in mammalian cells- has been described in rabbits (Leclerc et al. 1991) and dogs (Chapman et al. 1991), and was achieved by exposing DNA either in a dual balloon catheter system or a porous perfusion balloon-system. Increased enzyme-activity can be detected following a percutaneous approach, which was also proved by Lynch et al. (1991), who simply introduced transduced cells into a denudated artery and detected enzyme levels over 4 months of observation following transplantation. These recent developments suggest that human VSMC can be drastically affected by gene therapy.

4.3 Sense-Antisense Approach In this competitive approach, oligonucleotides are used to block messenger-RNA action. This antisense technology has been used to inhibit c-myc protein production in hematopoietic cells (Holt et al.

1988), resulting in an inhibition of proliferation. Simons and Rosenberg (1992) demonstrated that smooth muscle cell proliferation, results in an elevation of c-myb m-RNA levels, and the generation of the oncogene c-myb. This oncogene is critically important in the change of the fenotype and in cell growth regulation. Interfering in the process of intimal hyperplasia at the post-nuclear level might give us the possibility to block one specific cell response.

Simons et al. (1992) used the rat carotid arteries to investigate the role of c-myb and oncogene suppression by its complementary antisense oligonucleotide in neointimal formation in vivo. The sense oligonucleotide or the corresponding antisense molecule was applied locally to the injured vessel wall in a plurionic gel, and resulted in minimal intimal smooth muscle cell accumulation, in contrast to the controls. The same group also studied the effect of a mismatching antisense (two basepairs not complementary), compared to sense and to antisense oligonucleotide, on the ratio of intimal to medial cross sectional area. Respectively ratios were 1.05, 1.02 and 0.16, which indicates the high specificity and potency of the antisense oligonucleotide.

Alteration of proto oncogene expression is a very attractive concept, and the developments in this field might result in a complete interruption of the hyperplastic response of intimal tissue, at the level of the ultimate common pathway.

5. Conclusions

Despite 13 years of clinical experience and research in the field of restenosis after PTCA, there have been no major breakthroughs in pharmacologic interventions. Assessment of the value of drug trials that have been performed in the past is extremely difficult because of differences in selection of patients, methods of analysis and definition of restenosis. Recently our group has reviewed the influence of these three factors on the outcome and conclusion of restenosis studies (Beatt et al. 1990). Although there is no scientific proof that the tested drugs are effective, many clinicians continue to prescribe them to 'prevent restenosis'.

However, some positive results in selected patients have been reported with the use of fish oil, trapidil, verapamil and with lovastatin in post-angioplasty patients. Furthermore, we seem to have found an animal model that more closely mimics the restenotic lesion found in humans (Schwartz et al. 1991b). In the near future the results will be known of ongoing multicentre trials investigating ACE-inhibition, serotonin antagonists, hirudin, LMWH, angiopeptin and other promising drugs such as inhibitors of thrombin production, growth factor blockers, prostacyclin analogues and monoclonal antibodies against platelet membrane receptors (GP IIb/IIIa) and von Willebrand factor. The outcome of these trials may bring us closer to the solution of the restenosis problem.

Investigators are also looking for local drug delivery systems that allow adequate local drug concentrations without adverse systemic side effects. Wolinsky and Thung (1990) demonstrated the feasibility of delivering potentially therapeutic agents, ranging from small molecular weight dyes to proteoglycans like heparin into the vascular wall. For this purpose they used a perforated catheter, and an injection/inflation pressure up to 5 bar, and demonstrated that pharmacological agents can be selectively delivered to the arterial media and intima.

Several other experimental studies have been recently carried out using the microporous balloon technique (Van Lierde et al. 1991; Kaplan et al. 1991a; Hong et al. 1991). In the latter study, reduced platelet deposition at the site of angioplasty was observed with angiopeptin, without a systemic anticoagulant effect.

Despite these promising experiments, there remains a significantly higher incidence (55%) of rupture of the lamina elastica interna after the use of a microporous balloon compared with conventional balloons (29%) (De Scheerder et al. (1992). Hong et al. (1992) inflated a balloon with multiple channels to 6 atmospheres in rabbit iliac arteries. Using this drug delivery balloon they were able to deliver insulin and peroxidase without detectable media dissection or disruption, although damage of the lamina elastica interna was not reported. The group of Lambert (1992) recognised the problem of wall injury, caused by the jet and stream effects. For this purpose they designed a balloon with minimal pore size and maximal pore density. Balloon inflations during 30 seconds to 5 atmospheres in several artery models were performed. Analysis by light microscopy and scanning electron microscopy, revealed endothelial denudation, without clear subendothelial trauma. Proper deposition of the dye was tested by videodensitometric measurements, as well as by microscopic cross sectional analysis.

De Scheerder et al. (1992) furthermore reported the rapid loss of the drug from the vessel wall, possibly due to the lack of specific receptors. Only during the first 20 minutes after the start of drug delivery were the measured tissue concentrations greater than plasma concentrations.

A perfusion balloon with 32 pores on the surface which allowed pressure-mediated drug delivery has been evaluated using a dye inplace of a drug (Ruiz et al. 1992). No mechanical damage to the vessel wall was seen at the site of balloon inflation, while the test dye penetrated to the media and in one animal to the adventitia.

Wilensky et al. (1991b) employed micro-particles as carriers for drugs. This microcarrier drug delivery system could prohibit rapid elimination by the increased network of vasa vasorum in atherosclerotic lesions that causes early outward diffusion. For this study they injected polystyrene particles, 5 μm in diameter, which appeared to be deposited in the intimal and medial layers and the adventitia, and which could still be detected after an interval of 14 days. Recently, this delivery system containing dexamethasone was successfully applied in rat carotid arteries (Villa et al. 1993) (see section 3.4.1).

Red blood cells have also been investigated as micro carrier (Yellayi et al. 1991). Heparin was bound effectively to red blood cells in vitro during heating. This concept to deliver drugs to the arterial wall has to be explored further.

Cox et al. (1991) used balloon expandable stents, coated with heparin and/or methotrexate in coronary arteries of animals. Although they could not show significant differences in SMC proliferation, they demonstrated local release of the drug over a 3-week period. The concept of bio-absorbable stents is receiving attention in in vitro studies. Bier et al. (1991) studied a stent, constructed of purified type I collagen, which expands by hydration assisted by balloon inflation. Lumen diameter was moderately reduced without any substantial

blood flow reduction. Ebecke et al. (1991) used the bio-degradable polymer poly-l-lactide. This material was incorporated by incubation with pentosal and oligonucleotides. Release of the pharmacological agents was dependent of the molecular weight of the poly-l-lactide used.

These developments, combining temporary vessel dilatation and slow local release of pharmacological agents, seem to possess promising potential in the research against restenosis.

At this moment, enormous efforts are being put into the search for a treatment modality that will solve the problem of restenosis. Experimental and clinical research continues to attempt to find a drug that prevents restenosis in the long term. The more we discover regarding the underlying mechanisms, the more opportunities there are for further research. Whether this endeavour is only searching for 'the Holy Grail', or whether there is a feasible drug treatment that works on the process of restenosis, without serious adverse effects, remains uncertain.

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Chapter 2

Role of angiographically identifiable thrombus on long-term luminal renarrowing after coronary angioplasty

A quantitative angiographic analysis

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Background Experimental studies suggest that mural thrombus may be involved in post angioplasty restenosis. The aim of our study was to examine the role of angiographically identifiable thrombus in the clinical situation.

Methods and Results The study population comprised 2950 patients (3583 lesions). The presence of angiographically identifiable thrombus either before or after the procedure was defined as the presence of a generalized haziness or filling defect within the arterial lumen. Restenosis was assessed using both a categorical (>50% diameter stenosis at follow-up) and a continuous approach (absolute and relative losses). The study population included 160 lesions with, and 3423 lesions without angiographically identifiable thrombus. The categorical restenosis rate was significantly higher in lesions containing angiographically identifiable thrombus, 43.1% versus 34.4%, $p<0.01$; relative risk 1.449; CI 1.051-1.997. The absolute and relative losses were also higher in lesions containing angiographically identifiable thrombus (absolute loss 0.43 ± 0.66 versus 0.32 ± 0.52; relative loss 0.16 ± 0.26 versus 0.13 ± 0.21, both $p<0.05$). The higher restenosis in these lesions was due primarily to an increased incidence of occlusions at follow-up angiography in this group: 13.8% versus 5.7%, $p<0.001$. When lesions that went on to occlude by the time of follow-up angiography were excluded from the analysis, the restenosis rate between the two groups was similar by both the categorical (34.1% vs 30.4%, p=ns, relative risk 1.183; CI 0.824 to 1.696) and continuous (absolute loss, 0.23 ± 0.46 vs 0.24 ± 0.42, p=ns, relative loss 0.09 ± 0.17 vs 0.09 ± 0.16 p=ns) approaches.

Conclusions Our results indicate that the presence of angiographically identifiable thrombus at the time of the angioplasty procedure is associated with higher restenosis. The mechanism by which this occurs is through vessel occlusion at follow up angiography. Measures aimed at improving outcome in this group of patients should be focused in this direction.

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Since the introduction of coronary angioplasty by Grüntzig et al [1] and the subsequent refinements in equipment, the indications for the technique have been expanded to include patients with unstable angina [2-4] and acute myocardial infarction [5]. In these situations, however, angioplasty carries increased risks, thought to relate, in part, to the presence of thrombus. A number of studies have demonstrated that the presence of angiographically identifiable thrombus either before or after dilatation of a coronary stenosis carries an increased risk of acute occlusion [6,7]. The influence of thrombus on long-term restenosis, however, is

less clear. Experimental work suggests that local platelet deposition with the subsequent release of a number of chemotactic and mitogenic factors, such as platelet-derived growth factor and thrombin, [8] may mediate the fibroproliferative response. Recurrent platelet aggregation at the site of injury with associated vasoconstriction and the consequent increased frequency and severity of cyclic coronary blood flow variations may also play an important role in the subsequent development of neointimal proliferation [9]. Although one study suggested that thrombus formation and incorporation into the vessel wall may play a pivotal role in restenosis [10], this has not been confirmed by other investigators [11]. Few clinical studies have actually assessed the role of angiographically identifiable thrombus on subsequent restenosis. The aim of this study was to examine the role of angiographically identifiable thrombus on long-term restenosis in a large series of patients undergoing successful balloon angioplasty and routine follow-up QCA assessment.

Methods

Patients The study population was taken from the 3582 patients with significant primary stenoses in native coronary arteries who were prospectively enrolled into four major restenosis trials [12-15]. These demonstrated that active therapy had no effect on restenosis or clinical outcome in the first 6 months after balloon angioplasty, so for the purposes of this study the data for the active and placebo groups were pooled. Patients, men or women, were eligible for study entry if they were symptomatic or asymptomatic, had stable or unstable angina pectoris, and showed angiographically significant narrowing in one or more major coronary arteries. Patients with recent (<1 week) or evolving myocardial infarction and those with significant left main coronary artery disease were excluded from the study.

Angioplasty procedure and follow-up angiography Coronary angioplasty was performed with a steerable, moveable guide-wire system by the femoral route. Standard balloon catheters were used. The choice of balloon type and brand as well as inflation duration and inflation pressure were left to the discretion of the operator. Patients were followed up for 6 months, at which time a follow-up study was performed. If symptoms recurred within 6 months, coronary angiography was carried out earlier. If no definite restenosis was present and the follow-up time was <4 months, the patient was asked to undergo further coronary arteriography at 6 months.

Quantitative angiography Three coronary angiograms, in total, were obtained for each patient: before and after PTCA and at angiographic follow-up. To standardize the method of data acquisition and to ensure exact reproducibility of the angiographic studies, measures were taken as previously described and all angiograms were processed in a central angiographic core laboratory [12-15]. The angiograms were recorded in such a manner that they were suitable for QCA by the computer-assisted Coronary Angiography Analysis System (CAAS), which was described and validated earlier [16]. Because the computer algorithm is unable to measure total occlusions, a value of 0mm was substituted for the MLD and a value of 100% for the percent diameter stenosis before PTCA. In these cases, the post angioplasty reference diameter was substituted for vessel size.

Definitions Angiographically identifiable thrombus was defined as the presence of a filling defect within the coronary lumen, surrounded by contrast material, seen in multiple projections and in the absence of calcium within the filling defect [17,18]. Alternatively, the persistence of contrast material within the lumen or visible embolization of intralumi-

nal material downstream was also taken to represent intracoronary thrombus.

Total occlusion was present if no anterograde filling beyond the lesion was visible or if faint, late anterograde opacification of the distal segment was present in the absence of a discernible luminal continuity [19]. Occlusion at follow-up angiography was similarly defined.

Vessel size refers to the reference diameter of the relevant coronary segment and is represented by the interpolated reference diameter before PTCA. MLD is the point of maximal luminal narrowing in the analyzed segment.

Restenosis: Many criteria have been proposed for the assessment of restenosis [20-21]. For the purposes of this study, we used, firs, the categorical approach with the traditional cut-off point of >50% diameter stenosis at follow-up and second, a continuous approach using absolute and relative losses [21].

Absolute gain and absolute loss represent the improvement in MLD achieved at intervention and the absolute change during follow-up, respectively, measured in millimetres. Absolute gain is the MLD after PTCA minus MLD before PTCA. Absolute loss is the MLD after PTCA minus MLD at follow-up.

Relative gain and relative loss depict the improvement in MLD achieved at intervention and the change during follow-up, respectively, normalized for vessel size. Relative gain is [MLD after PTCA minus MLD before PTCA] divided by vessel size. Relative loss is [MLD after PTCA minus MLD at follow-up] divided by vessel size.

Absolute net gain is the MLD at follow-up minus MLD before PTCA.

Net gain index is the net gain normalized for the vessel size. Net gain index is [MLD at follow-up minus MLD before PTCA] divided by vessel size.

Statistical analysis Data were analyzed with the SAS statistical software package. A chi square test was used to assess differences in categorical variables. A Student's t-test was used to assess differences in continuous variables. To test the assumption that the variances were equal, the folded-form F statistic was used. Whenever this assumption was violated, the Cochran and Cox approximation of the t test was used. Differences in variables with an ordinal scale (severity of clinical outcome) were assessed with the Wilcoxon rank-sum test. The difference in event-free survival time between the two groups was evaluated by the Kaplan-Meier method with the log rank and Wilcoxon tests. To study the relationship between a binary outcome parameter (occlusion at follow-up, the occurrence of a clinical event) and multiple categorical and continuous determinants, multiple logistic regression analysis was used. To study the relationship between continuous outcome parameters and multiple categorical and continuous determinants, multiple linear regression analysis was used. Lesion characteristics were investigated with a lesion-based analysis and patient characteristics with a per patient analysis in which a single lesion was randomly selected in patients with multivessel angioplasty. Values of $p<.05$ were considered significant.

Results

Baseline patient characteristics, procedural results, and clinical follow-up

The study population comprised the 2950 patients (3583 lesions, 1.21 lesions per patient) who successfully completed the study and had follow-up QCA. The overall QCA restudy rate was 86% of all patients undergoing successful PTCA with a residual QCA stenosis of <50%. Of 3583 lesions in 2950 patients, 160 lesions in 158 patients complied with the angiographic definition of thrombus present either before or after PTCA.

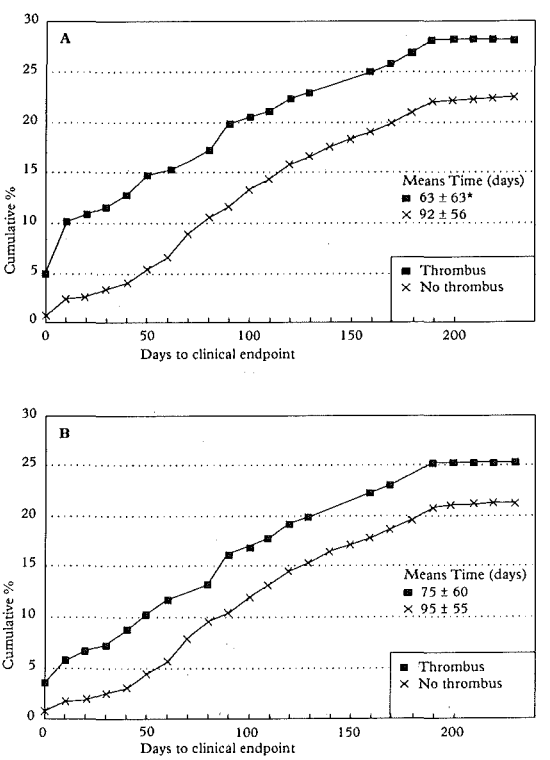
The two groups were comparable in terms of age and sex, but patients with angiographically identifiable thrombus at PTCA were more likely to have

Table I
Demographic data of patients with and without thrombus preangioplasty or post angioplasty included in analysis.

Clinical variable	Thrombus present	Thrombus absent
Patients, n	158	2792
Lesion, n	160	3423
Men, %	83	82
Age, y	57 ±9.3	55.3 ±9.3
Ever smoked, %	74	75
Current smoker, %	22	18
Hypertension, %	36	31
Diabetes, %	8	11
Hyperlipidemia, %	35	32
History of previous PTCA, %	1	4 *
History of previous MI, %	61	42 #
History of previous CABG,%	3.5	4.5
Pain at rest, %	28	34
No. of vessels diseased, %		
1 VD	61	59
2 VD	33	32
3 VD	6	9

MI indicates myocardial infarction; CABG, coronary artery bypass graft surgery; and VD, vessels diseased. * p<0.05, # p<0.001

Figure 1
a. Cumulative distribution curve of clinical end points over time for patients with and without the presence of thrombus at the time of angioplasty.
b. Cumulative distribution curve of clinical end points over time for patients with and without the presence of thrombus at the time of angioplasty (excluding lesions that went on to occlude at the time of follow up angiography).
*Numbers given are mean ± SD * p<0.05*



sustained a previous myocardial infarction and less likely to have had a previous PTCA (Table I). There were, however, substantial differences in lesion and procedural characteristics between the two groups (Table II). Thrombotic lesions were more likely to be located in the RCA than in the LAD and had a much higher proportion of total occlusions and multiple irregularities. They were also more likely to require a larger balloon and a greater number and duration of inflations. After the procedure, this group of lesions was also more likely to have a dissection (Table II).

Forty-four (28%) of the patients with angiographically identifiable thrombus present and 625 (22.4%) of the patients without thrombus had a clinical end point during follow-up (p=0.116). The individual components of death, myocardial infarction, coronary artery bypass graft surgery and repeat PTCA were 0%, 8.3%, 2.6% and 17.2% respectively, for lesions containing angiographically identifiable thrombus and 0.2%, 2.6%, 2.5% and 17.0% for lesions without thrombus (p=0.053). The mean time to clinical end point was significantly less in the angiographically identifiable thrombus group (63±63 vs 92±56 days, p<0.05, Figure 1a), and when we compared the pattern of occurrence of clinical end points by way of the log rank test, the probability value was 0.051, whereas the Wilcoxon test, which places more emphasis on early survival times, rendered a value of p=0.026, indicating the diverging survival curves in the beginning. When lesions that went on to occlude at the time of follow-up angiography were excluded from the analysis, there was no significant difference in the mean time to clinical end point (Figure 1b), and the log rank test gave a value of p=0.209 whereas the Wilcoxon test rendered a value of p=0.159, suggesting that the excess early events were related to the occlusions at follow-up angiography.

To exclude the possibility of a selection bias influencing our results, we also examined the incidence of thrombus-laden lesions and clinical end points in the 14% of the population in whom full QCA measurements were not available and who were therefore excluded from the study population. The incidence of thrombus in these patients (6.7%) was comparable to that in our study population (5.4%, p=ns). Of these patients with thrombus, 22.6% and of patients without thrombus, 22.6% had a clinical end point during follow-up (p=1.000). The individual worst clinical end point components of death, myocardial infarction, coronary artery bypass graft surgery, and repeat PTCA were 3.2%. 9.7%, 3.2% and 6.5%, respectively, for lesions with and 3.5%, 3.2%, 5.8% and 10.2% for lesions without thrombus (p=0.403).

QCA analysis Satisfactory QCA was performed in a mean of 2.12 matched angiographic projections per lesion had performed (Table III). The reference diameter did not change from before to after the procedure but was significantly larger in lesions containing angiographically identifiable thrombus, and this difference remained at follow-up (Table III). Although the MLD before angioplasty was significantly smaller in lesions containing angiographically identifiable thrombus, the MLD after angioplasty was similar. The residual percent stenosis after PTCA was higher in the angiographically identifiable thrombus group, as were the absolute and relative gains (Table III, Figure 2). At follow-up, although the MLD was similar in both groups, the percent stenosis was significantly higher

Table II
Baseline angiographic and procedural data of lesions.

Lesion type	Thrombus	No thrombus	Significance level
Number of lesions	160	3423	
Lesion location, %			0.0040
LAD	33.8	43.2	
LCX	20.6	25.0	
RCA	45.6	31.8	
Type of lesion %			
Multiple irregularities	13.0	7.6	0.0400
Total occlusion	20.0	6.8	0.0000
Tandemlesion	1.3	4.2	0.1120
Branch point in lesion	28.0	32.8	0.4460
Lesion calcification	16.9	12.1	0.0990
PTCA procedure			
Nominal size of largest balloon, mm	3.00 ±0.43	2.85 ±0.43	0.0001
Balloon-to-artery ratio	1.12 ±0.18	1.13 ±0.19	0.4345
Total number of inflations	4.2 ±2.9	3.53 ±2.3	0.0013
Total duration of inflation, s	380 ±325	308 ±266	0.0016
Maximum inflation pressure, atm	8.35 ±2.53	8.48 ±2.50	0.5423
Post PTCA result			
Dissection at the dilated site, %	54.4	33.1	0.0000

Table III
Quantitative analysis of 160 lesions with and 3423 lesions without thrombus before or after angioplasty included in analysis. DS indicates diameter stenosis.

Thrombus present	Thrombus	No thrombus	Significance level
No.	160	3423	
Reference diameter, mm			
Before angioplasty	2.77 ±0.54	2.60 ±0.54	0.0006
After angioplasty	2.80 ±0.53	2.65 ±0.51	0.0007
At follow-up	2.87 ±0.64	2.67 ±0.56	0.0000
MLD, mm			
Before angioplasty	0.88 ±0.52	1.01 ±0.39	0.0001
After angioplasty	1.80 ±0.35	1.75 ±0.36	0.0868
At follow-up	1.37 ±0.71	1.44 ±0.58	0.1552
Differences in MLD			
Absolurte gain, mm	0.82 ±0.53	0.75 ±0.41	0.0000
Relative gain	0.34 ±0.19	0.29 ±0.16	0.0002
Absolute loss, mm	0.43 ±0.66	0.32 ±0.52	0.0070
Relative loss	0.16 ±0.26	0.13 ±0.21	0.0339
Absolute net gain, mm	0.49 ±0.72	0.43 ±0.57	0.1970
Net gain index	0.18 ±0.27	0.17 ±0.23	0.5050
Loss index	1.57 ±13.59	0.46 ±2.44	0.0002
Percentage stenosis			
Before angioplasty	67.78 ±18.27	60.63 ±14.30	0.0000
After angioplasty	35.15 ±7.45	33.38 ±8.37	0.0039
At follow-up	51.71 ±23.70	45.71 ±19.00	0.0001
DS at follow-up > 50%	43.13	34.36	0.0280

in lesions containing thrombus (Figure 2, Table III), as were the categorical restenosis rate (43.1% vs 34.4% $p<0.01$; relative risk, 1.449; CI, 1.051-1.997) and the absolute and relative losses (Table III, Figure 3).

The higher restenosis rate in the angiographically identifiable thrombus group was predominantly due to an increased number of occlusions at follow-up angiography (13.8% vs 5.7%, $p<0.001$; Relative risk, 2.639; 95% CI, 1.645 to 4.233). When lesions that went on to occlude at follow-up angiography were excluded from the analysis, there remained a tendency for a higher categorical restenosis rate in the thrombus group (34.1% vs 30.4%; relative risk, 1.183; CI, 0.824 to 1.696), but this was no longer statistically significant ($p=0.411$). The absolute and relative losses were now also similar (0.23 ± 0.46 vs 0.24 ± 0.42 and 0.09 ± 0.17 vs 0.09 ± 0.16, respectively, both p=ns).

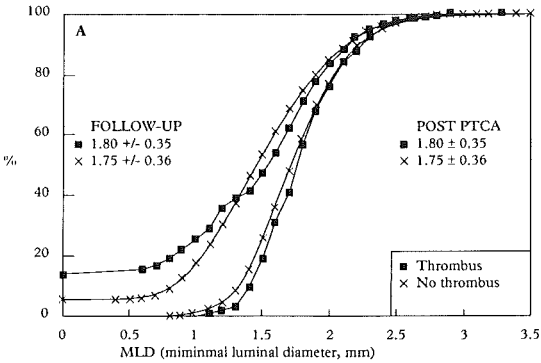
Multiple linear regression analysis We have previously demonstrated that vessel size, MLD before PTCA, absolute gain, and LAD location make a significant contribution to late angiographic outcome [22]. Adding thrombus to this model significantly improved its predictive value. Least-squares means for absolute loss were 0.404 for lesions with thrombus and 0.318 for lesions without thrombus. The probability value of adding the variable thrombus to the model was 0.037. Adding thrombus to the model when lesions that went on to occlude at follow-up angiography were excluded did not improve its predictive value. Least-squares means for absolute loss were 0.222 for lesions with thrombus and 0.243 for lesions without thrombus. The probability value of adding the variable thrombus to the model was 0.549.

To ascertain whether the trend towards a worse clinical outcome in patients with thrombus was related to differences in the underlying baseline characteristics, we corrected for these variables to see whether thrombus had an independent predictive value. We performed logistic regression with the above-mentioned baseline characteristics as covariates resulting in a value for the variable thrombus of $p=0.038$, implying that thrombus has a positive relationship with the probability of a clinical end point. Performing the analysis when lesions that went on to occlude at follow-up angiography were excluded gave a value of $p=0.183$, suggesting that the positive relation with the probability of a clinical end point was related to occlusion at follow-up angiography.

Uni and multi-variate analyses of occlusions at follow-up angiography The finding that the higher restenosis in lesions containing angiographically identifiable thrombus was predominantly due to an increased number of occlusions at follow-up angiography prompted us to examine the time to clinical and angiographic follow-up and a number of variables predictive of late occlusion in this group (Table IV). The time to clinical and angiographic follow-up was significantly shorter in lesions that occluded at the time of angiographic follow-up. These lesions had a higher incidence of total occlusion, a tighter stenosis before PTCA, a longer duration of inflation with the use of a smaller balloon in a smaller vessel with a tighter residual MLD, and a greater likelihood of a dissection after PTCA. Logistic regression analysis confirmed the presence of a total occlusion before

Figure 2

a. Cumulative distribution curve of MLD post PTCA and at follow up for patients with and without the presence of thrombus at the time of angioplasty.
b. Cumulative distribution curve of percentage stenosis post PTCA and at follow up for patients with and without the presence of thrombus at the time of angioplasty.
Numbers given are mean ± 1 SD
**p<0.05*



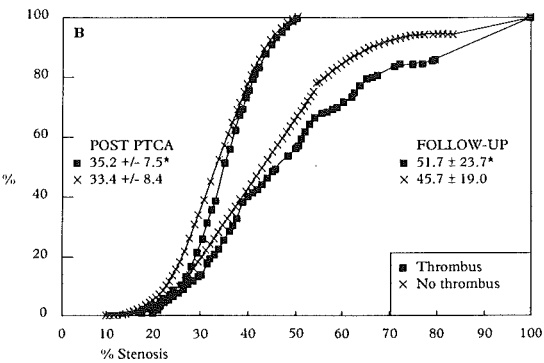
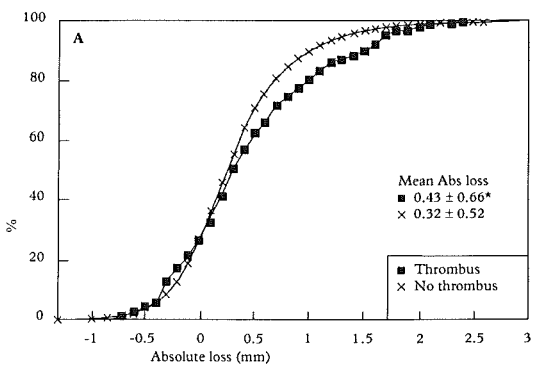


Figure 3

a. Cumulative distribution curve of absolute loss (change in MLD from before percutaneous angioplasty to follow up) for lesions with and without thrombus.
b. Cumulative distribution curve of relative loss (change in MLD from before percutaneous angioplasty to follow up corrected for vessel size) for lesions with and without thrombus.
Numbers given are mean ± 1 SD
**p<0.001*



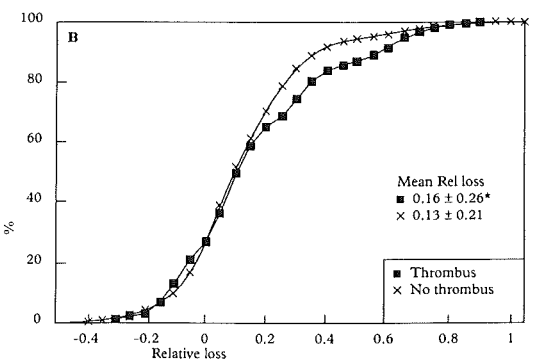


Table IV
Univariate analysis of patient-, lesion-, and procedure-related characteristics relevant to occlusion at follow-up angiography in 160 lesions containing thrombus.

Lesion type	Occlusion at follow-up (n=22)	No occlusion at follow-up (n=138)	Significance level
Anginal class, %			.365
None	4.6	11.6	
CCS class I	9.1	13.8	
CCS class II	40.9	26.8	
CCS class III	31.8	22.5	
CCS class VI	13.6	25.4	
Duration of angina, wk	106 ±219	63 ±132	0.326
Medication at screening, %			
Antivoagulants	0	0.7	1.000
Thrombocyte aggregation inhibitor	68.2	65.2	0.976
Aspirin	80.0	74.2	0.872
Dipyridamole	20.0	7.8	0.317
Laboratory investigations			
Hemoglobin	8.70 ±1.00	8.81 ±0.86	0.593
Hematocrit	0.42 ±0.04	0.42 ±0.04	0.863
Platelet count	274 ±55	267 ±80	0.691
Lesion location, %			0.085
LAD	13.7	37.0	
LCX	22.7	20.3	
RCA	63.7	42.8	
Lesion characteristics, %			
Concentric	26.3	36.6	0.542
Multiple irregularities	10.5	13.4	1.000
Branch point in stenosis	16.7	16.4	1.000
Coronary artery bend	15.6	24.1	0.615
Calcified lesion	31.8	14.5	0.088
Total occlusion	45.5	15.6	0.003 *
Degree of collateral supply			0.138
No collaterals	66.7	81.3	
Slight (mininal perfusion)	0	7.3	
Medium (partial perfusion)	26.7	7.3	
Major (complete perfusion)	0	1.0	
not assessed	6.7	3.1	
PTCA procedure			
Nominal size of largest balloon, mm	2.81 ±0.40	3.03 ±0.43	0.0264
Balloon-to-artery ratio	1.10 ±0.19	1.12 ±0.18	0.7172
Total number of inflations	4.5 ±3.2	4.2 ±2.9	0.6687
Total duration of inflation, s	530 ±484	356 ±289	0.0248 *
Maximum inflation pressure, atm	9.10 ±2.93	8.24 ±2.45	0.2159
Post PTCA result			
Dissection at the dilated site, %	77.3	50.7	0.036
Quantitative angiographic measurements			
Reference diameter, mm			
Before angioplasty	2.62 ±0.53	2.79 ±0.54	0.3101
After angioplasty	2.54 ±0.39	2.84 ±0.54	0.0036 *
MLD, mm			
Before angioplasty	0.55 ±0.56	0.93 ±0.49	0.0052
After angioplasty	1.66 ±0.30	1.82 ±0.35	0.0277

Table IV vervolg

Lesion type	Occlusion at follow-up (n=22)	No occlusion at follow-up (n=138)	Significance level
Differences in MLD, mm			
Absolute gain	1.11 ±0.60	0.89 ±0.51	0.1161
Relative gain	0.44 ±0.23	0.33 ±0.18	0.0360
Percentage stenosis			
Before angioplasty	79.00 ±20.40	65.99 ±17.33	0.0088
After angioplasty	34.35 ±8.38	35.28 ±7.35	0.6276
Lesion length post-PTCA, mm	6.14 ±1.99	6.26 ±2.35	0.8032
Days to follow-up	127 ±79	160 ±460	0.007

CCS indicates Canadian Cardiovascular Society angina classification. LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery. Values are mean ±SD.
* Retained in multivariate model.

Table V
Result of multiple logistic regression analysis to evaluate the respective contributions of clinical, angiographic, and procedural variables to occlusion at follow-up angiography in lesions containing thrombus.

Variable	Regression coefficiënt	Standard error of regression coefficiënt	P
Presence of total occlusion pre-PTCA	0.379	0.163	0.021
Total inflation time, s	0.002	0.0006	0.002
Reference diameter post-PTCA, mm	-1.634	0.630	0.009

PTCA and total inflation time (seconds) to be positively related and the reference diameter after PTCA (millimetres) to be negatively related to occlusion at follow-up angiography (Table V).

Discussion

Our study has specifically addressed the role of angiographically identifiable thrombus in long term restenosis in a large patient population with a control group, a high angiographic follow-up rate, and QCA at a pre-determined time interval. We have demonstrated, using both a categorical and a continuous approach, that restenosis is significantly increased by the presence of angiographically identifiable thrombus during coronary angioplasty. Furthermore, we have also shown that the mechanism for this is an increased rate of occlusion at follow-up angiography and that this is positively related to the presence of a total occlusion before PTCA and the total duration of balloon inflation and negatively related to the residual stenosis after intervention. If lesions that subsequently occlude at follow-up angiography are excluded from the analysis, then restenosis is similar in both groups. These findings support and expand on our current understanding regarding the role of thrombus in long-term luminal renarrowing and occlusion after successful PTCA.

Our findings support a role for thrombus in restenosis after successful PTCA in terms of both clinical and angiographic outcomes. They suggest that the contribution thrombus makes to restenosis relates to vessel occlusion by the time of follow-up angiography. The timing of this occlusion is unclear. If it occurred early, it is likely to have been the end result of an acute thrombotic process, whereas if it occurred late, it would be the final end result of the process of restenosis itself. We do not know when the occlusion at follow-up angiography occurred in our patients, so our results must be speculative. We suspect, however, that it occurred early. In support of this is the higher incidence of previous myocardial infarction in the thrombus group (successful dilatation of the infarct-related vessel is associated with a higher rate of early silent occlusion [23]) and the Wilcoxon test indicating early divergence in the survival curves when occlusions at follow-up angiography are included. Additional evidence comes from the much earlier occurrence of clinical and angiographic end points in the thrombus-laden lesions that had occluded by the time of follow-up angiography and the fact that the excess in clinical end points is driven by a much higher incidence of acute myocardial infarction. Our hypothesis that the occlusions occurred early is also supported by evidence in the literature suggesting that 2% to 8% of elective PTCA lesions [24] occlude during the first 24 hours, silently in many cases. Thus, although our data support a role for thrombus in vessel occlusion by the time of follow-up angiography and hence restenosis, they do not provide any strong evidence to support a role for angiographically identifiable thrombus in late myointimal hyperplasia. Further prospective studies are thus required to evaluate this important matter further.

Univariate regression analysis was suggestive of a number of procedural and angiographic variables related to occlusion at follow-up angiography. These included the presence of a total occlusion and a tighter stenosis before PTCA, a

longer duration of inflation with the use of a smaller balloon diameter in a smaller vessel with a tighter residual MLD, and a greater likelihood of a dissection after PTCA at the dilated site. Thus, the more difficult dilatation of a more complex lesion in a smaller vessel with a less satisfactory result would be more likely to occlude by the time of follow-up angiography. Multivariate regression analysis confirmed the presence of total occlusion before PTCA and a longer total inflation time to be positively related to the risk of subsequent occlusion and the reference diameter after PTCA to be negatively related. The relationship between total occlusion and subsequent risk of occlusion may be secondary to the highly thrombogenic surface generated by the successful dilatation of a total occlusion, without a pre existing endothelial lining [25]. Successful dilatation of a total occlusion may also expose flowing blood to activated thrombin bound to fibrin in the internal layers of a previously formed thrombus. The prothrombotic processes stimulated by the activated thrombin would be even more severe that those associated with the deeply injured artery and would further accelerate thrombosis after PTCA in these lesions [26,27] thus contributing to both enhanced local thrombus formation after successful dilatation of these lesions and an increased likelihood of thrombotic occlusion. The total inflation time may represent the more complex dilatation of a total occlusion, multiple irregularities, or a more complicated angioplasty. This is further supported by the higher incidence of dissections requiring prolonged inflation in lesions that occlude by the time of follow-up angiography. The negative relation between increasing vessel size and subsequent occlusion is probably representative of the local flow dynamics [28].

Our study has a number of limitations. First, it was a retrospective analysis of prospectively gathered data and is hence subject to the limitations inherent in any retrospective analysis. For example, there are significant differences in the baseline clinical, angiographic and procedural characteristics between the two groups that could have been responsible for, or associated with, the outcome of the procedure, including the presence of thrombus. Patients with angiographic evidence of thrombus before or after angioplasty had a significantly greater history of previous myocardial infarction and a significantly lower proportion of previous coronary angioplasty, both of which may have had an impact on the clinical and angiographic outcomes. There is evidence for a silent early occlusion after successful acute dilatation of infarct-related vessels [5,23] and that after stent implantation in coronary vessels supplying an infarcted segment, the low flow makes the vessel more prone to thrombotic occlusion [29]. Similar mechanisms may be operating in our study, but we do not know whether the vessel dilated was the infarct-related vessel, and we do not know the length of time since myocardial infarction, except that it was longer than 1 week. Similar arguments also apply to the history of previous PTCA. Again, we do not know whether the present procedure was performed at the same site, and it is not possible to draw conclusions about what effect it may have had on subsequent clinical and angiographic outcomes.

There were also significant differences in lesion location and lesion characteristics. There was a higher proportion of lesions containing thrombus in the RCA and less in the LAD. This may have had an impact on angiographic outcome in two ways. First, the RCA is significantly larger than the LAD, and this may

explain why the reference diameter in lesions containing thrombus was significantly larger. Second, there are significant differences between the two vessels in terms of local flow dynamics, vessel geometry and external compressive forces [30] that may have a substantial influence on the subsequent risk of occlusion [31]. Although lesion location was not a major risk factor in our multivariate analysis of occlusions at follow-up angiography, it is nonetheless interesting to note that the trend was for lesions which occluded to be in the RCA (p=0.085). Thus similar mechanisms may be operating in our study.

The type of lesion was also significantly different, with a greater proportion of total occlusions in the thrombus group. Successful dilatation of these may have enhanced local thrombus formation and may have contributed to the increased incidence of occlusion at follow-up angiography [23,32]. It may also partly explain the smaller MLD before PTCA and greater absolute and relative gains in this group of lesions. Differences in lesion location and characteristics could also have been responsible for the significant differences in the PTCA procedure. For example, the prevalence of RCA lesions could explain the larger nominal size of the largest balloon, whereas the greater number of inflations and total duration of inflation may reflect the more complex dilatation of a total occlusion, multiple irregularities, or a more complicated angioplasty.

Although we tried to compensate for these differences in baseline characteristics by using multivariate analysis and demonstrated that thrombus has a predictive value on restenosis and clinical outcome independent of the underlying clinical and angiographic characteristics, nonetheless, we cannot exclude the possibility of covert factors not available in the study influencing outcome. For example, we do not know what proportion of the angiographically identifiable thrombus group had a successfully treated occlusive dissection, a recognized risk factor for restenosis [33], and total occlusion as a late outcome [34].

Second, although the angiographic definition of thrombus we used is the standard definition found in the literature, [17,18] the individual sensitivity and specificity of the three criteria have, to the best of our knowledge, never been addressed. In addition, contrast angiography, although the gold standard for randomized studies, has a poor sensitivity for intracoronary thrombus [18]. When we used the above angiographic definition and coronary angioscopy as the gold standard, we found the specificity of contrast angiography to be good (100%) but the sensitivity to be poor (19.4%). This is in keeping with other evidence in the literature. Coronary angioscopy, for example, suggests a very high incidence of macroscopic mural thrombus, not identifiable by contrast angiography, after balloon angioplasty [35,36] whereas directional atherectomy suggests that thrombus may contribute to arterial narrowing in 8% to 25% of restenosis cases [37]. Thus, although our results apply to angiographically identifiable thrombus, they may not apply to patients with mural thrombus not visualized by contrast angiography.

Finally, the study relies on data pooled from four separate restenosis trials [12-15]. We believe that the pooling of data was justified, however, since the number of patients with angiographically identifiable thrombus present in each individual study was limited. Furthermore, the entry criteria for the studies were broadly similar, the data pooled were those common to all studies, and the angio-

graphic criteria were standardized, with one central angiographic core laboratory performing the QCA analysis in all studies. In addition, the resulting large study population provides a unique opportunity to obtain accurate QCA data at a predetermined time interval in a field in which few such data exist to date.

Clinical implications Our data support previous work suggesting that local thrombus formation may result in acute occlusion [7,38,39] and expand it to include late subacute occlusion and hence restenosis. This may have important clinical implications with regard to recent studies using monoclonal antibodies and synthetic peptides directed against the platelet glycoprotein IIb/IIIa receptor [40-42]. Although preliminary data suggest that they reduce the need for coronary revascularisation procedures in high-risk angioplasty patients [42], most of the reduction occurred in the first 30 days after intervention, and the effects were not verified at the angiographic level. Our data would suggest that perhaps some of their improved clinical outcome may relate to eliminating subacute occlusion in a subset of the population without necessarily affecting the restenosis process.

Conclusions

Our results indicate that the presence of angiographically identifiable thrombus at the time of the angioplasty procedure is associated with a higher rate of angiographic restenosis. The mechanism by which this occurs is through increased vessel occlusion at follow-up angiography. Measures aimed at improving outcome in this group of lesions should be focused in this direction.

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Chapter 3

Experiences of a quantitative coronary angiographic core laboratory in restenosis prevention trials

Victor A.W.M. Umans, Walter R.M. Hermans, Jean-Paul R. Herrman, Jaap Pameyer and Patrick W. Serruys

P.W. Serruys, D.P. Foley and P.J. de Feyter (eds.). Quantitative Coronary Angiography in Clinical Practice, Kluwer Academic Publishers, the Netherlands, 1993, 121-135

Introduction Since its introduction more than 14 years ago [1], percutaneous transluminal coronary angioplasty [PTCA] has been attended by a 17% to 40% incidence of restenosis, typically developing within 6 months of the procedure [2-5]. Each year the number of patients undergoing PTCA has increased and now approaches the number treated with coronary artery bypass grafting [CABG]. In the last 10 years, experimental models have given us more insight into the restenosis phenomenon and pharmacological agents have been developed aiming to prevent or reduce restenosis. Many of these agents have been investigated in clinical restenosis prevention trials [4-7] and although these agents were able to reduce restenosis in the animal model, most of the clinical trials failed to demonstrate a convincing reduction in the incidence of restenosis in man. In these clinical trials, the primary endpoint has been either angiograph-ic [change in minimal luminal diameter at follow-up; > 50% diameter stenosis at follow-up; loss > 50% of the initial gain] and / or clinical [death; nonfatal myocardial infarction; coronary revascularization; recurrence of angina requiring medical therapy; exercise test; quality of life]. The use of an angiographic parameter as a primary endpoint provides the necessary objectively whereby the patient population required for statistical analysis numbers between 500 and 700, whereas more than 2000 patients are necessary if a clinical endpoint is used [6].

Despite the widespread and long-standing use of coronary angiography in clinical practice, as well as the outstanding improvement in image acquisition, the interpretation of the angiogram has changed very little and is still reviewed visually. However, visual assessment is a subjective evaluation with a large inter- and intra observer variability and can therefore not be used in important scientific studies for example restenosis prevention trials [8-9]. QCA has the advantage of being more accurate and reproducible in the assessment of lesion severity, than visual or hand-held calliper assessments. At the Thoraxcenter, the computer-assisted Cardiovascular Angiography Analysis System [CAAS] using an automated edge detection technique was developed and validated [8,10]. Over the last 3 years, we have been the angiographic 'core laboratory' (using the CAAS-system) in 4 restenosis prevention trials with recruitment of patients in Europe, US and Canada. In order to obtain reliable and reproducible quantitative measurements over time from coronary [cine]-angiograms, variations in data acquisition and analyses must be minimized. (P.W. Serruys, D.P. Foley and P.J. de Feyter (eds.). Quantitative Coronary Angiography in Clinical Practice. Kluwer Academic Publishers 1993, the Netherlands, 121-135.)

Potential problems with angiographic data acquisition and analysis [Table I]

1. Pincushion distortion Pincushion distortion of the image intensifier introduces a selective magnification of an object near the edges of the image as compared with its size in the center [Figure I A]. An inaccuracy in the measurement of the minimal lumen diameter of the stenosis over time could be introduced if, for example, the stenosis after the angioplasty procedure is filmed in the center and at follow-up near the edges of the image intensifier. To overcome this potential problem, a cm grid has to be filmed in each mode of the image intensifier in all the catheterization rooms to be used before the clinic can start to recruit and randomize patients for a restenosis prevention trial. With this cm grid film, the CAAS system calculates a correction factor for each intersection position of the grid wires so that the pincushion distortion can be corrected for [Figure 2 B]. Fortunately, the newer generations of image intensifiers introduce significantly less distortion than the older ones from the early and mid 80's; the degree of distortion is even less when the lower magnification modes are used with multimode image intensifiers. At present time there are in our database pincushion correction factors of 734 different modes of magnification [113 clinics with 285 angiorooms] from all over Europe, United States and Canada.

2. Differences in angles and height levels of the x-ray gantry As it is absolute mandatory to repeat exactly the same (baseline) views of the coronary segments in studies to evaluate changes in lumen diameter over time, we have developed at the Thoraxcenter an on-line registration system of the x-ray system parameters such as parameters describing the geometry of the x-ray gantry for a particular cine-film run [rotation of U-arm and object, as well as distances from isocenter to focus, table height], and also selected x-ray exposure factors [kV, mA]. When repeat angiography is scheduled, the geometry of the x-ray system is set on the basis of the available data, so that approximately the same angiographic conditions are obtained. In a clinical study with repositioning of the x-ray system, it was found that the angular variability, defined by the standard deviation of the absolute differences of angular settings, was < 4.2 degrees and that the variability in the various positions of image intensifier and x-ray source was < 3.0 cm [8,11]. As on-line registration of the x-ray system settings is not available in all hospitals, we have developed a technician's worksheet that has to be completed during the PTCA procedure with detailed information of the procedure [view, catheter type, catheter size, balloon type, balloon size, balloon pressure, kV, mA, medication given] [Figure 2]. In this way minimization of differences in x-ray settings at follow-up angiography is ensured. Furthermore, each center intending to participate in one of the trials is required to provide 2 sample cine-angiograms from each of its catheterization rooms for verification of their ability to comply to our standards.

3. Differences in vasomotor tone of the coronary arteries As the vasomotor tone may differ widely during consecutive coronary angiographic studies, it should be controlled at all times. An optimal vasodilatative drug for controlling

Table I
Potential problems with angiographic data acquisition and analysis.

1	Pincushion distortion of image intensifier
2	Differences in angles and height levels of x-ray system settings
3	Differences in vasomotor tone
4	Variation in quality of mixing of contrast agent with blood
5	Catheter used as scaling device (angiographic quality, influence of contrast in catheter tip on the calibration factor, size of catheter)
6	Deviations in size of catheter as listed by the manufacturer from its actual size
7	Variation in data analysis

Table II
Influence of nitroglycerin on the mean diameter of non diseased segments in 202 patients in single projection.

Mean Diameter (mm)	Without Nitro Post-PTCA N = 34	With Nitro Post-PTCA N = 168	t-test
Pre-PTCA	3.12 ±0.63	2.74 ±0.63	
Post-PTCA	3.01 ±0.64	2.75 ±0.59	
Follow-up	3.18 ±0.55	2.82 ±0.63	
Delta (Post – Pre)	-0.11 ±0.27	+0.02 ±0.21	$p < 0.001$
Delta (Fup – Pre)	+0.06 ±0.22	+0.07 ±0.22	p = ns

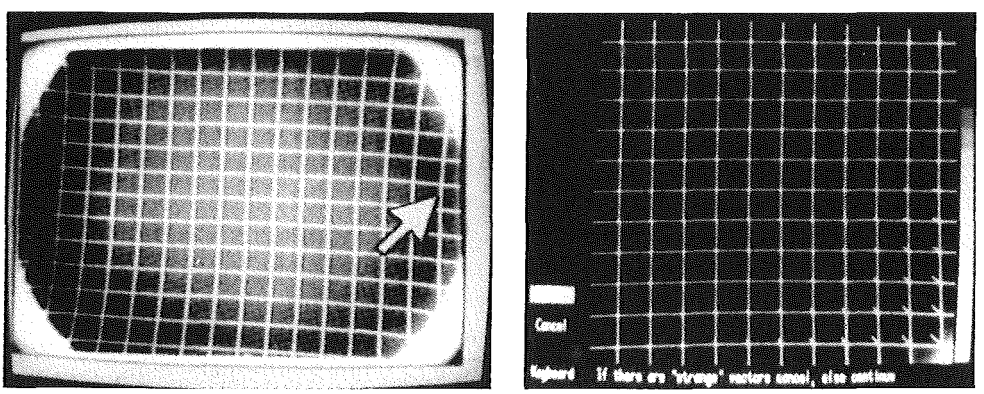

Figure 1
Example of pincushion distortion introduced by the image intensifier (A, see arrow) and of the calculated correction factor with the use of the filmed cm-grid (B).

Patient No 341 **finess 1** TWS PROCEDURE

ALLOCATION AND START OF PROCEDURE

39 **Date of procedure** 19 / 02 / 97 (dd/mm/yy)

40 **Angio room number** 3

41 **Angio film number** 970219

42 **Catheterization performed by (name):** Y. OZAKI

ANGIOPLASTY AT FIRST SITE

43 **To ensure maximum dilatation during filming of all study views, administer intra-coronary :** *(tick one)*

- [X] 1-3 mg Isosorbide dinitrate (ISDN)
- [ ] 0.2 mg Nitroglycerine (NTG)

44 **Number of segment on 1st site** 7

Listing of filming sequence and angulations

TABLE ANGULATION TO RIGHT ARM	PATIENT AXIS TO TABLE	TABLE ANGULATION TO LEFT ARM	FIELD SIZE	CA	CB	CA NB	MEDICATION (name, amount)
		1 60 -	- 9	1			ISDN 2cc
		2 60 - V	- 7	1			
3 45 0 -			- 7	1			
4 45 30 H V			- 7	1			
5 40 30 F V			- 7	1			
		6 40 30 F	- 7	1			
		7 40 30 H V	- 7	1			
8 45 30 H			- 7	1	1		
9 45 30 H			- 7	1		1	
10 45 30 H			- 7	1			2cc ISDN
		11 40 30 H	- 7	1			

continue on the following page

Figure 2
Example of a page of the technician's worksheet

the vasomotor tone of the epicardial vessel should produce a quick and maximal response without influencing the hemodynamic state of the patient. Only nitrates and calcium antagonists satisfy these requirements. On isolated human coronary arteries calcium antagonists are more vasoactive but they act more slowly; in the in-vivo situation, however, the nitrates are more vasoactive than the calcium antagonists [12-15].

We have measured in 202 patients the mean diameter of a normal segment of a non-dilated vessel in a single view pre-PTCA, post-PTCA and at follow-up angiography 6 months later. In cases where a stenosis of the left anterior descending artery [LAD] had been dilated, a non-diseased segment in the left circumflex artery [LC] was analyzed and vice versa; where dilatation of a stenosis in the right coronary artery [RCA] was performed, a non-diseased segment proximal to the stenosis was used for analysis. All patients were given intracoronary [either 0.1 to 0.3 mg of nitroglycerin or 1 to 3 mg isosorbide dinitrate [ISDN] before PTCA and before follow-up and all but 34 received similar dosage before the angiogram immediately after PTCA. Table II summarizes the results of the analyses; a decrease in mean diameter of -0.11 $\pm$0.27 [mm] was observed in the segments of patients studied without intracoronary nitrates post-PTCA, whereas a small increase was seen of +0.02 $\pm$ 0.21 [mm] in the group with intracoronary nitrates prior to post-PTCA angiography [$p < 0.001$]. No difference in the mean diameter between pre-PTCA and follow-up angiography was measured.

In summary, the vasomotor tone should be controlled in quantitative coronary angiographic studies. This is only achieved by means of a vasodilator drug that produces fast and complete vasodilation without any peripheral effects. Therefore, we strongly advocate the use of 0.1 to 0.3 mg nitroglycerin or 1 to 3 mg of ISDN pre-PTCA, after the last balloon inflation before repeating the views used pre-PTCA and at follow-up angiography.

4. Influence of contrast agent on vasomotor tone of epicardial coronary agents Jost et colleagues have clearly demonstrated that the vasodilative changes in vessel dimensions due to contrast medium administration are significantly smaller with the use of a non ionic rather than ionic contrast medium [16]. Therefore, in quantitative coronary angiographic studies, non ionic contrast media with iso-osmolality should be applied.

It has been suggested to administer the contrast medium by an ECG triggered injection system. This is however not [yet] feasible during routine coronary angioplasty even in a setting of a clinical trial.

5. Catheter used as scaling device for measurements of absolute diameters *Angiographic versus microcaliper measured size of catheter* The image quality of the (x-ray radiated) catheter is dependent on the catheter material, concentration of the contrast agent in the catheter and kilovoltages of the x-ray source. Reiber et al. in 1985 showed that there was a difference of +9.8% in angiographically measured size as compared with the true size for catheters made from nylon. Smaller differences were measured for catheters made from woven dacron [+0.2%], polyvinylchloride [-3.2%] and polyurethane [-3.5%] [17]. It was concluded that nylon

Table III

Comparison of the true sizes of 7F, 8F, 9.5F, 10F and 11F catheter segments with angiographically measured dimensions (measurements were averaged over the two different fillings (water and contrast medium), each at two different kilovoltages (60 and 90 kV).

	True size (mm)	Angiographically measured size (mm)	Average diff (%)
7F Catheters			
Schneider	2.26	2.23±0.03	-1.16
Scimed	2.21	2.36±0.07	6.77
USCI	2.31	2.29±0.18	-1.03
8F Catheters			
Medtronic	2.58	2.61±0.05	1.58
Schneider	2.63	2.67±0.01	1.60
Scimed	2.58	2.66±0.06	3.01
USCI	2.59	2.69±0.03	3.76
9F Catheters			
Schneider	2.95	2.94±0.03	-0.06
9.5 F Catheter			
DVI	3.17	3.21±0.18	1.18
10 F Catheter			
Medtronic	3.25	3.30±0.04	1.42
Schneider	3.29	3.39±0.07	3.15
11F Catheter			
DVI	3.66	3.52±0.11	-3.93

Mean value ±standard deviation

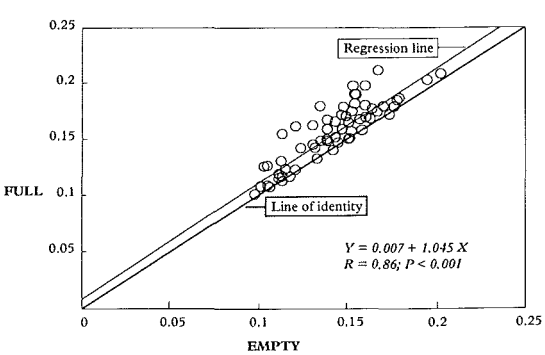

Figure 3

Relationship between the calibration factor (mm/pixel) calculated using an contrast empty (flushed) catheter versus a contrast filled catheter. A considerable number of measurements with the contrast filled catheter are above the line of identity.

catheters could not be used for quantitative studies. Recently our group has characterized the angiographic properties of newer generation catheters (Table III). Also for these catheters small differences were found between the true size and the angiographically measured size (average difference: -1.16% to 6.77%). Therefore, it was concluded that these catheters may be used for quantitative studies when the CAAS edge detection algorithm is applied.

Influence of variation in contrast filling of the catheter on calibration It was also demonstrated that catheters made from woven dacron, polyvinylchloride and polyurethane when flushed with saline had, identical image contrast qualities whereas differences in image contrast at various fillings [air, contrast with 3 different concentrations [Urografin-76, Schering AG, Berlin, Germany; 100%, 50%, 25%] of the catheters acquired at different kilovoltages was seen [17].

In addition, we measured the calibration factor in 95 catheters from 15 different clinics to compare contrast with filled saline catheter. Figure 3 summarizes our results. In a considerable number of cases, a difference in calibration factor was present with an average calibration factor of 0.143 ± 0.020 [mm/pel] for the flushed [contrast empty] catheter versus 0.156 ± 0.030 [mm/pel] for the catheters filled with contrast [$p < 0.001$]. This means that with the use of a contrast filled catheter instead of a flushed catheter, the minimal luminal diameter will have an apparent increase in diameter value of ± 0.05 mm pre-PTCA, ± 0.15 mm post-PTCA and ± 0.20 mm for the reference diameter.

For this reason we strongly advise the clinics to flush the catheters before each cine-run to have an 'identical flushed catheter' for calibration throughout the study period.

Size of the catheter Until recently only 7F and 8F catheters have been used for follow-up angiography and from earlier studies it is known which of the catheters are preferred for quantitative analysis [17,18]. However, 5F and 6F catheters are available and increasingly being used for follow-up angiography. Koning et al. have carried out a study to determine whether these catheters can be used for calibration purposes (Internal Report), (Table IV). They found that the differences between the true and angiographically measured diameters of the 5F and 6F catheters in all cases were lower for 6F than for the 5F catheters. Secondly, the Argon catheters showed the largest overall average difference, followed by the Edwards catheters and the 5F USCI catheter. The Cordis catheters, the 6F right Judkins Medicorp, the 6F Schneider and 6F USCI have the lowest average differences between the true and measured diameters. However, none of the catheters satisfy earlier established criteria [17], being that the average difference of the angiographically assessed and true diameter is lower than 3.5% and that the standard deviation of the measured diameters be smaller than 0.05 mm, under the following conditions: filled with 100% contrast concentration, filled with water, acquired at 60 kV and 90 kV. On the basis of these results, it was concluded that 5F or 6F catheters should not be used for QCA studies using the CAAS-system at the present time.

Table IV

Comparison of the true sizes of the 5F and 6F catheter segments with angiographically measured dimensions (measurements were averaged over the three different fillings (water, contrast medium concentrations of 185 and 370 mg I/cc), each at two different kilovoltages (60 and 90 kV).

	True size (mm)	Angiographically measured size (mm)	Average diff (%)
5F Catheters			
Argon	1.66	1.85 ±0.09	11.3
Cordis	1.73	1.79 ±0.15	3.2
Edwards	1.66	1.80 ±0.08	8.5
Mallinckrodt	1.73	1.72 ±0.14*	-0.8
Schneider	1.69	1.79 ±0.07	6.1
USCI	1.61	1.75 ±0.14	8.5
6F Catheters			
Argon	1.98	2.14 ±0.07	8.1
Cordis	2.01	2.03 ±0.11	1.1
Edwards	1.96	2.10 ±0.07	7.1
Medicorp (left)	1.97	2.07 ±0.04	5.1
Medicorp(right	1.99	2.02 ±0.10	1.6
Mallinckrodt	1.97	1.91 ±0.15*	-2.9
Schneider	1.94	2.00 ±0.09	3.0
USCI	1.99	2.06 ±0.08	3.4

Mean value ±standard deviation, * measurements of the Softouch tip will be more favourable

Table V

Intra- and inter-observer variability of 96 catheter diameter measurements with an electronic microcaliper.

Intra-observer variability

	N	Overall mean	Mean of diff	P-value	S.d. of diff
9F	30	2.75	0.008	NS	0.026
8F	114	2.56	0.009	NS	0.028
7F	132	2.25	0.001	NS	0.008
6F	12	1.94	-0.002	NS	0.006

Inter-observer variability

	N	1 vs 2 Mean diff	S.d. diff	1 vs 3Mean diff	S.d. diff	2 vs 3 Mean diff	S.d diff
9F	20	0.00	0.04	0.00	0.02	0.00	0.04
8F	76	0.00	0.03	0.00	0.02	0.00	0.03
7F	88	0.00	0.01	-0.01	0.02	0.00	0.02
6F	8	-0.02	0.03	-0.01	0.00	0.01	0.03

S.d. = standarddeviation; diff = differenc

6. Deviations in the size of the catheter as listed by the manufacturer In our experience, the size of the catheter as specified by the manufacturer often deviates from its actual size, especially disposable catheters. If the manufacturer cannot guarantee narrow ranges for the true size of the catheter, all catheters should be measured by a micrometer. Therefore, all catheters used during the angioplasty procedure and at follow-up are collected, labelled and sent to the angiographic core laboratory for actual measurement.

As the actual measurement can be hampered by individual variation, we have evaluated the inter- and intraobserver variability of catheter measurements at the Core Laboratory. A total of 96 catheters with different sizes [6F to 9F] were measured by 3 different analysts independent of each other. One month later, all three analysts measured the same catheters for a second time, unaware of the results from the first time (Table V). The intraobserver variability was excellent with a mean difference of less than 0.01 mm and a standard deviation of the difference of less than 0.03 mm for all catheter sizes. Similarly the interobserver variability between the 3 analysts showed a mean difference of less than 0.03 mm and a standard deviation depending on the size between 0.00 and 0.04 mm. We conclude that the catheter can be measured with an excellent accuracy and precision.

7. Variation in data analysis *Minimal luminal diameter* From the contours of the analyzed segment,following pincushion correction and calibration, a diameter function can be determined by computing the distances between the left and right edges. From these data a number of parameters can be obtained.Direct measurements include 1) minimal luminal diameter, 2) lesion length 3) obstruction and reference area. Interpolated measurements include the reference diameter while percent diameter stenosis and percent area stenosis are derived measurements.

Particularly, the minimal luminal diameter is of great importance as it presents to the inverse fourth power in the formulas describing the pressure loss over a coronary obstruction. Moreover to determine the effect of interventions on the severity of coronary obstructions, one should compute the changes in minimal luminal diameter and not those in percent diameter stenosis, as the reference position in general will also be affected by intervention.

A major limitation of edge detection (aside from the technical quality of the cine-film) is the analysis of the post-angioplasty result. In particular, dissections are a frequent occurrence following PTCA and the resulting haziness, irregular borders or extravasation of contrast medium makes edge detection difficult. There is no ideal solution to this problem. If a dissection is present on the post-angioplasty angiogram, the computer 'decides' whether the extraluminal defect is included or excluded in the analysis, thereby avoiding subjective bias.

To determine the accuracy and precision of the post-angioplasty luminal assessment by edge detection, a consecutive series of 117 end-diastolic post-PTCA cine-frames were analyzed by two independent analysts. The agreement by the standard deviation of the between-analysis difference was 0.21 mm. Therefore, quantitative coronary angiography shows that a small discrepancy exists in the post-PTCA luminal assessment between two analyses. This observa-

tion suggests that the edge detection is an acceptable method for objectively assessing the result of coronary balloon angioplasty.

Reference diameter Although the absolute minimal luminal diameter is one of the preferred parameters for describing changes in the severity of an obstruction as a result of an intervention, percent diameter stenosis is a convenient parameter to work with in individual cases. The conventional method of determining the percent diameter stenosis of a coronary obstruction requires the user to indicate a reference position. It is clear that this computed percent diameter stenosis of an obstruction depends heavily on the selected reference position.In arteries with a focal obstructive lesion and a clearly normal proximal arterial segment the choice of the reference diameter is straightforward and simple. However in cases where the proximal part of the arterial segment shows combination of stenotic and ectatic areas, the choice may be difficult. To minimize these variations, we have implemented many years ago an interpolated technique, which is not user defined to determine the reference diameter at the actual stenosis site without operator interference. This basic idea behind this technique is the computer estimation of the original diameter values over the obstructive region (assuming that there was no coronary disease present) based on the diameter function. Following this approach the reference diameter is taken as the value of the polynomial at the position of the minimal luminal diameter. The interpolated percent diameter stenosis is then computed by comparing the minimal diameter value at the site of the obstruction with the corresponding value of the reference diameter function.

Length of analyzed segment Anatomic landmarks such as bifurcations are used for the manual definition of start and end points of arterial segments so as to minimize the problem of non identical analyses. For that purpose, drawings are made by the investigator of all different views suitable for quantitative analysis, pre-PTCA, post-PTCA and at follow-up. In addition, a hard-copy is made of every drawing, to enable analysis of the exact same segments at follow-up angiography.

Manual corrections For those parts along the detected arterial segment, where the observer does not agree with the automatically detected boundaries, manual correction by means of a writing table are possible. If the manual corrections are performed after the first iteration of the edge detection procedure, the system is allowed to find an optimal path within these limits during second iteration. It has been our experience that in almost all cases the contour will then follow the desired path at these locations. An advantage of this approach is that the final contour will still be based on the available edge detection information within the limitations set by the observer. This type of correction may be set as 'soft' correction. In those situations where the soft correction still does not result in the desired contour after the second iteration, the user may apply a final 'hard' correction. The computer registers for both the left- and right- hand contours the length of the arterial segments that were manually corrected, expressed as percentages of the total length of the analyzed contour sides.

Frame Selection Usually, an end-diastolic cine-frame is selected for the quantitative analysis of a coronary obstruction to avoid blurring effect of motion. If the obstruction is not optimally visible in that particular frame [e.g. by overlap by another vessel] a neighbouring frame in the sequence is selected. However, since a marker is not always present on the cine-film, the visually selected cine-frame may not be truly end-diastolic. Beside that, individual analysts may choose different frames even when the same selection criteria are followed. In addition, it is possible that the frames are selected from different cardiac cycles, in relation to the moment of contrast injection. Reiber et al have critically assessed this problem in 38 films whether selection of the frame [3 frames preceding, 3 frames immediately following the frame and the same frame as chosen by the senior cardiologist as the reference end-diastolic frame, but one cardiac cycle earlier or later] resulted in significant differences in the measurements. They found no significant difference in the mean and the standard deviation of the differences for the obstruction diameter, interpolated reference diameter, percent diameter stenosis, extent of the obstruction and area of atherosclerotic plaque obtained in various frames with respect to the 'select reference frame'. Therefore, it is concluded that the selection of a true end-diastolic cineframe for quantitative analysis is not very critical and that in case of overlap it is possible to select a neighbouring frame [19].

Quality control in the MERCATOR trial

In the MERCATOR-trial – a restenosis prevention trial with a new angiotensin converting enzyme inhibitor cilazapril – in which 26 clinics have participated, quantitative coronary angiography was used to determine the primary endpoint as defined by the rate and extent of restenosis. Before the clinics could start to recruit patients for the study, they had to supply 2 sample cinefilms for analysis to demonstrate that they could comply with the required standards. Of all participating clinics 1 or more cm-grid films of all modes of all image intensifiers were received at the core laboratory to allow correction for pincushion distortion of the image intensifiers. All clinics received a set of radiopaque plates to be able to make it clear on the film whether nitroglycerin or isosorbide dinitrate was given before the contrast injection, which field size of the image intensifier was used, the balloon pressure and balloon size used etc. In a period of 5 months (June 1989 -November 1989), a total of 735 patients were recruited with a minimum of 8 patients and a maximum of 56 patients per clinic. Five of the 735 patients were not included in the final analysis of the trial because their cinefilm could not be quantitative analyzed; in 1 patient the film developing machine broke down so that no post-PTCA film was available for analysis; in 2 patients analysis was not possible due to a large coronary artery dissection; in 1 patient no matching views were available and in 1 patient poor filling of the vessel had occurred [due to the use of a catheter with side holes] making comparison with the baseline film unreliable.

In 2 patients pre-PTCA, 34 patients post-PTCA and in 4 patients at follow-up angiography intracoronary nitroglycerin or isosorbide dinitrate had not been administered as assessed by the absence of the plate on the film and nothing had

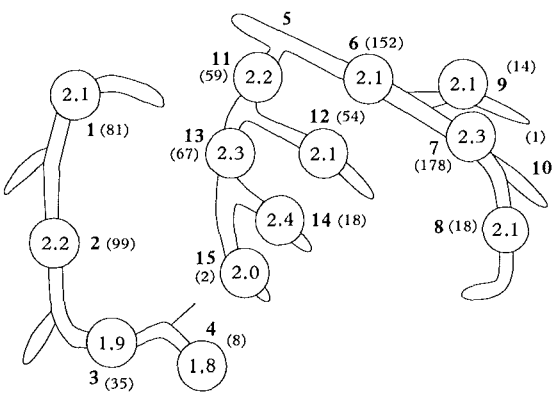

Figure 4

The average number of matched projections (pre-PTCA, post-PTCA and at follow-up) that were used for quantitative analysis in the MERCATOR trial per segment are given in the circles. The numbers between the brackets are the total number of stenoses for that particular segment.

been recorded in the column 'medication given during the procedure'. In 26 patients, a 5 or 6 French catheter was used at the time of follow-up angiography. In 8% of the views pre-PTCA, 12% of the post-PTCA views and 12% of the follow-up views, the images had to be analyzed with a contrast-filled catheter because no flushed catheter was available. Figure 4 shows the average number of matched views available for QCA analysis per segment dilated.

Qualitative assessment

In addition to quantitative measurements, an angiographic core laboratory can assess qualitative or morphologic factors, such as type of lesion (according to the AHA classification), description of the eccentricity of the lesion and type of dissection after the procedure, using modified NHLBI criteria, to establish the roles of these descriptors in the restenosis process. Recently, we have studied the inter-observer variability for the description of the lesion and the type of dissection [20,21]. Using the Ambrose classification there was an agreement of 80% between the two assessors of the core laboratory and for dissection there was an agreement of 87%. At the present time, no additional data is available but will become available in the near future,

Conclusion

The use of quantitative coronary angiography is an objective and reliable method to evaluate changes in arterial dimensions over time. An angiographic core laboratory plays a crucial role in minimizing the problems of data acquisition and data analysis as well as the overall quality of the trial. Beside that an angiographic core laboratory may help demonstrating the reproducibility of qualitative factors and their role in the occurrence of acute and late complications of PTCA.

Furthermore, in our experience it has been possible to standardize angiographic data acquisition from 82 different clinics in Europe, United States and Canada.

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Chapter 4

Radiologic quality of coronary guiding catheters:

A quantitative analysis

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Abstract Quantitative Coronary Angiography (QCA) is a validated and widely accepted method to investigate changes in arterial dimension over time. Calibration of measurements is enabled by the use of the coronary catheter as a scaling device. The dimensions and laminar composition of coronary catheters, however, have changed significantly over recent years and the suitability of the current generation of coronary catheters for calibration purposes has not been validated. We therefore recorded 57 coronary guiding catheters on cinefilm, and compared their automated quantitative measurements (Cardiovascular Angiography Analysis System; CAAS), with their true values (precision micrometer). We found an overall underestimation of quantitatively derived dimensions, ranging from -8.9% to +4% for water-filled catheters and from -15.5% to -3.9% for contrast-filled catheters. In conclusion, while the current generation of coronary guiding catheters shows a wide variety in radiologic quality, it can be clearly detected by the CAAS system, and is suitable for calibration of QCA measurements (with the exception of the DVI atherectomy catheter), provided that calibration is done on contrast-empty catheters. (Catheterization and Cardiovascular Diagnosis 1994;33:55-60)

Quantitative coronary angiography (QCA) is increasingly being used for both on-line analysis to guide interventional procedures as well as for off-line analysis in the study of restenosis and progression/regression. Despite the widespread and long-standing use of coronary angiography, as well as recent improvement in radiographic acquisition, the interpretation of angiograms in clinical practice has changed little over the years and is still assessed visually. Visual assessment, however, is subjective with a large inter- and intraobserver variability and should therefore not be incorporated in scientific research of restenosis prevention and progression-regression [1-3]. Computer-assisted or automated QCA is not only objective but also has the advantage of being more accurate and reproducible than visual or hand-held calliper measurements.

At the Thoraxcenter, the computer-assisted Cardiovascular Angiography Analysis System (CAAS) using an automated edge detection technique was developed and has recently been updated [4]. The CAAS has been used in the angiographic 'core laboratory' in several multicenter pharmacological and device studies [5-7]. In order to obtain reliable and reproducible quantitative measurements of coronary cine-angiograms, variations in data acquisition associated with the use of different coronary guiding catheters must be minimized. The actual size of individual catheters often differs significantly from the nominal size stated by the manufacturer. In order to obtain the true external diameter of the catheter, all catheters are measured by a digital micrometer, with a high degree of accuracy and precision (0.001 mm) [8]. This provides an accurate basis for cali-

bration of the measurements of the catheter image obtained by QCA.

Combining micrometer measurements and radiological size results in a calibration factor (mm per pixel) which allows objective and absolute measurements of coronary artery dimensions. Consequently, QCA measurements based on catheter calibration have been previously shown to be highly reproducible [9].

The automated QCA measurement of a guiding catheter and the subsequent calibration factor are significantly influenced by the material of the catheter, the presence of radiographic contrast in the catheter lumen, and the settings of the X-ray equipment. QCA validation studies of previously available catheters have been reported [10-14] but the recent development of catheters of new materials in tri-laminar composition with altered radiopacity made reinvestigation of the radiographic quality of coronary guiding catheters necessary.

Methods

Catheters Mid-portions of a total of 57 coronary guiding catheters (Table I) were filmed. We selected frequently used and commonly available catheters from Scimed (Triguide series), Medtronic (Sherpa), Bard (Illumen), Cordis (6Fr Diaventional and 8 Fr Brite tip), Schneider (Soft touch) and DVI (atherectomy catheters).

Image acquisition and processing A monoplane Philips Poly Diagnost C2 machine equipped with an MCR X-ray tube and powered by an Optimus CP generator (Philips Medical Systems , Best, The Netherlands) was used for all radiographic imaging. The 5" (12.5cm) field mode of the image intensifier (focal spot 0.5 mm) was selected. Catheters were taped on a block of polymethylmethacrylate (PMMA or perspex) 25*250*250 mm, and filmed in the isocenter of the X-ray system. The distances from X-ray source to catheter and from X-ray source to the image intensifier were 75 and 100 cm respectively.

Each catheter was filmed at two kilo-volt levels. The X-ray system automatically adjusts the kilovoltage level to 60 and 90 kV upon the interposition of 125 or 250 mm PMMA polyethylene blocks, respectively. The incorporation of the PMMA polyethylene blocks results in more appropriate kilovolt levels and a scatter medium which more closely imitates the X-ray scatter in the human thorax during cineangiography.

Catheters were filmed at two filling conditions; filled with water and filled with 100% radiographic contrast (Iopamidol;370 mg iodine/ml, Bracco, Italy). A centimeter grid was filmed in exactly the same plane for scaling purposes.

All catheters were imaged and acquired on 35-mm cinefilm (CFE Type 2711, Kodak, Paris, France) at a frame rate of 12.5 images/sec, using an Arritechno 90 cine camera (Arnold & Richter, Munich, Germany) with an 85 mm lens. The cinefilms were processed by a Refinal developer (Agfa-Gavaert, Leverkusen, Germany) for 4 minutes at 28°C. The film gradient was measured in all cases to ensure that the optical densities of interest were on the linear portion of the sensitometric curve.

QCA Edge detection For each individual catheter 8 cineframes were quantitatively analysed (water/60kV, water/90kV, Iopamiro/60 kV, Iopamiro/90kV, with two cineframes in each setting). All cineframes were analyzed using the new generation Cardiovascular Angiography Analysis System (CAAS II) (Pie Medical Maastricht, the Netherlands) [4]. The principles of computer assisted quantitation of coronary cine-angiograms using this system have been previously described in detail [15]. Briefly, the selected cineframes were digitized by a high resolution digital camera. The calibration factor for this study was determined by a manual definition of three pairs of points on horizontal lines of a centimeter grid in the center of the image. The calibration factor is thus expressed in mm per pixel. Catheter contours are then determined automatically with the edge detection algorithm over a length of approximately 15 mm. This edge detection is based on the

weighted sum of the first and second derivative functions applied to the digitized brightness profile information along scan lines perpendicular to the local centerline directions of the catheter. All contour positions were corrected for the pincushion distortion induced by the image intensifier.

Calibration The true size of the catheter, was measured by an experienced analyst as the mean of two orthogonal measurements of each individual catheter by an electronic precision-micrometer (Mitutoyo OP-1HS Tokyo, Japan; accuracy 0.001 mm) [8].

Data Analysis The mean ± standard deviation of the QCA measurements of cinefilm-recorded catheters was calculated for each individual catheter from four cineframes comprising either contrast-filled catheters or water-filled catheters. This was averaged per French size and per manufacturer. The mean of two digital micrometer measurements was taken as the true size. Radiographically measured size was compared to the true size and expressed as percent deviation, a positive percentage deviation meaning a larger QCA measurement compared to the true catheter size, and a negative percentage deviation meaning a smaller QCA measurement compared to the true catheter size.

Results

The data are averaged over the 60 and 90 kilo volt level and presented per manufacturer and french size, but subdivided in measurements of contrast-filled and contrast-empty catheters. Micrometer-derived diameters differed significantly from the manufacturer's nominal size. The micrometer measurements of the external diameter were on average 0.025mm smaller than the nominal diameter listed by the manufacturer ($p<0.0001$).

Quantitative analysis of each catheter, either filled or empty of contrast, results in a lower value, compared to assessments by digital micrometer ($P<0.0001$) (Fig. 1). This deviation is less in water-filled catheters (ranging between -8.9% and +4%), and more distinct in contrast-filled catheters (ranging between -15.5% and -3.9%). Overall the deviation results in an average weighted underestimation of -2.9% for catheters filled with water, and -7.1% for contrast-filled catheters, compared to micrometer measurements. Applying the edge detection procedure on water-filled catheters results consistently in a larger diameter (0.11mm ± 0.07), when compared to analysis of contrast-filled catheters ($P<0.0001$) (Figs. 1 and 2) and thus generates less underestimation of the catheter diameter on cinefilm.

Discussion

Assessment of the true catheter size One of the vital components of the calibration process is an accurate micrometer measurement of the coronary guiding catheter [12, 15, 16]. This is a mandatory step in the calibration since actual sizes of the catheter often differ from the nominal catheter sizes specified by the manufacturer. In our study it appears that catheters are 0.025mm smaller in diameter in comparison to the dimensions listed by the manufacturer ($P<0.0001$) (Table II). Very low standard deviations demonstrate the high reliability of the digital micrometer assessment. Previously, this method has been validated [14]. Inter-observer variability between 3 analysts measuring a total of 96 catheters ranging from 6 to 9 French, showed a mean difference of less than 0.03mm and a stan-

Table I
Summary of the Manufacturer, Composition of Material, Number and Sizes of the Catheters Studied.

Material	N	Size (Fr)	Manufacturer
Teflon/Polyurethane/Ultrax	7	9.5/11	DVI
Polyurethane/Steel braid/Teflon	6	8/10	Medtronic
Teflon/Steel braid/Polyurethane	13	7-10	Schneider
Teflon/Steel braid/Trilon	9	7/8	Scimed
Teflon/Kevlar braid/Pebax	12	7/8	Bard
Trilon/Steel braid/Nylon	10	6/8	Cordis

Table II
Catheter Dimensions and Average Deviation

		Catheter Dimensions (mm) and Average Deviation (%)				
			cine-film			
Material		micrometer	water-filled		contrast-filled	
Size	N	mm	mm	%	mm	%
TEFLON/PU/ULTRAX						
9.5fr	3	3.17±0.01	3.01±0.06	-5.2	2.77±0.08	-12.6
11fr	4	3.66±0.02	3.32±0.16	-8.9	3.08±0.05	-15.5
PU/STEEL/TEFLON						
8fr	2	2.58±0.02	2.45±0.00	-4.8	2.39±0.01	-7.1
10fr	4	3.25±0.02	3.12±0.01	-4.0	3.01±0.02	-7.5
TEFLON/STEEL/PU						
7fr	3	2.26±0.02	2.10±0.01	-6.9	2.10±0.03	-7.2
8fr	4	2.63±0.01	2.50±0.02	-5.0	2.45±0.02	-6.7
9fr	5	2.95±0.02	2.75±0.03	-6.5	2.72±0.03	-7.5
10fr	1	3.29	3.18±0.03	-3.4	3.09±0.00	-6.1
TEFLON/STEEL/TRILON						
7fr	4	2.21±0.02	2.30±0.05	4.0	2.12±0.06	-3.9
8fr	5	2.58±0.02	2.56±0.06	-1.0	2.41±0.07	-6.7
TEFLON/KEVLAR/PEBAX						
7fr	6	2.25±0.03	2.30±0.02	2.4	2.16±0.04	-3.9
8fr	6	2.59±0.01	2.55±0.02	-1.9	2.49±0.02	-4.3
TRILON/STEEL/NYLON						
6fr	5	1.95±0.00	1.91±0.01	-2.0	1.81±0.02	-7.2
8fr	5	2.67±0.01	2.59±0.03	-2.9	2.48±0.02	-7.1
TOTAL	57	guiding catheters		2.9%		-7.1%

Micrometer (mm), catheter dimensions measured by a digital precision micrometer which is considered to be the true size; Water-filled, QCA dimensions of water-filled catheters; Contrast-filled, QCA dimensions of contrast-filled catheters.
Materials: PU, poly urethane; Steel, stainless steel wire braid
The percentage deviation is the difference from QCA derived dimensions in comparison to the true size ($P<0.0001$ for both filling states). Consistently the analysis of contrast-filled catheter image results in a significant smaller diameter ($P<0.0001$ vs water-filled).

dard deviation depending on the size between 0.00 and 0.04mm. The intraobserver variability showed a mean difference of less than 0.01mm and a standard deviation of the difference of less than 0.03mm for all sizes. This indicates that it is possible to measure the true size of a catheter with a high degree of accuracy and precision [8].

Analysis of contrast-filled and contrast-empty catheters Additional reliability of calibration can be achieved by the acquisition of the catheter image after flushing with saline or filling with blood in the same projection and field of view of the image intensifier, positioning the catheter in the centre of the radiographic image and operating a correction for pincushion distortion [8]. In this study, overall deviation of angiographically measured dimensions of contrast-empty catheters was approximately -2.9% ($P<0.0001$, range -8.9% to +4%) (Table II). When free of contrast, the edge detection algorithm reliably detects the external border of the guiding catheter. The weighted sum of the first and second derivative will result in a slightly smaller diameter than the true diameter. However, the presence of the misleading high density column of contrast medium inside the catheter lumen will cause a much higher underestimation of the catheter dimension since the edge detection will trace the contours of the contrast column, i.e., the internal diameter of the catheter, instead of the external diameter which is actually measured by the digital precision micrometer (Fig. 1). In our study, analysis of contrast-filled catheters resulted in an average underestimation of -7.8% (range -15.5% to -3.9%), and thus a subsequent overestimation of calibration factor and coronary dimensions of 8.5% ([[100/(100-7.8)]-1]*100). This clearly underlines the importance of calibration methodology, not only for longitudinal studies, but also for on-line clinical practice.

In a previous study we have compared quantitatively analyzed cineframes using contrast-filled catheters and water-filled catheters (either filled with blood or flushed with saline) for calibration [14]. The measured calibration factor was 0.156 ± 0.030 mm per pixel with the contrast-filled catheter, and 0.143 ± 0.020 mm per pixel with the catheter filled with saline or blood ($p<0.001$) [8, 17]. The use of calibration obtained from contrast-filled catheters would have resulted in an average overestimation of 9.1% (range -1%/30%) of the measurements obtained from cineframes recording a contrast empty catheter.

Catheter material and composition The use of catheters of low radiopacity should be avoided in QCA studies [12, 16]. Our study has highlighted the wide variation in radiopacity of coronary guiding catheters. Every guiding catheter must possess a minimal degree of radiopacity, in order to be visible on fluoroscopic or cinefilm images. For calibration purposes, a sharp contrast-gradient near the outer edge is vital for accurate contour detection. The outer layer of material must overcome the peak in the density profile caused by stainless steel braidings. Analysis of catheters with a highly radiopaque outer layer, or without presence of a 'distracting' stainless steel braiding in the middle layer (DVI and Bard), may result in less underestimation of the angiographic catheter size. Both may contribute to the low deviation from the true size, and even positive values in the Bard

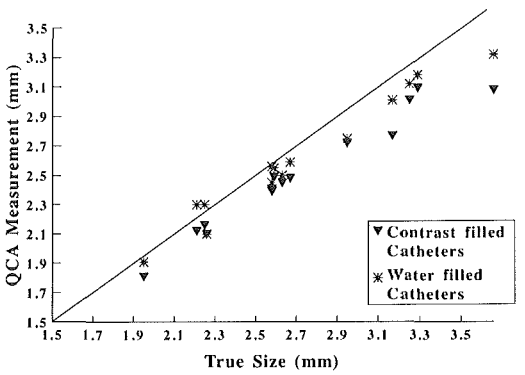

Figure 1
QCA measurements plotted against the true size, as measured by a precision micrometer. Diameters of contrast-filled catheters are underestimated more than water filled catheters ($p<0.0001$).

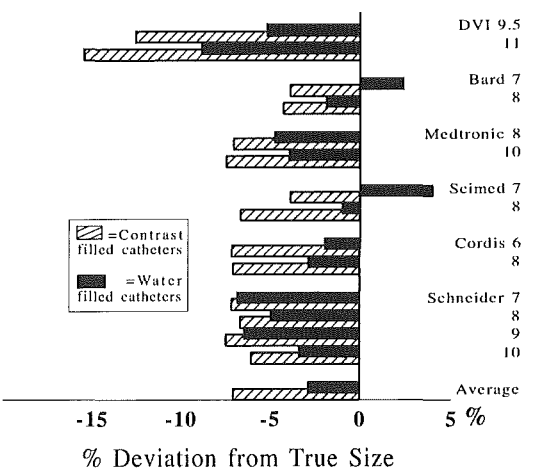

Figure 2
Schematic presentation of the percentage deviation from the true size for contrast-empty and contrast-filled coronary guiding catheters per manufacturer and per French size.

catheters. The atherectomy catheters, which also lack a steel braiding as well, introduce an extreme divergence in obtained values. These catheters, even filled with water give a deviation of -5.2 to -8.9% depending on their French size. The consistently poor reproducibility of the computer assisted measurements of each individual atherectomy catheter is expressed in a wide standard deviation, and is an indication for the relative radiolucency of the Ultrax outer layer. Average deviations from the true size of the remaining manufacturers was -4.2 and -5.9% for Medtronic and Schneider (polyurethane) catheters, and +1.2, +0.3, and -2.4% for Scimed (Trilon), Bard (Pebax), and Cordis (nylon) catheters, respectively, which is in concordance with the observations of Fortin et al. [18].

We measured the shafts of coronary guiding catheters, according to the protocol used by Reiber et al [12]. The rationale of this approach is the following: catheter material at the tip does not vary from the material used in the catheter shaft, with the exception of some diagnostic catheters [13]. In general it is the addition of barium sulphate or other radiopaque substances to the nylon and nylon-like compounds in especially the outer layer of the coronary catheters and not the compound itself that defines radiopacity and radiographic qualifications.

Conclusion

Correct acquisition of the angiographic catheter dimension is critical for reliable calibration of QCA measurements, but when these conditions are respected, this technique can give accurate and reproducible results. The use of coronary guiding catheters of poor radiographic quality, and catheters of different brands in a long-term angiographic follow-up study should be minimized. In a multicenter study, this issue is ideally standardized by providing each participating centre with similar guiding catheters, or diagnostic catheters of high quality which are used before and after angioplasty, as well as during the follow-up catheterization procedure.

We conclude that coronary guiding catheters can only be analyzed reliably only when recorded in contrast-empty state. Although the rationale for calibrating angiographic cine-images on a contrast empty catheter is evident, some angiographic core-labs persist in calibrating their cine-angiograms on a contrast filled catheter. The reason for this practice is apparently that these angiographic core-labs are using a QCA-system, which is unable to detect the edge of an empty catheter. This inability is related to the lack of resolution and potentially to the algorithm used in this system.

Acknowledgements

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Chapter 5

Coronary arteriography for quantitative analysis:

Experimental and clinical comparison of cinefilm and video recordings

Yukio Ozaki, David Keane, Jean-Paul R. Herrman, David Foley, Jürgen Haase, Ad den Boer, Carlo di Mario, Patrick W. Serruys

American Heart Journal 1995;129:471-5

Abstract Although use of videotape for the recording of coronary angiograms continues to grow, the validity of quantitative coronary angiographic analysis of video images remains unknown. To estimate the reliability of angiographic images recorded on videotape, experimental and clinical angiograms were recorded simultaneously on both 35 mm cinefilm and super-VHS videotape with normal images and with spatial filtering of the images (edge enhancement) on a digital cardiac imaging system. The experimental angiographic studies were performed with plexiglass blocks and stenosis phantom 0.5 to 3.0 mm in diameter. The clinical angiograms were recorded in twenty patients undergoing percutaneous transluminal coronary angiography (31 frames before and 20 frames after percutaneous transluminal coronary angiography). The cinefilm and corresponding videotapes were analyzed off-line with the new version of the coronary angiography analysis system. For the experimental study, measurements of minimal luminal diameter obtained from cinefilm, normal video-tape, and edge enhanced videotape were compared with the true phantom diameter. In the clinical study, the agreement between measurements obtained from cinefilm and measurements from normal-image videotape and edge-enhanced videotape was examined. In the phantom series the accuracy and precision of quantitative coronary angiography measurement for cinefilm were -0.10 mm ±0.08 mm, for normal-image videotape -0.11 mm ±0.18 mm and for edge-enhanced videotape -0.10 mm ±0.11 mm. In the clinical series, the differences between measurements from cinefilm and normal-image videotape were 0.14 mm ±0.20 mm and from cinefilm and edge-enhanced videotape were 0.04 mm and ±0.13 mm.
In the experimental phantom study, the use of cinefilm resulted in the most precise measurements. In the clinical study, edge-enhanced videotape provided the highest agreement with measurements obtained from cinefilm. These indings suggest that cinefilm is more reliable than video as a recording medium for quantitative coronary analysis in scientific studies; however, for routine practice, videotape with edge enhanced images may provide an acceptable alternative. (American Heart Journal 1995;129:471-5)

Coronary angiography continues to be the gold standard for coronary artery imaging in clinical practice. With the increasing demand for quantitative coronary angiography (QCA) and the development of digital acquisition systems, there has been a substantial growth in the deployment of videotape as a recording medium because of its easy handling, instant replay capability, and low cost [1-

4]. In some institutions videotape has replaced 35mm cinefilm as the original imaging medium. However, despite proposals for the replacement of cinefilm by videotape, the suitability of video recordings for QCA analysis has not been established. To evaluate the potential application and reliability of videotape recording for QCA for clinical studies, recordings of experimental phantom stenoses [5-8] and clinical coronary artery stenoses before or after balloon angioplasty were assessed with the new version of a computer based coronary angiography analysis system (CAAS II) [5].

Methods

Experimental image acquisition of phantom stenoses.
For the in-vitro validation we used radiolucent plexiglass cylinders (50 mm length, 20 mm in outer diameter) with precision-drilled concentric circular lumens (tolerance 0.01 mm) of 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 mm in diameter. The length of each phantom stenosis channel was 20 mm and the adjacent 'normal' channel length of the proximal and distal segments was 30 mm. The plexiglass channel (including the artificial stenosis) was filled with contrast medium (iopamidol 370; 370 mg iodine/ml (Bracco, Milano, Italy)). Digital and cinefilm acquisition was performed with additional plexiglass blocks (12.5 cm anteriorly and 5 cm posteriorly). These plexiglass blocks provide a more appropriate kilovolt-level and a scatter medium that more closely approximates the radiological scatter of the human thorax during angiography. Angiograms were performed with the 5-inch field of an image intensifier, with separate recordings and two different focal spots (0.5mm, which was used for most of the experimental and clinical series, and 0.8mm) and at an image acquisition rate of 25 frames/sec. The radiographic system settings were kept constant (kilovolt, milliampere, x-ray pulse width) in each projection. All phantoms were imaged at the radiographic isocenter of the X-ray gantry [9] and acquired simultaneously on 35-mm cinefilm (Kodak CFE Type 2711, Paris, France) and digitally (Philips Digital Cardiac Imaging (DCI) system; Philips, Best, The Netherlands).

Coronary arteriographic procedure before and after percutaneous transluminal coronary angiography (PTCA) Coronary angiography was performed in multiple projections with 8F polyurethane catheters (Cordis, Miami, Fla.) in 20 patients before and after PTCA at the Thoraxcenter, Rotterdam. To control vasomotion, intracoronary isosorbide dinitrate (1-3 mg) [10] was administered before manual injection of contrast medium (iopamidol 370; 370 mg iodine/ml) at 37°c. The 5-inch field of the image intensifier was selected and the radiographic settings were kept constant (kilovolt, milliampere, x-ray pulse width) in each projection. All clinical images were simultaneously acquired digitally by DCI and on 35-mm cinefilm with frame rates of 25 images/sec.

Image processing and spatial filtering (edge-enhancement) in the DCI Both experimental and clinical angiographic images were stored on a 474 MB Winchester disk. The DCI system uses a matrix size of 512 x 512 pixels (average horizontal pixel size 200 μm; density resolution 8 bits = 256 gray levels). The images were processed with the automated coronary analysis software package of the DCI system [8,11,12]. Edge-enhanced images were produced by spatial filtering on the DCI system. The algorithm of spatial filtering operates by substituting new pixel values for the original pixel values on the coronary angiogram [13]. A visible horizontal edge of the coronary artery is formed when a string of horizontally connecting pixels displays values which are different from those immediately above or below. Similarly, a vertical edge of the coronary artery is formed when a string of vertically connecting pixels have values different from those immediately to the right or to the left. Oblique edges are generated through combinations of horizontal and vertical components. The default edge-enhancement mode of the DCI system was used. Spatial filtering amplifies the pixel differences between the opacified vessel and its

background, thereby providing a more distinct border to the coronary artery. Images with and without edge-enhancement were then directly relayed to the video recorder (Panasonic 7330, Osaka, Japan) and recorded on S-VHS tape (Fuji-film double coating SE-60, Sizuoka, Japan).

Stenosis characteristics Twenty-one experimental frames and the 51 pre-PTCA and post-PTCA clinical frames were selected for quantitative analysis, and their minimal luminal diameter was measured. Of the clinical angiograms, the 31 pre-PTCA frames consisted of 16 left anterior descending, 10 left circumflex, and 5 right coronary artery lesions. Of the remaining 20 post-PTCA frames evaluated, 10 showed left anterior descending, 8 left circumflex, and 2 right coronary artery lesions. All pre-PTCA lesions had >50% diameter stenosis.

Correction of pincushion distortion Before the performance of the calibration and analyses of the stenoses, computerized correction for pincushion distortion was applied by the recording and subsequent off-line digitization of a centimeter grid placed in front of the image intensifier.

Calibration of images For the experimental in vitro series, the measurements of the phantom stenoses were calibrated with a contrast-filled 3.0 mm-diameter circular channel in a plexiglass cylinder as a scaling device. This calibration frame was digitized and traced by the automated contour detection algorithm before the series of analyses of the in vitro phantoms was begun. In the clinical study, cinefilm and videotapes were calibrated by the use of the recorded contrast-free catheter tip as a scaling device. The nontapering catheter tip was measured with a precision micrometer (Mitutoyo No.293-501, Tokyo, Japan; accuracy 0.001 mm).

Quantitative analysis in CAAS II Cinefilms and corresponding frames of videotapes with and without spatial filtering (edge enhancement) were quantitatively analyzed off-line by using the computer-based Cardiovascular Angiographic Analysis System (CAAS II; Pie Medical, Maastricht, The Netherlands) [5,14-19]. In the experimental study a sufficiently long segment of the plexiglass cylinders including the stenosis phantom was selected for analysis. In the clinical study frames without foreshortening or overlapping side branches were selected. Arterial dimensions of clinical frames were measured at specific distances from identifiable branch points in end-diastolic frames. The entire cineframe of size 18 x 24 mm is digitized at a resolution of 1329 x 1772 pixels in the CAAS II system. During image acquisition of videotape a time-base corrector was implemented to ensure high quality stand-still images [1]. The video signal from tapes with and without spatial filtering (edge-enhancement) were digitized at a resolution of 512 x 512 pixels by the CAAS II system. In the CAAS II system, the edge detection algorithm is based on the first and second derivative functions applied to the digitized brightness profile along scanlines perpendicular to a model using minimal cost criteria [20,21]. The contour definition is carried out in two iterations. First, the user defines a number of center-line points within the arterial segment that are interconnected by a straight line and serve as the first model. Subsequently, the program recomputes the centerline, determined automatically as the midline of the contour positions that were detected in the first iterations. A computer-derived estimation of the original dimensions of the artery at the site of the obstruction was used to determine interpolated reference values for arterial diameter and area. Manual correction of the automatically detected contours was neither necessary nor performed in either the experimental phantom nor the clinical studies (in routine practice at angiographic core laboratories, subjective manual correction of the detected contours at the minimal luminal diameter is only occasionally performed in the case of complex dissections with parallel luminal extravasation, which was not present in our clinical angiograms)

Linear regression analysis of QCA measurements obtained from cinefilm (CINE), normal-image videotape (VIDEO-N) and edge-enhanced videotape (VIDEO-E), against true phantom diameter. MLD, minimal luminal diameter.

Figure 1A
Shows cinefilm versus phantom.

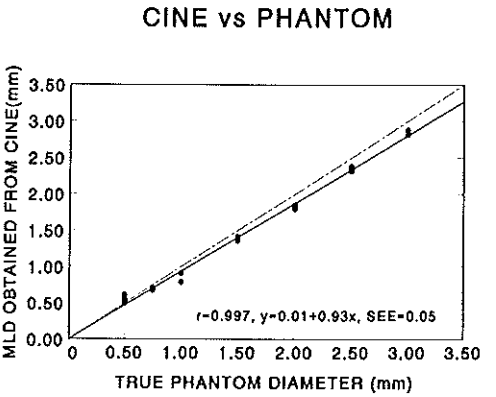


Figure 1B
Normal-image videotape versus phantom.

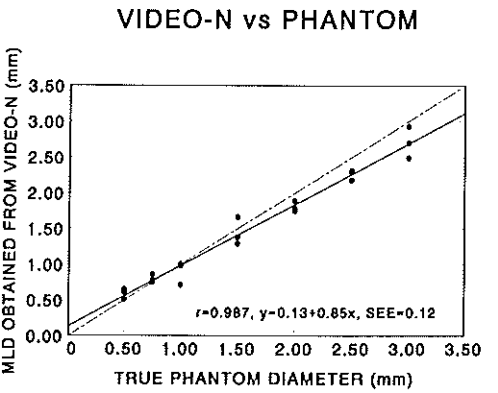


Figure 1C
Edge-enhanced videotape versus phantom.

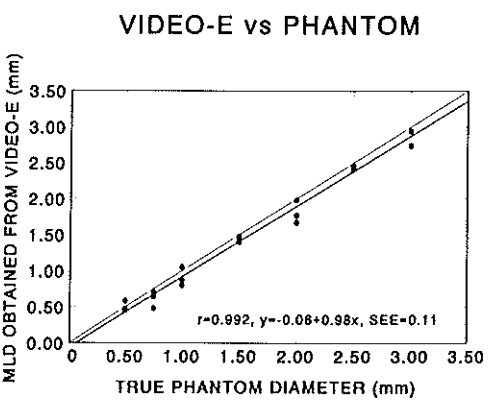
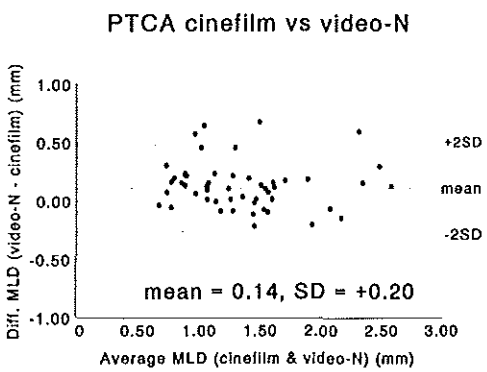


Table I
Comparison of phantom diameter and minimal luminal diameter obtained from cinefilm, normal-image videotape, and edge-enhanced videotape in experimental in vitro phantom study.

Recording medium	Accuracy	Precision	Difference	Correlation	Linear regression analysis	SEM
Cinefilm	-0.10	±0.08	P<0.01	0.997	y=0.01+0.93x	0.05
Normal-image videotape	-0.11	±0.18	p<0.05	0.987	y=0.13+0.85x	0.12
Edge-enhanced videotape	-0.10	±0.11	p<0.01	0.992	y=-0.06+0.98x	0.11



Agreement between measurements obtained from cinefilm and video recordings according to statistical approach proposed by Bland and Altman (22).

Figure 2 A
Difference between normal-image videotape and cinefilm measurements has been plotted against their mean.

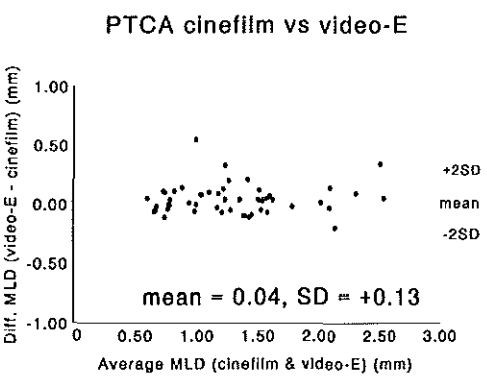


Figure 2 B
Difference between edge-enhanced videotape and cinefilm measurements has been plotted against their mean.
Abbreviations as in Fig. 1.

Statistics In the experimental study, the individual measurements of minimal luminal diameter were compared with the true phantom diameter using the paired Student t-test and linear regression analysis. The mean of the signed differences between the true phantom diameters and the individual minimal luminal diameter values derived from measurements of cinefilm and of videotape were considered an index of accuracy and the SD of the differences an index of precision. In the clinical study, the mean ± SD of the signed differences between measurements of minimal luminal diameter derived from cinefilm and measurements of minimal luminal diameter derived from videotape were used as an index of agreement between measurements from the different recording media. This statistical approach to the comparison of two measurement systems has been previously recommended by Bland and Altman [22].

Results

In vitro results The results of QCA measurements obtained from cinefilm, videotape with normal image, and videotape with spatial filtering (edge enhancement) and their comparisons with the true phantom diameters are summarised in Table I and displayed graphically in Fig. 1. Of the three recording modes, cinefilm was found to provide the most precise measurement results.

Clinical results The results of measurements from normal-image videotape are plotted against those from cinefilm in Fig. 2A [22]. The agreement between the two sets of measurements was poor (0.14mm ±0.20mm ($p<.01$)). The results of measurements from edge-enhanced video are plotted against those from cinefilm in Fig. 2B [22]. Although the agreement between the two sets of measurements was better with edge-enhanced video than with normal-image videotape, the difference between the edge-enhanced videotape and cinefilm measurements still achieved statistical significance (0.04mm ±0.13mm ($p<.05$)).

Discussion

We have demonstrated that of the three image recording modes studied, cinefilm produces the most precise QCA measurements. The reasons for this finding may relate to the high resolution afforded by cinefilm frame analysis (1329 x 1772 pixels) compared to the limited resolution provided by the analysis of a single video field (312 lines per field – each video frame consists of two interlaced fields). The noise introduced by the video recording process and by the videotape itself may contribute to the lower precision of the QCA measurements. This video-induced noise was not overcome by our compensatory steps of recording on super-VHS videotape to reduce the signal/noise ratio [3] and deploying a time-base corrector during image acquisition to overcome jitter [1]. As was expected, the introduction of a systematic noise such as that associated with video recording did not exert a significant effect on the accuracy of our QCA measurements.

Our studies of both experimental and clinical images indicate that video recording with on-line spatial filtering results in more reliable QCA measurements than videotape without enhancement. These findings support the view that on-line spatial filtering (edge enhancement) before the introduction of video noise provides a more distinct border to the coronary vessel (or phantom steno-

sis) which is more faithfully tracked by the off-line QCA edge-detection algorithm than the vessel border of an unenhanced image.

Study implications Financial considerations have now become an important factor in the administrative and technical decision-making process of most cardiac catheterization laboratories. A single videotape can store the complete angiographic records of approximately 40 patients at a cost of less than $1 per patient; cinefilm increases the cost per patient to $40. Our findings suggest that the adoption of videotape with edge enhanced images may present an acceptable alternative to cinefilm for routine purposes and possibly for QCA purposes under certain circumstances. It should be recognized, however, that the addition of any noise or imprecision to the system of off-line QCA will increase the SD of the angiographic results of the study. In turn, this might increase the number of patients needed for detection of a statistically significant difference among two study populations under comparison [23]. Thus the inclusion of videotape in the design of a restenosis prevention or progression-regression trial may present a false economy by virtue of a concomitant increase in the number of patients required and subsequent greater total study costs.

Study limitation The edge-enhancement of images and subsequent QCA analysis in our study was performed by the Philips DCI system and CAAS II system. Further studies will be required to confirm whether our findings can be generalized to other hardware or software systems. It is conceivable that if an on-line edge-enhancement algorithm was inaccurate, a systematic underestimation or overestimation of vessel diameters could be translated to subsequent off-line QCA measurements.

Conclusion

Despite the application of on-line edge enhancement, the selection of super-VHS video tape and the deployment of a time-base corrector in the processing of video images for off-line QCA, cinefilm continues to present a more reliable image recording medium of coronary angiograms for scientific studies. For routine practice, however, videotape with edge-enhanced images may provide an acceptable alternative.

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Chapter 6

Inter- and intra-observer variability in the qualitative categorization of coronary angiograms

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Abstract The ABC classification of the American College of Cardiology and the American Heart Association is a commonly used categorization to estimate the risk and success of intracoronary intervention, as well as the probability of restenosis. To evaluate the reliability of qualitative angiogram readings, we randomly selected 200 films from single lesion angioplasty procedures.
A repeated visual assessment (≥ 2 months interval) by two independent observers resulted in kappa values of inter and intra-observer variability for the ABC lesion classification and for all separate items that compile it. Variability in assessment is expressed in percentage of total agreement, and in kappa value, which is a parameter of the agreement between two or more observations in excess of the chance agreement. Percentage of total agreement and kappa value was 67.8% and 0.33 respectively for the ABC classification, indicating a poor agreement. Probably this is due to the deficiency of strict definitions. Further investigation has to demonstrate whether improvement can be achieved using complete and detailed definitions without ambiguity, and consensus after panel assessment. (International Journal of Cardiac Imaging 1996; 12:21-30)

In 1988 the task force of the American College of Cardiology and the American Heart Association (ACC/AHA) on assessment of diagnostic and therapeutic cardiovascular procedures presented guidelines for percutaneous transluminal coronary angioplasty (PTCA) [1], which were recently updated for current experience and technology [2]. They proposed a lesion classification, based on morphologic features that presumably influence the chance of successful outcome of coronary angioplasties and the risk of acute closure (table I). Lesions are categorized into type A (success ≥ 85%, low risk of abrupt closure), type B (success 60-85% and/or moderate risk of abrupt closure), and type C (success ≤ 60% and/or high risk of abrupt closure). In 1990, Ellis and colleagues modified the type B lesions into type B1 stenoses (only one adverse type B characteristic), and into type B2 stenoses (two or more adverse type B characteristics). This subdivision was based on multivariate analysis, indicating the cumulative weight of unfavourable lesion characteristics [3]. The lesion scoring system is in widespread clinical use nowadays in order to attempt risk stratification for PTCA patients and selection of the interventional devices available. It is well known that visual estimates of lesion severity and several lesion features are less reliable as e.g. quantitatively evaluated characteristics [4]. To measure the inter and intra observer variability of the qualitative items that compose the ABC lesion classification, and the TIMI flow grade [5] (table II), two experienced corelab readers (VU and JH) performed a double independent and blinded reading of coronary angiograms.

Methods

Patient Identification All cinefilms were made in the period between september 1992 and may 1993, in patients with proven coronary artery disease in native arteries as shown by a diagnostic angiofilm. All patients were treated for unstable angina pectoris, according to the Braunwald classification [6].

Assessment of Coronary Angiograms

To assess the reliability of qualitative angiogram readings, we randomly selected 200 angiograms from the pool of cinefilms available in the cardiovascular research laboratory Cardialysis. All the cinefilms recorded an intracoronary intervention of a single lesion. Those films were assessed independently by 2 observers. To assess intra-observer variability, the same set of cinefilms was analyzed at least 8 weeks later by the two observers who were blinded for the results of the first analysis. Except for concentricity, we used the definitions for morphologic characteristics from the original ACC/AHA task force project [1,2]. We used the more differentiating Ambrose classification [7] to assess concentricity aspects and regularity of the lesion. Concentric and tandem lesions are smooth, like type I eccentric lesions. We defined multiple irregularities as concentric and irregular, and type II eccentric lesions as eccentric and irregular. We additionally scored TIMI flow. The assessors were blinded for clinical data, and therefore could not differentiate between TIMI flow grade 0 c.q. total occlusion existing less or longer than three months (ergo a type B or type C characteristic). We added total occlusion to the four categories of the modified lesion classification.

Statistical Analysis Calculations revealed that a group size of 200 coronary cinefilms would be more than sufficient to achieve a reasonable to precise kappa-value (see appendix).

The degree of agreement was measured as percentage of total agreement, and using the kappa statistics, which is a parameter of the agreement between two or more observations in excess of the chance agreement [8]. If there is perfect agreement, then kappa = +1.00 and in case of pure chance agreement, then kappa = 0.00. It is usual to consider kappa values greater than 0.75 to represent excellent agreement beyond chance, and values below 0.40 to represent poor agreement beyond chance and to values between 0.40 and 0.75 to represent fair to good agreement beyond chance. Kappa value was calculated as (observed-expected)/(1-expected).

Double data entry secured accuracy. BMDP was used as statistical software package.

Results

In the first assessment one film was not assessable, therefore a total of 199 films were analyzed. The inter-observer variability between observer 1 and 2 (table III) showed poor agreement for ABC lesion classification (k=0.33) and the modified (A, B1, B2, C) classification (k=0.29), length of lesion (k=0.35), and branch point involvement in stenosis (k=0.39). Fair to good agreement was found for Ambrose classification (k=0.48), relationship to coronary artery bend (k=0.48), vessel calcification (k=0.53), TIMI flow grade (k=0.57), and thrombus (k=0.60). Perfect agreement was found for ostial lesion (k=1.00) and tortuosity (k=1.00).

Percentage of total agreement was found lowest in Ambrose classification (49.7%), and lesion length (67.8%). The agreement on modified (A, B1, B2, C) ABC classification was only 47.7%, and on the original tri-modal ABC score improved to 67.8%. Kappa however raised only from 0.29 to 0.33. Agreement for calcification and branch point involvement was reached in 89.4 and 80.9% of the

Table I
Lesion Specific Characteristics of Type A,B, and C Lesions [1,2]

Type A lesions (minimally complex)	
discrete (<10 mm length)	less than totally occlusive
concentric	little or no calcification
readily accessible	not ostial in location
nonangulated segment, <45°	no major branch involvement
smooth contour	absence of thrombus
Type B lesions (moderately complex)	
tubular (10 to 20 mm length)	moderate to heavy calcification
eccentric	total occlusions < 3 months old
moderate tortuosity of proximal segment	some thrombus present
bifurcation lesion requiring double guide wires	ostial in location
moderately angulated segment, >45°,<90°	irregular contour
Type C lesions (severely complex)	
diffuse (>20 mm length)	inability to protect major side branches
total occlusion > 3 months old	extremely angulated segment, >90°
excessive tortuosity of proximal segment	degenerated vein grafts with friable lesions

Ellis and colleagues modified the type B lesions into type B1 stenoses (only one adverse type B characteristic), and into type B2 stenoses (two or more adverse type B characteristics). This subdivision was based on multivariate analysis, indicating the cumulative weight of unfavourable lesion characteristics [3].

Table II
Assessment of TIMI Flow Grade

0:	no flow,
I:	penetration with minimal perfusion (contrast material passes beyond the area of obstruction but 'hangs up' and fails to opacify the entire coronary bed distal to the obstruction for duration of the cine run)
II:	delayed perfusion (opacification of the coronary bed distal to the obstruction, but rate of entry and/or clearance of the contrast medium is reduced)
III:	complete perfusion

Table III
Inter-observer Variability Between Observers 1 and 2 (N=199)

Length of lesion Observer 1	Observer 2 1<10 mm	10-20 mm	>20 mm	N.A.	Total
<10 mm	110	6	1	0	117
10-20 mm	43	14	3	0	60
>20 mm	3	5	0	2	10
N.A.	0.	1	0	11	12
Total	156	26	4	13	199

Kappa value = 0.35, 95 % confidence interval 0.25-0.35, agreement is 67.8% .
N.A. = Not applicable

Ambrose lesion type / Observer 1	Observer 2: Conc.	Ecc. IA	Ecc. IB	Ecc. IIA	Ecc. IIB	Mult. irreg.	Tandem	N.A.	Total
Concentric	48	1	1	2	0	3	1	0	56
Eccentric type	2	3	1	1	7	0	0	0	14
IA Eccentric type IB	44	14	34	1	3	3	0	1	100
Eccentric type IIA	1	0	1	0	1	3	0	0	6
Eccentric type IIB	2	1	1	0	0	0	0	0	4
Multiple irregularities	1	1	0	0	0	1	0	0	3
Tandem lesion	1	0	0	0	0	1	2	0	4
N.A.	1	0	0	0	0	0	0	11	12
Total	100	20	38	4	11	11	3	12	199

Kappa value = 0.48, 95 % confidence interval 0.43-0.53, agreement is 49.7% .
Conc. = concentric, Ecc. = eccentric, Mult. irreg. = multiple irregularities, Tandem = tandem lesion, N.A. = Not applicable

TIMI flow grade / Observer 1	Observer 2: TIMI 0	TIMI I	TIMI II	TIMI III	Total
TIMI 0	8	3	0	0	11
TIMI I	0	4	1	1	6
TIMI II	0	3	11	9	23
TIMI III	0	1	13	145	159
Total	8	11	25	155	199

Kappa value = 0.57, 95 % confidence interval 0.45-0.68, agreement is 84.4%

Ostial lesion / Observer 1	Observer 2: No	Yes	Total
No	190	0	190
Yes	9	0	9
Total	199	0	199

Kappa value = 1.00, agreement is 95.5%

Tortuosity / Observer 1	Observer 2: No	Moderate	Severe	Total
No	196	1	0	197
Moderate	1	0	0	1
Severe	1	0	0	1
Total	198	1	0	199

Kappa value = 1.00, agreement is 98.5%

Branchpoint involved in the stenosis Observer 1	Observer 2 No	Yes	Total
No	143	6	149
Yes	32	18	50
Total	175	24	199

Kappa value = 0.39, 95 % confidence interval 0.26-0.52, agreement is 80.9%

Relationship to coronary artery bend Observer 1	Observer 2 No	Yes	Total
No	186	4	190
Yes	4	4	8
Bad quality	1	0	1
Total	191	8	199

Kappa value = 0.48, 95 % confidence interval 0.22-0.74 , agreement is 95.5%

Presence of thrombus Observer 1	Observer 2 No	Yes	Total
No	190	3	193
Yes	2	4	6
Total	192	7	199

Kappa value = 0.60, 95 % confidence interval 0.34-0.86, agreement is 97.5%

Presence of calcium Observer 1	Observer 2 No	Yes	Total
No	164	12	176
Yes	8	14	22
Bad quality	1	0	1
Total	173	26	199

Kappa value = 0.53, 95 % confidence interval 0.38-0.68, agreement is 89.4%

Lesion type Observer 1	Observer 2 A	B1	B2	C	T.O	Bad quality	Total
A	20	2	0	0	0	0	22
B1	28	39	6	2	0	0	75
B2	15	34	28	3	0	0	80
C	1	3	4	0	0	2	10
T.O.	0	1	1	0	8	2	12
Total	64	79	39	5	8	4	199

Kappa value based on A, B1, B2 and C classes = 0.29, 95 % confidence interval 0.21-0.37, agreement is 47.7% .
Kappa value based on A, B and C classes = 0.33, 95 % confidence interval 0.25-0.41, agreement is 67.8% .
T.O = totally occlusive coronary artery

Table IV
Intra-observer Variability for Observer 1 and 2 (N=197)
Each cell gives the number of observations for observer 1 (top) and observer 2 (bottom).

Length of lesion Observer 1	Observer 2 <10 mm	10-20 mm	>20 mm	N.A.	Total
<10 mm	92	23	0	0	115
	144	9	1	0	154
10-20 mm	19	39	2	0	60
	9	13	3	1	26
>20 mm	0	4	6	0	10
	1	0	3	0	4
N.A.	0	0	0	12	12
	2	1	1	9	13
Total	111	6	8	12	197
	156	23	8	10	197

Kappa value observer 1 = 0.57, 95 % confidence interval 0.47-0.67, agreement is 75.6% .
Kappa value observer 2 = 0.61, 95 % confidence interval 0.51-0.71, agreement is 85.8% .
N.A. = Not applicable

Ambrose lesion type Observer 1	Observer 2 Conc.	Ecc. IA	Ecc. IB	Ecc. IIA	Ecc.IIB	Mult. irreg.	Tandem	N.A.	Total
Concentric	37	3	16	0	0	0	0	0	56
	91	1	6	0	0	0	0	1	99
Eccentric type	0	7	6	1	0	0	0	0	14
	2	11	4	2	0	0	0	0	19
IA Eccentric type IB	8	7	84	0	0	0	0	0	99
	9	5	22	1	0	0	1	0	38
Eccentric type IIA	0	6	0	0	0	0	0	0	6
	2	0	0	1	1	0	0	0	4
Eccentric type IIB	0	3	0	0	0	0	0	0	3
	2	4	1	2	1	1	0	0	11
Multiple irregularities	1	0	0	0	0	1	1	0	3
	0	1	0	0	0	10	0	0	11
Tandem lesion	0	0	0	0	0	0	4	0	4
	0	0	0	0	0	0	3	0	3
N.A.	0	0	0	0	0	0	0	0	12
	1	1	1	0	0	0	0	9	12
Total	46	26	106	1	0	1	5	12	197
	107	23	34	6	2	11	4	10	197

Kappa value observer 1 = 0.61, 95 % confidence interval 0.53-0.69, agreement is 67.5% .
Kappa value observer 2 = 0.66, 95 % confidence interval 0.59-0.73, agreement is 75.1% .
Conc. = concentric, Ecc. = eccentric, Mult. irreg. = multiple irregularities, Tandem = tandem lesion, N.A. = Not applicable

TIMI flow grade Observer	Observer 2 TIMI 0	TIMI I	TIMI II	TIMI III	Total
TIMI 0	11	0	0	0	11
	7	1	0	0	8
TIMI I	1	4	1	0	6
	1	7	1	2	11
TIMI II	0	0	9	13	22
	0	3	14	7	24
TIMI III	0	0	3	155	158
	0	2	8	144	154
Total	12	4	13	168	197
	8	13	23	153	197

Kappa value observer 1 = 0.70, 95 % confidence interval 0.59-0.81, agreement is 90.9% .
Kappa value observer 2 = 0.66, 95 % confidence interval 0.56-0.76, agreement is 87.3% .

Ostial lesion Observer 1	Observer 2 No	Yes	Total
No	188	1	189
	197	0	197
Yes	1	7	8
	0	0	0
Total	189	8	197
	197	0	197

Kappa value observer 1 = 0.87, 95 % confidence interval 0.72-1.02, agreement is 99.0% .
Kappa value observer 2 = 1.00, agreement is 100.0% .

Tortuosity Observer 1	Observer 2 No	Moderate	Severe	Total
No	194	1	0	195
	193	3	0	196
Moderate	0	1	0	1
	1	0	0	1
Severe	0	0	1	1
	0	0	0	0
Total	194	2	1	197
	194	3	0	197

Kappa value observer 1 = 0.80, 95 % confidence interval 0.47-1.13, agreement is 99.5% .
Kappa value observer 2 = 1.00, agreement is 98.0% .

Branchepoint involved in the stenosis Observer 1	Observer 2 No	Yes	Total
No	143	4	147
	163	10	173
Yes	22	28	50
	7	17	24
Total	165	32	197
	170	27	197

Kappa value observer 1 = 0.61, 95 % confidence interval 0.50-0.72, agreement is 86.8% .
Kappa value observer 2 = 0.62, 95 % confidence interval 0.47-0.77, agreement is 91.4% .

Relationship to coronary artery bend

Observer 1	Observer 2 No	Yes	Total
No	188	0	188
	189	0	189
Yes	2	6	8
	3	5	8
Bad quality	1	0	1
	0	0	0
Total	191	6	197
	192	5	197

Kappa value observer 1 = 0.85, 95 % confidence interval 0.69-1.01, agreement is 98.5% .
Kappa value observer 2 = 0.76, 95 % confidence interval 0.55-0.97, agreement is 98.5% .

Presence of thrombus Observer 1	Observer 2 No	Yes	Total
No	190	1	191
	187	3	190
Yes	2	4	6
	2	5	7
Total	192	5	197
	189	8	197

Kappa value observer 1 = 0.72, 95 % confidence interval 0.46-0.98, agreement is 98.5% .
Kappa value observer 2 = 0.65, 95 % confidence interval 0.42-0.85, agreement is 97.5% .

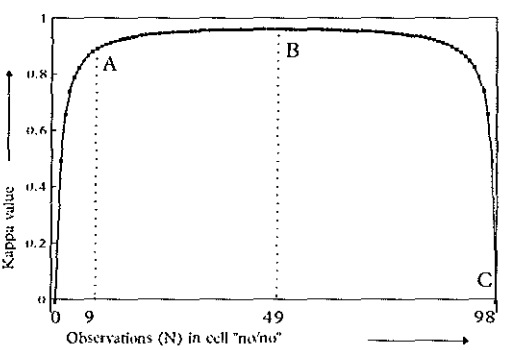
Presence of calcium Observer 1	Observer 2 No	Yes	Total
No	172	2	174
	162	9	171
Yes	14	8	22
	13	13	26
Bad quality	1	0	1
	0	0	0
Total	187	10	197
	175	22	197

Kappa value observer 1 = 0.46, 95 % confidence interval 0.28-0.64, agreement is 91.4% .
Kappa value observer 2 = 0.48, 95 % confidence interval 0.59-0.73, agreement is 88.8% .

Lesion type Observer 1	Observer 2 A	B1	B2	C	T.O	Bad quality	Total
A	11	10	1	0	0	0	22
	47	16	0	0	0	0	63
B1	11	42	20	1	0	0	74
	13	54	8	2	0	1	78
B2	1	19	58	1	0	0	79
	4	12	21	1	1	0	39
C	0	3	1	6	0	0	10
	1	0	0	4	0	0	5
T.O.	0	0	0	0	12	0	12
	0	0	0	0	7	1	8
Bad quality	0	0	0	0	0	0	0
	0	2	1	1	0	0	4
Total	23	74	80	8	12	0	197
	65	84	30	8	8	2	197

Kappa value based on A, B1, B2 and C classes observer 1 = 0.49,
95 % confidence interval 0.40-0.57, agreement is 65.5% .
Kappa value based on A, B1, B2 and C classes observer 2 = 0.55,
95 % confidence interval 0.47-0.63, agreement is 67.5% .
Kappa value based on A, B and C classes observer 1 = 0.61, 9
5 % confidence interval 0.54-0.68, agreement is 85.3% .
Kappa value based on A, B and C classes observer 2 = 0.59,
95 % confidence interval 0.52-0.66, agreement is 77.7% .
T.O = totally occlusive coronary artery

Figure 1
The graph plots the number of observations in the 'no/no' cell on the X-axis against the kappa-value on the Y-axis, and illustrates the dependency of the kappa value to the distribution of the readings. In this example agreement between two observers is kept constant at a level of 98%. The smaller boxes give the exact number of observations at three points in the graph, on which the kappa value and agreement are based.



A

	no	yes	total
no	9	1	10
yes	1	89	90
total	10	90	100

Kappa-value = 0.89
Agreement = 98%

B

	no	yes	total
no	49	1	50
yes	1	49	50
total	50	50	100

Kappa-value = 0.96
Agreement = 98%

C

	no	yes	total
no	98	1	99
yes	1	0	1
total	99	1	100

Kappa-value = -0.010
Agreement = 98%

cases. In all the other items the percentage of total agreement is over 90%.
Both observers demonstrated excellent agreement in intra-observer variability for tortuosity, relationship to coronary artery bend and ostial localization. All other studied lesion characteristics showed fair to good agreement for intra-observer variability (table IV).

Discussion

Kappa statistics are a well known method of evaluating agreement between observers. This method is most useful when observations are frequent and have a Gaussian distribution. However the limitation arises when observations are relatively rare or even exceptional. One single outlying observation can then dramatically affect the kappa values. Figure 1 illustrates the relationship between kappa value and the distribution of observations over the cells. It shows clearly that percent agreement can remain constantly high (98%), while kappa value ranges from -0.010 to 0.96. The graph also depicts the possible abrupt change in kappa value when the majority of observations is concentrated in only one cell. Kappa value can change from -0.010 to 0.490 when one observation is differently positioned over the cells (figure 1).
It is well known that visual estimates of lesion characteristics are less accurate in comparison to quantitatively derived parameters. Several variability and quality control studies have been conducted. Beauman and Vogel [9] compared visual estimations of lesion severity to quantitative analyses of percent diameter stenosis of coronary and phantom obstructions. Quantitatively assessed coronary arteries comprising a 50% diameter stenosis, and 50% phantom stenoses recordings were visually scored in ranges from 15 to 80 percent, and 30 to 95 percent respectively. Determination of the reference diameter showed that only 41% of the estimations were within 10% of the limits of the quantitatively derived diameter.
Another study [10] in 50 lesions reports the inter observer agreement of 73% for stenosis length (defined as the length of that portion of the stenosis that had a ≥30% reduction in luminal diameter using the adjacent normal vessel diameter as a 'yardstick' or unit) and 64% for lesion eccentricity (defined as asymmetrically positioning in one or more views), resulting in kappa values of respectively 0.38 and 0.25.
A report from our corelab [11] from 1990 reported the discordance in interobserver measurements in 151 lesions of 21% for lesion eccentricity (24% in our study), 29% for branch point involvement (18% in our study), 14% for location in a bend (3.5%), 2% for presence of thrombus (2.5%), 10 % for presence of calcification (10%), and 25% for the lesion type according to the ACC/AHA classification (32%).
A recently presented study in 403 coronary lesions [12] demonstrated an excellent agreement for type C lesions (k=0.85). Good agreement was shown for TIMI flow (k=0.73), ABC classification (k=0.48), angulation and side branch (k= 0.48 and 0.40 respectively). Poor achievement was reached in eccentricity, tortuosity, lesion calcification, and in the distinction of discrete, diffuse and tubular lesion length.

Mild discrepancies between the two assessors can be explained by insufficient quality in image acquisition, when e.g. overlap or foreshortening hampers assessment. Especially very proximal lesions in the left anterior descending artery are sometimes very difficult to explore visually, and are therefore subject of discordant descriptions.

Another source of incongruous assessment can be the experience of the angiographist. In this study one of the two MD's is interventional cardiologist (VU), while the other is a permanent assessor in the core lab of Cardialysis (JH).

An issue of essential relevance which contributes to the poor agreement within and between investigators is a clear description of the definitions of items to be assessed. The original and updated combined AHA/ACC [1,2] paper mentions the individual items on which the ABC classification is based, without detailed delineation of these elements. Many dissimilar definitions are in use throughout the literature. Although basically comparable, they differ in details, and cause discrepancies in cinefilm readings. Length of lesion e.g. can be interpreted e.g. as the length of plaque, related to the pre-defined size of the catheter on the image. An adjusted definition is the length where the stenosis ≥70% of the lumen diameter, or ≥50% , or ≥30% . This can then be expressed in absolute diameters or in terms of normal lumen diameter ratio [10]. Lesion length can also be defined as the calliper measurement of the distance from the proximal to the distal shoulder of the lesion in the projection that best elongated the stenosis. Cut-off points were chosen as <10 and >20 mm [3]. We propose to use the definitions as listed in table V. They leave a minimum of space for different explanation and interpretation.

Panel assessment gives a substantial improvement in inter and intra observer agreement [9]. It is clear that the weighted sum of several simultaneous observations eliminates the most extreme disagreements, where the isolated assessor can develop his own interpretation and thus deviate from the original definitions.

Serial observations as in pre-readings, with knowledge of the results of the first observer's judgement may result in higher kappa-values for qualitatively assessed lesion characteristics. The mechanism of improved agreement in case of pre-reading however differs from improved agreement following panel assessment. In serial readings, the first judgement is merely dominant and respected by the second reviewer, who tends to compliance, implicating an improved outcome.

Conclusions

The data demonstrate a substantial discordance of agreement between two observers and also a partial lack of reproducibility of the results. These findings may be attributed to, among others: quality of data acquisition on film; experience of angiography assessors; and mainly lack of strict definitions. Further investigation, preferably by panel assessment might be performed, only after agreement upon complete and detailed definitions for each angiographic variable. Besides, if we want to estimate procedural success rates and the risk for procedural complications we have to debate operator experience and clinical variables.

Table V
List of Proposed Definitions to be Used for Qualitative Assessment of Coronary Angiograms.

Symmetry; Lesions are judged for their symmetry depending on their appearance in any of multiple projections. Eccentric lesions are asymmetrically positioned in one or more views.
Categories: Concentric, Eccentric.
Roughness; The stenosis was judged to be rough if its luminal edge was irregular, or had a sawtooth component [3].
Categories: Discrete/smooth, Irregular contour.
Length of lesion; Estimation of the length of that portion of the stenosis that has a ≥50% reduction in luminal diameter. A contrast empty catheter tip is used for 'visual calibration'.
Categories: < 10 mm, 10 – 20 mm, > 20 mm, NA.
TIMI flow grade; [5]
0: no flow,
I: penetration with minimal perfusion (contrast material passes beyond the area of obstruction but 'hangs up' and fails to opacify the entire coronary bed distal to the obstruction for duration of the cine run)
II: delayed perfusion (opacification of the coronary bed distal to the obstruction, but rate of entry and/or clearance of the contrast medium is reduced)
III: complete perfusion
Occlusion; Total obstruction without anterograde flow TIMI 0. The distal vessel may or may not be filled by through retrograde or anterograde collateral (bridging) flow [13].
Categories: No total occlusion, Total occlusion.
Branch point; A branch point is considered present if any part of the lesion > 30 % narrowed is adjacent to a branch vessel that has a diameter of 25% or more of the diameter of the vessel being scored [3].
Categories: Branch point involved, Branch point not involved
Bifurcation stenosis; The stenosis was recorded as a bifurcation stenosis if a branch vessel of medium or large size originated within the stenosis and iof the side branch was completely surrounded by significant stenostic portions of the lesion to be dilated [3].
Ostial lesion; When it involved the origin of the proximal LAD, LCX or RCA. When 'ostial' and 'bifurcation' occurred together they were counted as only one ACC/AHA class B characteristic [3].
Categories: Ostial, Not ostial located.
Bend point; A bend point is considered present if in any angiographic projection orthogonal to the lesion, any part of the lesion is located in a portion of the vessel that has a ≥ 45 degrees angulation at end diastole. CASS Registry [10] and ACC/AHA classification [1,2]
Categories: Not located in a bend point, Mild bending, bend point > 45, <90 degrees, Severe bending, bend point > 90 degrees.
Calcifications; Calcifications are present if fixed radiopaque densities having the appearence of calcification are noted in any projection in the area of the stenosis to be dilated.
Categories: None, Little, Heavy calcification.
Intra coronary thrombus; Intra coronary thrombus is defined as; presence of intraluminal non calcified central filling defect or lucency surrounded by contrast material seen in multiple projections, or persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream [14].
Categories: Absent, Present.
Tortuosity; Stenoses distal to two bends are in general scored as moderately tortuous, and those distal to three or more bends were considered to be associated with excessive tortuosity.
Categories: No tortuosity, Moderately tortuosity, Excessive tortuosity.
Tandem lesions were defined as adjacent separate lesions, more than three lumen diameters apart. This lesion does not include multiple separated lesions in different portions of the same vessel.
Categories: Tandem lesion, No tandem lesion.

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Appendix

The decision to review 200 angiofilms was based on the degree of agreement between the angiographic corelab Cardialysis and the investigators in the Helvetica study with regard to lesion type according to the ACC/AHA classification. This agreement was accomplished by a non-blinded review of cine-angiofilms by two assessors in the angiographic corelab. Kappa values for this study population were consequently calculated for each lesion type separately (A, B1, B2 and C) and across all categories. The overall kappa value was 0.64 (90% CI 0.61 to 0.68).

To calculate the kappa value within the core lab, we used the inter rater data generated from the corelab and investigator with a standard deviation of 0.60 (standard error 0.02 in 905 lesions). The expected kappa was set at 0.70, since it is assumed that the generated kappa value of the core lab will be greater than that calculated between core lab and the investigator (0.64). The lower accepted kappas were assumed to be 0.69, 0.68, 0.67, 0.66, and 0.65. The number of lesions to be reviewed, assuming a one-sided significance level of 0.05, power of 0.90 or 0.80, is listed in table VI. Based on these calculations we decided to review 200 angiofilms.

Table VI
Number of Patients or Lesions Needed to be Reviewed
(Alpha = 0.05, Standard Deviation = 0.60).

lower kappa limit	number of patients needed	power = 90%	power = 80%
	0.69	1700	1230
	0.68	425	308
	0.67	189	137
	0.66	107	77
	0.65	68	49

Chapter 7

Argatroban during percutaneous transluminal coronary angioplasty:

Results of a dose verification study

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Abstract Background Thrombin is a key enzyme in thrombogenesis. In animals, specific anti-thrombotic therapy at the time of percutaneous transluminal coronary angioplasty reduced the incidence of subacute occlusion and inhibited the restenosis response. Argatroban is a highly selective synthetic thrombin antagonist that binds in a competitive manner. This is a report of a dose verification study, assessing safety and feasibility of intravenous argatroban administration in patients undergoing percutaneous transluminal coronary angioplasty.

Methods Before angioplasty an intravenous bolus of 30μg/kg was administered, followed by a continuous infusion of 3.5 μg/kg/min for 72 hours. Bolus injection was repeated and infusion rate was increased in order to achieve an activated coagulation time (ACT) of over 300 seconds. Following interim-analysis, the bolus and initial infusion rate for the subsequent treatment groups was determined. Study endpoints were the occurrence of adverse events, coagulation tests, and qualitative angiogram reading. Patients were monitored by continuous 12-lead electrocardiographic recording over 24 hours, and underwent control angiography 18-24 hours following angioplasty.

Results Four treatment groups, comprised 2,8,9, and 11 patients, respectively, were studied. The first two patients were excluded from analysis, since the initial dose was ineffective to attain an ACT authorizing coronary angioplasty. The group with the highest dosage received a 250 μg/kg intravenous bolus of argatroban, followed by a 4 hour infusion of 15 μg/kg/min. At 4 hours the infusion rate was lowered to 3.8 μg/kg/min and continued for 68 hours without adjustment for catheter removal. The adverse event profile included myocardial infarction, aorto-coronary bypass graft, bail-out procedures and repeat coronary angioplasty. Thrombin-time (TT), activated partial thromboplastin time (aPTT) and pro-thrombin time (PT) were significantly related to argatroban plasma concentration, as demonstrated by regression analyses (R-square 0.64, 0.71, and 0.84 respectively). Prothrombin fragment 1 and 2 and thrombin-antithrombin III complex did not fit into a mathematical model, but showed slightly increased levels after reduction or cessation of the infusion rate.

Conclusions This dose verification study, including 30 patients at four dose levels, indicated that argatroban infusion in coronary angioplasty patients can be administered safely, and results in an adequate and predictable level of anticoagulation. (Journal of Thrombosis and Thrombolysis 1996;3:367-375)

Angioplasty of coronary narrowings greatly enlarges the arterial lumen. Nevertheless, the short term therapeutic benefit of percutaneous transluminal coronary angioplasty (PTCA) can be limited by formation of mural thrombus and subsequent abrupt closure of the coronary artery. In addition, organization of mural thrombus and proliferation of smooth muscle cells lead to vessel restenosis, also adversely influencing medium- and long-term result. The central role of thrombin in these events is a result of its effects on the clotting cascade, leading to fibrin and thrombus formation; on platelet aggregation, causing the liberation of growth factors; and finally its direct mitogenic effect on smooth muscle cells [1]. A low thrombin level is sufficient to activate phospholipid membrane bound factor V, which is part of the pro-thrombinase complex, and can therefore strongly accelerate thrombin formation [2]. Interference with this positive feedback loop by potent and specific thrombin inhibition is a very effective antithrombotic strategy [3, 4].

Argatroban, ((2R,4R)-4-methyl-1-[N^2-((3-methyl-1,2,3,4-tetrahydro-8-quinolonyl) sulphonyl)-arginyl]-2-piperidine carboxylic acid) is an arginine derivate that binds competitively to thrombin [5]. Compared with heparin, it has several potential advantages. Argatroban is synthetic and therefore has no natural inhibitors. It does not need antithrombin III as a co-factor, and it is non-antigenic. Heparin is a mixture of heterogenous sized depolymerized molecules, and therefore its bioactivity may vary depending on the particular lot. In contrast, the anticoagulant effect of argatroban is concentration dependent [6] and comprises both free and clot bound thrombin.

Specific antithrombin therapy using argatroban has been shown to be superior to heparin treatment in numerous experimental animal models of arterial thrombogenesis [7-19]. At doses causing comparable prolongation of activated partial thromboplastin time (aPTT), argatroban has been demonstrated to be significantly more effective than heparin for the prevention of platelet rich thrombotic occlusion in an animal model [20]. Several human trials with argatroban have been completed using dosage regimens up to 25 μg/hr i.v. for 2 hours [21] or 20 μg/hr i.v. for 2 hours after a 10 μg bolus [22]. These studies have demonstrated a moderate dose-dependent 4.5-fold increase in thrombin time, and a 1.6-fold prolongation of aPTT with no effect of concomitant aspirin administration. The doses administered in human studies were low compared to those used in animal models, and at these low doses, the elimination half time is 24 minutes [23]. Based on these findings it was felt safe to investigate this drug in patients with a hypercoagulable condition such as patients with unstable angina or undergoing PTCA.

Recently argatroban was infused over 4 hours in patients experiencing unstable angina pectoris [24]. Infusion of the drug (0.5 to 5.0 μg/kg/min) resulted in a dose dependent increase in the aPTT and effectively prevented recurrences of ischemic episodes. Notably, 9 out of 43 patients experienced an episode of unstable angina shortly after drug administration was terminated. The authors attributed this to a rebound phenomenon.

There has been limited experience with the use of argatroban in PTCA patients (Fitzgerald, data not published). In his study, 12 patients were randomi-

zed to receive either a bolus of 30 μg/kg argatroban, followed by an intravenous infusion of 2 μg/kg/min over 2 hours, or a bolus injection of 10000 IU of heparin and a subsequent 4 hour infusion of 1000 IU/hour.

In order to further define the optimal dose of argatroban for patients undergoing coronary angioplasty, the pre-Argaplasty trial was initiated. This dose verification study was undertaken to assess safety and feasibility of administration of argatroban in patients undergoing balloon angioplasty and to broaden our understanding of the response to the coagulation system to this agent.

Methods

Study design This study was an open, non-randomized dose verification study, carried out in three hospitals in the Netherlands. The study was conducted according to G.C.P. regulations. Patients were allocated to study medication after giving written informed consent. This study assessed the safety and feasibility of the use of three different doses of argatroban (group A, B and C respectively) in patients undergoing PTCA. Acetyl salicylate (250 mg intravenous) was administered just prior to angioplasty, and 160 mg acetyl salicylate was administered orally for the next 3 days.

Patient selection Patients with stable or unstable angina pectoris due to one or more angiographically significant lesions in the native coronary system amenable to balloon angioplasty were considered eligible for this study. The main exclusion criteria were acute myocardial infarction or thrombolytic therapy within two weeks prior to PTCA; current treatment with oral anticoagulant drugs; and history of peptic ulcer disease, upper gastro-intestinal bleeding or stroke.

Coronary angiograms and angioplasty Coronary angiograms were obtained immediately before and after balloon angioplasty, and repeated 18-24 hours afterwards. To standardize the method of data acquisition and to ensure the exact reproducibility of the angiograms performed after the intervention and at follow-up, measures were taken as described earlier [25, 26]. Choice of angioplasty device, balloon pressure, and duration of inflation were left to the discretion of the operator. All angiograms were read at Cardialysis B.V., Rotterdam, the Netherlands.

Argatroban dose regimen and dose adjustment argatroban was provided by Synthélabo Recherche (L.E.R.S.), Bagneux Cédex, France. It's clearance and half-life are 5.0 ±0.5 ml/kg/min and 24.4±3.5 minutes, respectively, in healthy volunteers. As argatroban is a photo-sensitive drug, it was dispensed in opaque ampoules containing 0.5 mg/ml. The maximum total daily authorized dose was 10 mg/kg bodyweight.

The first eight patients (group A) were to receive a bolus of 30 μg/kg argatroban, followed by a 3.5 μg/kg/min infusion during the next 72 hours. When the ACT exceeded 300 seconds, angioplasty was authorized approximately 45 minutes after administration of the bolus and the start of the argatroban infusion. For an ACT less than 300 seconds, an extra 30 μg/kg bolus was administered, and the rate of infusion was increased by 0.5 μg/kg/min increments until the ACT exceeded 300 seconds. Following PTCA, the infusion rate was adjusted to maintain the aPTT between 100 and 120 seconds (2.5 to 3 times normal value) according to the adjustment algorithm shown in table I. Following interim-analysis, the bolus and initial infusion rate for the adjacent treatment groups (group B and C) were determined.

The femoral sheath was removed after reduction of the infusion rate to a predetermined level of 2.0 μg/kg/min, in order to obtain an aPTT of less than 80 seconds (less than twice normal value). One hour after sheath removal, infusion rates were restored to their previous setting. The patients were kept on strict bed rest for the next 24 hours.

Table I
Argatroban adjustments after the angioplasty procedure according to aPTT values.

aPTT (seconds) times x normal value (NV)	Discontinuation (duration in minutes)	Dose change in µg/kg/hour
<80 sec <2 times NV	-	+1 µg/kg/min increase
80-100 sec 2 to 2.5 times NV	-	+0.5 µg/kg/min increase
101-120 sec 2.5 to 3.0 times NV	-	No change
121-140 sec 3.0-3.5 times NV	-	-0.5 µg/kg/min decrease
141-160 sec 3.5 to 4.0 times NV	15 min	-1 µg/kg/min decrease
>160 sec >4 times NV	30 min	-2 µg/kg/min decrease

Table II
Demographic data on the studied population per dosage group.

	Group B (N=8)	Group C (N=9)	Group D (N=11)
Male gender	6 (75%)	5 (56%)	6 (55%)
Caucasian	7 (88%)	9 (100%)	11 (100%)
Age (range)	53 (37-70)	57 (33-74)	59 (49-77)
Weight (range)	76 (62-105)	65 (60-85)	72 (56-92)
Previous MI	2 (25%)	2 (22%)	5 (45%)
Previous PTCA	1 (13%)	5 (56%)	5 (45%)
Previous CABG	0 (0.0%)	1 (11%)	1 (9%)
Hypertension	3 (38%)	5 (56%)	4 (36%)
NIDDM	0 (0.0%)	2 (22%)	1 (9%)
Hypercholesterolaemia	3 (38%)	4 (44%)	2 (18%)
Current smoker	2 (25%)	1 (11%)	2 (18%)
Stable angina	4 (50%)	6 (67%)	6 (55%)
Unstable angina [40]	4 (50%)	3 (33%)	5 (45%)
class I	0	1	3
class II	4	2	1
class III	0	0	1
Two vessel disease	1 (12%)	2 (22%)	2 (18%)

Categorical variables are presented in absolute values and percent N (%). MI = myocardial infarction; PTCA = percutaneous transluminal coronary angiography; CABG = coronary artery bypass graft surgery; NIDDM = non-insulin dependent diabetes mellitus; unstable angina class I-III = according to the Braunwald classification [40].

Study endpoints; evaluation of efficacy and safety Study endpoints were the occurrence of major adverse cardiac events and bleeding complications, electrocardiographic changes as detected by continuous 12-lead recording, coagulation test results and qualitative angiogram interpretation. Efficacy was evaluated on the basis of occurrence of death, non-fatal myocardial infarction, revascularization procedures, and bleeding complications. All deaths were considered cardiac unless documented to the contrary. Myocardial infarction was diagnosed on the basis of new Q-waves (Minnesota Code [27]) or an increase of creatinine kinase more than twice the upper limit of normal with a concomitant increase in myocardial bound fraction. Bailout stenting was considered equivalent to coronary artery bypass grafting (CABG) if patency of the target vessel could not be maintained otherwise. If a stent was implanted electively after the initial PTCA (i.e. not comprising a bail out procedure), this was considered to be equivalent to repeat angioplasty. Repeat angioplasty was defined as re-insertion of a guiding catheter followed by angioplasty at the previously dilated site. The occurrence of typical anginal symptoms or electrocardiographic evidence of myocardial ischemia at rest or during exercise in case of atypical anginal symptoms and an angiographic diameter stenosis of > 50 percent by visual inspection was required to justify repeat angioplasty or bypass surgery.

Safety was evaluated on the basis of bleeding, which was classified as major if it was overt and led to a haemoglobin fall of at least 5 g/dl or 15% haematocrit; required transfusion of five or more units of whole blood or packed cells; or occurred intracranial, retroperitoneal, or into a major joint [28]. Minor bleeding was defined as overt, gross haematuria, or haematemesis, or if it led to a fall in haemoglobin of at least 3 grams per decilitre, or at least 10% haematocrit. Haematuria was classified as macroscopic when clinically evident, and as microscopic in the presence of more than 4 erythrocytes per high-power field.

Secondary endpoints Coronary angiograms were analyzed for TIMI flow assessment [29], as documented at re-catheterization 24 hours following balloon angioplasty. Continuous 12-lead electrocardiography over 24 hours was analyzed for ischemic episodes of recurrent ischemia, defined as at least 0.1 mV ST-segment elevation for at least one minute. Finally, blood samples were taken at various points in time to assess the dose response relationship of various coagulation parameters. Determination of ACT was done extemporaneously in the catheterization laboratory on whole blood samples. Bleeding time was performed at screening, 12 hours and 72 hours after commencement of the study medication. Blood samples for activated partial thromboplastin time (aPTT), pro-thrombin time (PT), thrombin-time (TT, thrombin concentration 4 to 20 IU/ml), thrombin-antithrombin-complex (TAT) and pro-thrombin fragment one and two ($F_{1.2}$) were drawn in the arm contra to that receiving medication. Those samples were centrally assessed in a haemostasis core-lab.

Statistical analysis Mainly descriptive statistics were carried out. Continuous variables were expressed as medians with their range, while categoric variables were expressed as absolute numbers and percentages. Two patients (group A) were not included in the statistical analysis since the initial dose regimen, as specified in the protocol, was ineffective in terms of ACT. Regression analyses of the different coagulation parameters on argatroban plasma levels were performed. Linear models were fitted and expanded with a logarithmic, square or cubic term.

Results

Patient population and dose regimen The first two patients (group A) enroled in the trial received a bolus of 30 μg/kg argatroban followed by an infusion of a rate of 3.5 μg/kg/min in order to obtain an ACT before balloon angioplasty of 300 seconds. However, despite repeated bolus injections and multiple increases in the

Table III
Qualitative angiographic data on the studied population per dosage group.

Treatment group (Number of lesions)	Group B (N=9)	Group C (N=10)	Group D (N=13)
Restenotic lesion	1 (11%)	4 (40%)	4 (31%)
Total occlusion	2 (22%)	1 (10%)	1 (8%)
Type of lesion (AHA/ACC)			
Type A	1 (11%)	1 (10%)	1 (8%)
Type B1	3 (33%)	0	4 (30%)
Type B2	5 (56%)	9 (90%)	7 (54%)
Type C	0	0	1 (8%)
TIMI flow post-PTCA			
TIMI 0	0	1 (10%)	1 (8%)
TIMI I	0	0	0
TIMI II	0	1 (10%)	0
TIMI III	9 (100%)	8 (80%)	12 (92%)
Dissections			
Absent	7 (78%)	5 (50%)	7 (53%)
Type A	1 (11%)	1 (10%)	1 (8%)
Type B	0	3 30%)	4 (31%)
Type C	1 (11%)	1 (10%)	0
Type D	0	0	1 (8%)
Thrombus post-PTCA	0	1 (10%)	0
TIMI flow at 24 hrs			
TIMI 0	0	0	1 (8%)
TIMI I	0	1 (12%)	0
TIMI II	0	0	0
TIMI III	9 (100%)	7 (88%)	10 (84%)
Not applicable	0	0	1 (8%)

Categorical variables are presented in absolute values and percent N (%).

infusion rate to 5.0 μg/kg/min, the ACT level did not exceed 200 seconds. Medication in these patients was replaced by routine (i.e. heparin) treatment.

Following detailed review of the first two patients, the next dosage group (group B) was assigned to receive a bolus of 60 μg/kg, followed by a 4 hour infusion of 8 μg/kg/min. At 4 hours the infusion rate was lowered to 4 μg/kg/min and was continued for 68 hours, so that the maximum daily dose would not be exceeded. In the absence of bleeding, no dosage adjustments were made. After the re-catheterization, the infusion rate was transiently reduced for catheter removal to 2.0 μg/kg/min to achieve an aPTT level of less than twice the normal value. One hour after sheath removal, the infusion rate was restored. Eight patients were treated according to this schedule. Major bleeding was not observed, ACT increased only moderately, and based on the results of $F_{1.2}$ and TAT, a higher administration level was investigated.

In parallel with a phase I study carried out in 9 healthy volunteers [unpublished data], the third dosage group (group C) received a bolus of 250 μg/kg, followed by a 4 hour infusion of 10 μg/kg/min. At 4 hours the infusion rate was lowered to 3.8 μg/kg/min and continued for 68 hours. This dosage group comprised nine subjects. For sheath removal the same procedure was followed as in the foregoing patients. Evaluation of clinical data indicated satisfying safety levels, as bleeding was not observed. Furthermore, haemostatic parameters revealed that at 45 minutes thrombin generation was not totally controlled, and that reduction of infusion rate to 2 μg/kg/min for the sheath removal was accompanied by in-creased thrombin conversion and activity. Interim analysis demonstrated that the aPTT level, at an infusion rate of 3.8 μg/kg/min, was around twice the normal value. Based on these data, the patients in the fourth dosage group (group D) received a bolus of 250 μg/kg, followed by a 4 hour infusion of 15 μg/kg/min. At 4 hours the infusion rate was lowered to 3.8 μg/kg/min and was continued for 68 hours without adjustment for catheter removal. This dosage regimen group was comprised of 11 patients who received a total daily dose of 8.4 mg/kg.

Demographic, clinical and angiographic data of the studied patients are summarized in tables II and III respectively. None of the patients had a history of bleeding or stroke. The small number of patients does not allow for comparison of the groups; however, the strict inclusion criteria ensure that they are very similar.

Study endpoints (Table IV) In group B, one patient experienced insignificant bleeding at the puncture site 7 hours after sheath removal during a 4 μg/kg/min infusion, and therefore the study medication was discontinued. One case of phlebitis at the site of the indwelling infusion needle was reported, which occurred 58 hours after the commencement of the study medication.

In treatment group C, two patients had a failed PTCA (one of which was a total occlusion before the attempt) and after discontinuation of argatroban infusion were referred for elective bypass surgery. Another patient showed recurrent stenosis (>50%) with thrombus at the 24 hours control angiogram. Repeat PTCA (under argatroban therapy) was successful. In this group no bleeding complications were observed.

Table IV
Occurrence of clinical events per dosage group.

	Group B (N=8)	Group C (N=9)	Group D (N=11)
Death	-	-	-
MI	-	-	1 (9%)
CABG	-	2 (22%)	-
Bail-out	-	-	1 (9%)
Repeat PTCA	-	1* (11%)	3* (18%)
Any event	0 (0%)	3 (33%)	2 (18%)
12-lead ECG ST monitoring	8 (100%)	9 (100%)	11 (100%)
Patients with ST-episodes	-	1 (11%)	3 (27%)
Number of ST-episodes#	-	0.9	2.9
Duration of episodes (median in min:sec)	-	2:33	32:07
Bleeding complications			
Epistaxis	-	-	1 (9%)
False aneurysm	-	-	1 (9%)
Bleeding at puncture site	1 (13%)	-	1 (9%)

Group B: bolus of 60 µg/kg, followed by a 4 hour infusion of 8 µg/kg/min.
At 4 hours the infusion rate was lowered to 4 µg/kg/min and was continued for 68 hours.
Group C: a bolus of 250 µg/kg, followed by a 4 hour infusion of 10 µg/kg/min.
At 4 hours the infusion rate was lowered to 3.8 µg/kg/min and continued for 68 hours.
Group D: a bolus of 250 µg/kg, followed by a 4 hour infusion of 15 µg/kg/min.
At 4 hours the infusion rate was lowered to 3.8 µg/kg/min and continued for 68 hours.

* The repeat PTCA in group C and one repeat PTCA in group D was triggered by the recatheterization
24 hours after the start of the infusion.
# Normalized to 24 hours recording time during treatment
MI = myocardial infarction; CABG = coronary artery bypass graft surgery;
PTCA = percutaneous transluminal coronary angiography.

In treatment group D, one patient experienced a myocardial infarction due to a subacute re-occlusion. Repeat PTCA was attempted but not successful. Another patient showed recurrent stenosis (>50%) on the 24 hours control angiogram. Repeat PTCA was not satisfactory and a stent was implanted and argatroban was replaced with routine anticoagulants. During treatment with heparin and Acenocoumarol, this patient had prolonged epistaxis, necessitating blood transfusion. A second repeat PTCA was performed in this patient for occlusion at the stented segment. In addition, a false aneurysm was reported in one patient, and prolonged insignificant bleeding at the puncture site in another.

There were no reported death or cases of haematuria in any of the four dose groups. Results of TIMI flow assessment and 12-lead electrocardiographic monitoring are reported in tables III and IV respectively. The low number of patients enroled does not justify solid conclusions on angiographic and clinical results. Individual coagulation parameters may yield specific information on the unstable syndrome or complexity of the lesion, but this relationship remains speculative.

Dose-response relationship of argatroban plasma level and coagulation parameters The relationships between argatroban plasma level and coagulation parameters (ACT, aPTT, TT and PT) are presented in figure 1. A clear dose-response relationship is documented for each parameter. Table V presents the results of linear regression analysis. Inclusion of a squared variable in the model (e.g: $y = a + bx + c^2$) for TT improved the R-square value from 0.639 to 0.653. Additional inclusion of a cubed variable (e.g: $y = a + bx + c^2 + dx^3$) in the aPTT-model only marginally improved the R-square value from 0.715 to 0.725. Although these improvements in the models for TT and aPTT are statistically significant, we suggest restriction to the linear and square model respectively for pragmatic reasons. Logarithmic strategy in the aPTT-model resulted in an R-square value of 0.64 ($P < 0.001$), which is inferior to the square model. No clear relationship could be detected between the argatroban plasma level and $F_{1.2}$ and TAT concentrations (Figure 2); however, a substantial number of measurements exceeded the normal range (1.29 nmol/L for $F_{1.2}$ and 3.2 µg/L for TAT).

Discussion

Argatroban was developed in Japan (Mitsubishi Chemical) where authorization for its use in the treatment of peripheral arterial disease was obtained in 1991 (MD-805, available as Novastan). The rationale of this study was based on the finding that specific antithrombin therapies can inhibit mural thrombosis after deep arterial injury and prevent the growth of pre-existing thrombus on deeply injured arteries exposed to both high and low shear stresses [8]. Experimentally, injured arteries remain highly thrombogenic for several hours to days following balloon injury [30-33]. Therefore it was judged prudent to commence argatroban administration before the angioplasty procedure and continue during the next 72 hours. The restriction of argatroban to intravenous administration limits its use to hospital setting.

The initial dosage was based on previous studies in cardiac patients. Gold

Table V
Relationship between coagulation parameters and argatroban plasma level.

Parameter	Formula	P-value	R-square
BT	-	NS	0.00
ACT	190 + 36.8 * APL	0.000	0.54
aPTT	33.2 + 23.9 * APL – 2.25 * APL^2	0.000	0.71
TT	5.53 + 23.7 * APL	0.000	0.64
PT	11.4 + 4.88 * APL	0.000	0.84

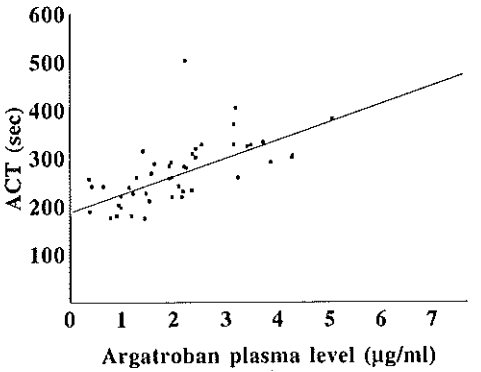
APL = Argatroban plasma level; BT = bleeding time; ACT = activated coagulation time; aPTT = activated partial thromboplastin time; TT = thrombin time; PT = pro-thrombin time.

Table VIA
Thrombin-antithrombin III complex (normal range 0.60-3.20 µg/L) by dosage group (median and range shown in brackets).

Time	N	Group B	N	Group C	N	Group D
- 1/2 hr	8	4.1 (2.2-18.5)	9	2.6 (2.0-10.5)	11	3.9 (2.0-11.0)
1 hr	8	4.0 (2.0-6.0)	9	2.7 (2.0-31.5)	11	3.3 (2.0-17.0)
2 hrs	7	5.0 (2.4-12.0)	8	2.3 (2.0-4.2)	11	4.8 (2.4-161.0)
24 hrs	8	5.7 (4.5-20.0)	7	5.0 (4.0-6.8)	9	8.0 (4.3-52.5)
72 hrs	7	3.2 (2.4-13.0)	7	2.6 (2.0-4.2)	8	3.8 (2.9-11.0)
73 hrs	7	4.8 (2.3-28.0)	7	3.0 (2.0-6.3)	8	5.5 (2.2-36.0)
74 hrs	7	4.3 (3.2-125.0)	7	3.6 (2.0-13.0)	8	5.0 (2.0-10.6)

Table VIB
Prothrombin fragment 1 and 2 (normal range 0.57-1.29 nmol/L) by dosage group (median and range shown in brackets).

Time	N	Group B	N	Group C	N	Group D
- 1/2 hr	8	0.93 (0.43-2.10)	9	1.10 (0.45-1.50)	11	1.30 (0.55-3.40)
1 hr	8	0.76 (0.50-1.30)	9	1.10 (0.80-3.20)	11	1.80 (0.90-3.40)
2 hrs	7	1.30 (0.60-2.20)	8	1.35 (0.90-2.20)	11	2.00 (0.60-7.00)
24 hrs	8	1.80 (0.95-3.80)	7	1.90 (1.45-2.60)	9	2.90 (0.85-4.00)
72 hrs	7	0.70 (0.47-1.05)	7	1.05 (0.90-1.30)	8	1.35 (0.85-2.10)
73 hrs	7	0.95 (0.65-2.20)	7	0.95 (0.70-1.30)	8	1.45 (0.75-5.00)
74 hrs	7	1.00 (0.70-11.25)	7	0.90 (0.62-1.80)	8	1.13 (0.34-2.50)



The relationship between argatroban plasma level and the result of coagulation tests: ACT = activated clotting time; aPTT = activated partial thromboplastin time; TT = thrombin-time; PT = pro-thrombin time. The lines within the graphs outline the predicted levels of the models delineated in table V.

Figure 1A
ACT in seconds.

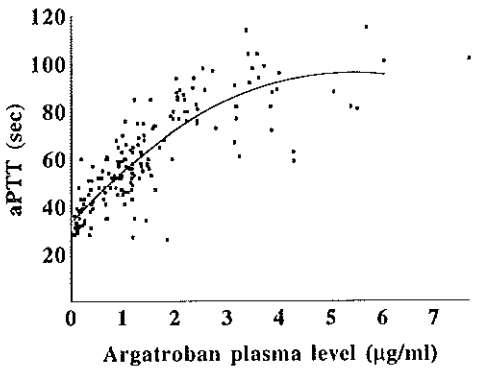


Figure 1B
aPTT in seconds.

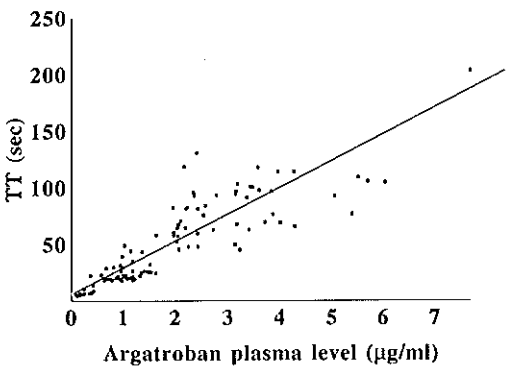


Figure 1C
TT in seconds (thrombin concentration 20 IU/ml).

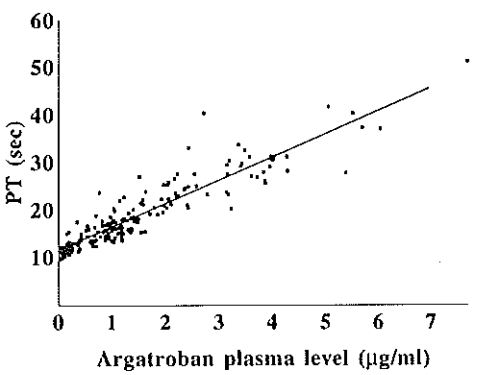


Figure 1D
PT in seconds.

The relationship between argatroban plasma level and the result of prothrombin fragment 1 and 2 (F1.2) and thrombin-antithrombin III (TAT) measurements.

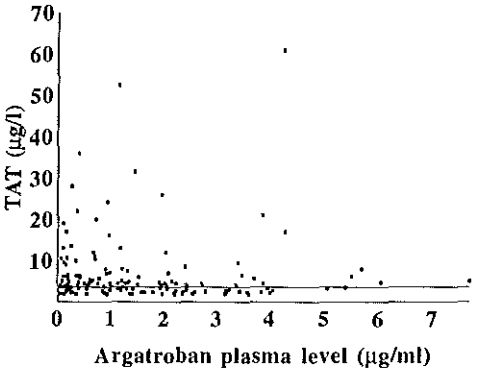


Figure 2A
$F_{1.2}$ in nmol/L; the upper normal limit is marked at 1.29 nmol/L .

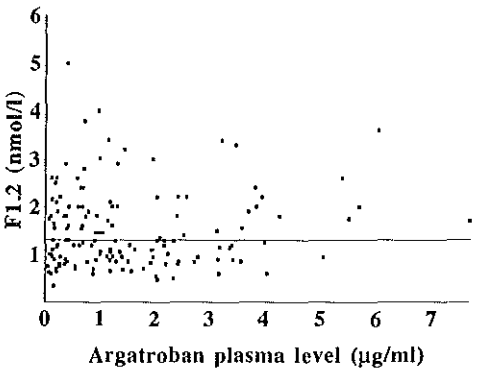


Figure 2B
TAT in µg/L, the upper normal limit is marked at 3.2 µg/L .

The results of $F_{1.2}$ and TAT measurements related to the infusion period.

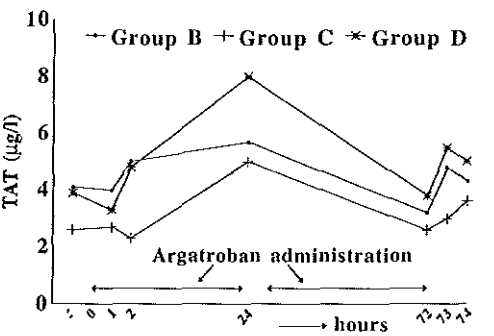


Figure 3A
Median $F_{1.2}$-levels in in nmol/L .

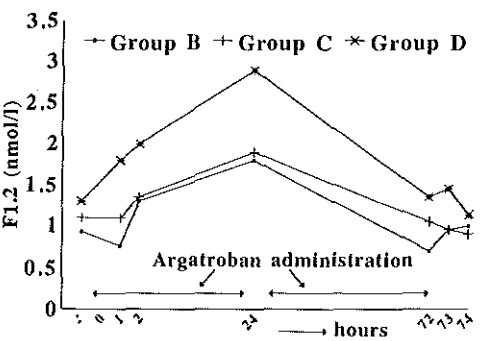


Figure 3B
Median TAT-levels in in µg/L .

and his colleagues infused argatroban (0.5 to 5.0 μg/kg/min) over 4 hours in patients with unstable angina pectoris [24]. This dose regimen was effective against the recurrence of ischemic episodes. Fitzgerald (not published) treated angioplasty patients with an initial bolus of 30 μg/kg argatroban, followed by an intravenous infusion of 2 μg/kg/min over 2 hours. Based on toxicity studies in animals, the total daily dose should not exceed 10 mg/kg bodyweight.

A significant correlation was observed between the argatroban plasma level and the dose response curves for several coagulation parameters. The predictability of the aPTT and the PT in particular, is very good and can be described mathematically with statistical significance. However, the predictability of bleeding time is low. Measurements of ACT with the Hemochron system yields values approximately 30% higher than the results assessed with HemoTec ACT assessment [34], which may have influenced the R-square value. The dose response curves of argatroban and coagulation tests demonstrate the feasibility of accurate dosing of this compound in contrast to heparin, with which non-specific plasma protein binding, variable bio-availability, non-linear dose dependent clearance and important individual differences among patients frequently result in underdosing or overdosing.

Levels of $F_{1.2}$ and TAT reflect the degree of pro-thrombin conversion and thrombin activity, respectively. Sampling was performed from separate peripheral vein punctures to avoid bias caused by the indwelling catheter [35, 36]. These measurements indicate the systemic status of the coagulation system. Sampling from the great cardiac vein may have been more accurate, representing a less diluted effect on the local stimulus to the coagulation system. Absolute inhibition of thrombin generation by thrombin blockade remains an Utopian concept, since argatroban requires an insignificant pro-thrombin conversion before it can display its antithrombin activity. Zoldhelyi et al. [37] recently reported the failure to block thrombin generation in patients, despite a 10,000-fold molar excess of free hirudin over the thrombin-hirudin complex. Furthermore, these coagulation products are extremely sensitive, to such an extent that only perfect atraumatic vein punctures can provide blood with unaffected, non-elevated levels.

Conceivably, a superior marker for thrombin blockade would be the fibrinopeptide A concentration [38]. This peptide is a product of thrombin induced fibrinogen conversion and is therefore a direct mirror of the performance of non-blocked thrombin.

Rebound is described after cessation of i.v. argatroban infusion in patients with unstable angina pectoris [24, 39]. In this study we did not uncover any clinical evidence for a rebound phenomenon in this cohort of patients. However, 24 hours after start of the infusion (just prior to sheath removal when the infusion was transiently interrupted), a substantial elevation of TAT and $F_{1.2}$ levels was observed (table VI, figure 3). A lesser increase in TAT and $F_{1.2}$ levels was detected one and two hours after the termination of the argatroban infusion (73 and 74 hours after the start of the infusion, respectively). This disparate response to cessation of argatroban administration may reflect the process of vessel wall passivation. It has to be emphasized that repetitive vena punctures within a short period

of time may result in subsequent less optimal sampling quality, and therefore demonstrate artificially elevated levels of TAT and $F_{1.2}$.

Conclusions

This dose-finding study demonstrates that argatroban infusion in patients undergoing PTCA may be safely administered, and produces an adequate and predictable level of anticoagulation. This trial provides useful and consistent information on the response of the coagulation system when exposed to argatroban. Indirect parameters indicate ongoing thrombin conversion (pro-thrombin fragment 1 and 2), and activity (thrombin-antithrombin complex). An apparent safe and adequate dosage regimen was identified and this dose is now undergoing further evaluation in a double blind, double dummy, 2:1 randomized and heparin controlled study. The effect of argatroban effect on the restenosis process following coronary angioplasty must be determined in a larger investigation.

Acknowledgements

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Chapter 8

Evaluation of recombinant hirudin (CGP 39 393/™REVASC) in the prevention of restenosis after percutaneous transluminal coronary angioplasty

Rationale and design of the HELVETICA¶ trial, a multicentre randomized double blind heparin controlled study

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¶ HELVETICA: Hirudin in an European restenosis prevention trial versus heparin treatment in PTCA patients. The institutions and investigators participating in the HELVETICA trial are listed in the appendix in chapter 9

European Heart Journal 1995(supplement L);16:56-62

Abstract One of the main areas of interest in interventional cardiology is the understanding, and ultimate prevention of restenosis after an initially successful percutaneous transluminal coronary angioplasty. Restenosis is the recurrence of luminal narrowing following angioplasty, and still frustrates the late results in the treatment of angina pectoris. Experimental, pathological and clinical studies suggest that restenosis may occur via activation of the coagulation cascade, platelet activation and thrombus formation. Thrombin itself is identified as the most potent platelet activator, and has a pivotal role in the coagulation system. Futhermore, thrombin directly mediates smooth muscle cell proliferation by stimulating thrombin receptors at the smooth muscle cell surface. Thrombus indirectly induces excessive intimal smooth muscle cell proliferation by means of released mitogens (growth factors), which may contribute to late restenosis. Therefore direct and irreversible thrombin blockade by hirudin is deemed to be effective in the prevention of restenosis following angioplasty.

The HELVETICA trial is a multicentre randomized double blind heparin controlled study, designed to compare the effects of two dose regimens of recombinant hirudin (CGP 39 393/™REVASC) with those of heparin on event-free survival, safety and tolerability and luminal renarrowing using quantitative coronary angiography no later than 26 weeks after the coronary angioplasty procedure. (European Heart Journal 1995(supplement L);16:56-62)

In spite of the immediate therapeutic success of coronary angioplasty, restenosis, the recurrence of luminal narrowing following percutaneous transluminal coronary angioplasty (PTCA) still hampers longterm outcome in the treatment of obstructive coronary artery disease [1]. Experimental, pathological and clinical studies suggest that several responses to luminal enlargement, such as vessel wall injury, occur, and there is evidence that thrombin and thrombus trigger of the restenosis process.

Exposure of the collagen of the media as well as the material of the squeezed atheromatous plaque activate the coagulation system and various coagulation factors (V, VIII, XIII) ensure rapid thrombin generation via an autocatalytic reaction and a positive feedback loop [2]. Thrombin converts soluble fibrinogen into fibrin and induces fibrin cross-linking by activation of factor XIII.

Thrombin itself is also a very potent platelet activator. Platelet deposition and platelet degranulation at the site of endothelial denudation early after angioplasty (minutes to days) can induce coronary spasm and the formation of mural thrombus, which may promote restenosis [3-8]. Due to thrombin activation, platelets release various pro-coagulant factors and mitogens (growth factors), which add respectively, to the formation of thrombus and encourage intimal smooth muscle cell proliferation, contributing to late (7 to 150 days) restenosis [3,9-17].

Thrombin receptor stimulation at the smooth muscle cell surface may directly mediate smooth muscle cell proliferation.

The incidence of restenosis ranges from 17% to 40%, depending on the angiographic, clinical or physiological definition and study design. Many pharmacological interventions have been used in prospective randomized trials to prevent restenosis [18,19], but despite a sufficient number of patients and therefore statistical power, complete angiographic follow-up, and quantitative angiographic analysis, to date no compound has been able to reduce or control luminal renarrowing after initially successful PTCA. To assess their respective abilities in the prevention of restenosis after coronary angioplasty in patients with unstable angina, hirudin, with its direct and highly selective action was compared with heparin in a multicentre, double blind, randomized trial.

Potential role of recombinant-hirudin (CGP 39 393/™REVASC) in prevention of restenosis Hirudin, a potent, selective and direct thrombin inhibitor, composed of only 65 amino acids, was originally extracted from the salivary gland of the medicinal leech. The recombinant hirudin CGP 39 393/™REVASC is expressed in yeast and differs from the natural hirudin only by the absence of a sulphate group in the Tyr-63 position. The favourable element of hirudin in comparison to other serine protease inhibitors is its ability to irreversibly block thrombin at multiple sites [20] without the need for circulating cofactors such as antithrombin III, thereby preventing direct activation of clotting factors V, VII and VIII. Furthermore hirudin is able to inhibit clot-bound thrombin, and can thus restrict further thrombus formation which may occur during thrombolysis [21].

This recombinant molecule has demonstrated its powerful anti-thrombotic qualities in venous shunt and arterial thrombosis models [22,23], and reduced platelet deposition to only a single layer following balloon injury to carotids of pigs [24]. Sarembock et al. [25] showed significantly less restenosis 28 days after dilatation assessed by quantitative angiography and histopathology in hypercholesterolaemic rabbits following administration of ™REVASC as compared to heparin. The group of Buchwald [26] demonstrated a significantly lower platelet count and fibrin deposition on implanted stents in minipigs with the use of hirudin compared to heparin.

Recombinant-hirudin (CGP 39 393/™REVASC) in patients undergoing coronary angioplasty The effect of recombinant hirudin (CGP 39 393/™REVASC) in angioplasty patients was recently evaluated in a multicentre double blind pilot study [27]. One hundred and thirteen patients were randomized in a 2:1 fashion, to receive either 20 mg recombinant hirudin as an intravenous bolus prior to angioplasty followed by a 24 h infusion of 0.16 mg/kg/hr (N=74), or to 10,000 IU of heparin bolus i.v. followed by a 12 IU/kg/hr infusion for 24 h (N=39). In the heparin treated group, four patients experienced a periprocedural myocardial infarction and/or emergency coronary artery bypass graft (CABG), compared to only one emergency CABG, and one elective CABG 3 weeks after PTCA in group allocated to recombinant hirudin. Haemorrhage at

Table I
Inclusion and exclusion criteria in the HELVETICA study.

Inclusion criteria

Cooperative male or female patients, aged 30-75 years, who had developed the following conditions within the past 3 months: new onset of angina pectoris or worsening of the angina pattern (i.e. worsening of angina pattern by two classes according to the classification of the Canadian Cardiovascular Society class [32] or need for additional antianginal medication) and/or angina at rest may be considered for inclusion in the trial [modified from 28].

Patients should be designated to undergo a percutaneous transluminal coronary angioplasty (balloon, directional or rotational atherectomy or excimer laser) of one or more coronary vessels as a result of diagnostic angiography, during which stenosis of greater than 50%, suitable for dilation, was observed. Patients must give written informed consent.

Exclusion criteria

General exclusion criteria

Women of childbearing potential or nursing mothers. Women are considered to be of childbearing potential unless they are post-hysterectomy, one or more years post-menopausal or one or more years post-tubal ligation.

Previous participation in the present trial.

Major non-cardiac surgical intervention planned within the next 6 weeks.

Life expectancy of less than one year.

Factors making follow-up difficult or unlikely (e.g. not fixed address, psychological instability, drug or alcohol addiction etc.).

Body weight > 100 kg.

Criteria related to the angioplasty

Unprotected left main disease.

Planned multistage procedure.

Planned implantation of a stent.

Planned angioplasty solely of a coronary bypass graft.

Factors contraindicating angioplasty or coronary angiography (e.g. graft or severe atherosclerosis of the lower limbs, severe iodine allergy).

Cardiovascular and haematologic criteria

Myocardial infarction with a rise in creatine kinase (CPK) 3 times (or CK-MB 2 times) above the upper normal limit within the past 2 weeks.

Evolving myocardial infarction.

Cardiopulmonary resuscitation manoeuvres within the past month.

Cerebro-vascular accident within the past 6 months.

Known pericarditis.

Known bacterial endocarditis.

Known left heart thrombus.

Cuff systolic blood pressure < 90 mmHg.

Cuff diastolic blood pressure (measured at the disappearance of sounds) above 100 mmHg and/or systolic blood pressure above 180 mmHg.

History of gastrointestinal, pulmonary or intraocular bleeding.

Known diabetic retinopathy.

Signs or symptoms of acute internal or external haemorrhage.

Known congenital or acquired haemostatic disorders and/or liver disease.

History of heparin-induced thrombocytopenia.

Known significant anaemia (haemoglobin < 110.0 g/L [=6.2 mmol/L]).

Known thrombocytopenia (< 100,000 platelets/mm3).

Other exclusion criteria

Major surgery, biopsy or puncture of a non-compressible vessel within the past month.
Severe trauma within the past 3 months.
Lumbar puncture (within the past 7 days).
Known intolerance to acetyl salicylic acid.
Acute or chronic renal impairment (defined as an increase of serum creatinine above 1.5 mg/100mL (130 μmol) or a fall in creatinine clearance below 90 mL/min)..
Known severe hepatic disease.
Documented peptic ulcer or gastritis within the last 6 months.
Known cavitary lung disease.
Known inflammatory bowel disease.
Participation in another study with an investigational drug within the past month.

Previous and concomitant treatments

The use of corticosteroids, oral anticoagulants, non-steroidal anti-inflammatory drugs (with the exception of acetyl salicylic acid), dipyridamol, sulphinpyrazone, ticlopidine, melphalan, vincristine, l-asparaginase is prohibited 48 hours prior to randomization. Oral anticoagulants and/or anti-platelet agents (with the exception of acetyl salicylic acid) may only be started or reinstituted 7 days after the angioplasty.

The use of any investigational drug (including hirudin, natural or recombinant) within 30 days prior to the start of the trial is prohibited.

The use of fish-oil is prohibited during the course of the trial.

Lipid-lowering drugs are allowed only when they are indicated on the basis of the patient's lipid levels according to the recommendations of the European Consensus Group.

As a general rule, beta-blockers, calcium-antagonists and nitrates (both sublingual and oral, short- and long-acting) should be discontinued at hospital discharge to determine whether the patient has become asymptomatic after angioplasty.

Calcium antagonists are allowed at randomization only when they are already being taken before randomization. Their discontinuation should, in all cases, be attempted at hospital discharge. A calcium-antagonist may be given orally or intravenously in case of spasm during the angioplasty session.

Beta-blockers may be continued as a secondary prevention in patients with a history of myocardial infarction prior to trial entry or as antihypertensive agents, provided that beta-blockade had been started before hand. In such cases, the dose should be kept constant. Other cardiac medications may be used on strict indication only.

the arterial puncture site was seen in four recombinant hirudin-treated patients, for which blood transfusion was needed, while in the heparin-allocated group one patient experienced visual impairment because of a cerebral infarction. Follow-up angiography at 24 h revealed a complete perfusion in all hirudin treated patients, compared to 92% in the heparin treated group. Based on this very recent publication, it was concluded that recombinant hirudin can safely be administered to patients undergoing balloon angioplasty for stable angina pectoris.

The HELVETICA trial; hirudin in an european restenosis prevention trial versus heparin treatment in PTCA patients.

Aim of the trial. The HELVETICA study is a double-blind, randomized, and heparin-controlled multicentre trial, which is carried out in Belgium, France, Germany, Italy, Spain, Sweden and the Netherlands. The primary objective of the present trial was to compare the effects of two dose regimens of recombinant-hirudin to that of unfractionated heparin on the event-free survival in patients planned to undergo PTCA. Cardiac death, non-fatal myocardial infarction, CABG or bail out procedure (e.g. stent), repeat angioplasty or elective stent implantation were counted as clinical events.

The secondary objectives were to compare the safety and tolerability of two CGP 39 393/™REVASC dose regimens to those of heparin and to compare the effects on the quantitative cineangiographic measurement of the minimal luminal diameter (MLD) assessed at 6 months after PTCA.

Design and treatment Eligible patients, whether already on heparin (i.e. unstable angina) or not, who had developed recent onset or worsening angina or angina at rest within the past 3 months [28] (Table I) and who were to undergo PTCA were randomly allocated just prior to angioplasty, to one of the two recombinant hirudin regimens, or to the control (heparin) group. The treatment groups are listed below.;

Unfractionated sodium heparin treatment group Subjects were given a bolus of 10,000 IU heparin i.v., followed by a continuous i.v. infusion of 15 IU/kg/h. The infusion period was followed by a placebo administered subcutaneously twice daily for 3 consecutive days.

Treatment groups Subjects were given a bolus of 40 mg recombinant hirudin i.v., followed by a 0.2 mg/kg/h continuous i.v. infusion. In one group the infusion period was followed by administration of 40 mg subcutaneously twice daily for 3 consecutive days, in the other by administration of placebo subcutaneously twice daily for 3 consecutive days.

An optional blinded i.v. bolus could be given 1 h after the first i.v. bolus. This would comprise heparin in the heparin treatment group and placebo in both recombinant hirudin groups. The operator remained blinded for coagulation parameters, and therefore infusion rate could not be adjusted.

The height of bolus and infusion rate in both the r-hirudin groups was based on the analysis of coagulation markers (thrombin-antithrombin complex (TAT)

and pro-thrombin fragment 1 and 2 ($F_{1.2}$)) in the pilot trial [27]. Concentrations of $F_{1.2}$ appeared to be somewhat higher in the hirudin group directly and 6 h after angioplasty (a median of 1.015 versus 0.795 nanomole/litre (nmol/L) and 0.95 versus 0.8 nmol/L respectively). Although these differences did not reach statistical significance, this finding was nevertheless suggestive of enhanced thrombin generation during this period in hirudin treated patients compared to heparin treated patients. Therefore the bolus dose was doubled from 20 mg in the pilot trial to 40 mg in the present study, and the infusion rate of 0.16 mg/kg/h in the pilot phase was increased to 0.20 mg/kg/h in the present study.

Heparin administration before randomization had to be discontinued at least 30 min before initiation of trial medication. Blinded trial medication was used to flush the various devices and materials during and after the PTCA procedure.

Randomization is stratified for previous (i.e. last 24 h) heparin administration. Within-centre balance between treatments is obtained by randomizing in blocks.

Acetyl salicylic acid (100 to 500 mg once daily) if not already being taken was started immediately and was continued for 14 days. Oral anticoagulants and/or other antiplatelet agents could be reinstituted or started 7 days after PTCA according to local routine.

Sample size considerations The sample size considerations are based on the assumption that the event free period is exponentially distributed with a constant hazard rate and that each patient is followed-up for 30 weeks. Based on the results of previous trials [29] over a 6-months observation period, the event rate in the control group was expected to be 30%. This corresponds to a hazard rate = 0.06. To detect at least a 30% reduction in the event rate in the hirudin group (hazard rate = 0.037) at a level alpha of 2.5% (adjusted) with a power of 80% a sample size of 357 patients per treatment group was required (observation period 6 months) [30]. A sample size calculated on the event rate at 26 weeks yielded 368 patients per treatment group.

Trial chronology All out- or in-patient candidates for PTCA were screened for potential participation in the study. Inclusion and reason for exclusion (Table I) were documented in order to compare the study population with the entire PTCA population in the 30 participating centres. After written informed consent, patients were randomized according to the allocation given by the central telephone allocation service. Heparin administered for unstable angina had to be discontinued at least 30 minutes before initiation of trial medication. Bolus injection and a weight-adjusted infusion of study medication were started simultaneously before angiography was begun. The i.v. infusion was continued for 24 h, without dose adjustments. Pre- and post- angioplasty coronary angiography are performed according to the requirements for quantitative analysis. The sheath was removed no less than 3 h after the end of the infusion period, just prior to the first subcutaneous injection of 40 mg hirudin or placebo twice daily for 3 consecutive days. A follow-up visit was required 2 weeks after the PTCA. A visit 26

weeks following PTCA documented anginal status, 12-lead electro cardiogram, current medication and any adverse experience. A symptom-limited bicycle ergometry was performed before the control angiogram was made. It was decided that final clinical follow-up for evaluation of clinical endpoints would occur 30 weeks after PTCA to allow sufficient time for performance of justified (based on recurrence of anginal symptoms or demonstrated exercise-induced ischaemia) elective reintervention or CABG as determined by clinical and angiographic follow-up.

Laboratory findings on coagulation parameters At predetermined time points, blood samples for assays of activated partial thromboplastin time (APTT), $F_{1.2}$ and plasma hirudin concentrations were taken. Blood samples were retrieved by separate venepunctures at screening, just prior to start of the infusion, 6 and 24 h after angioplasty and just prior to and 3 h after the last subcutaneous injection. Assays for APTT and prothrombin fragment $F_{1.2}$ were analyzed centrally at the Department of Haemostasis and Thrombosis in the Academic Medical Center, Amsterdam, The Netherlands. Assays for plasma hirudin concentrations were analyzed by BPK Ciba (Basle, Switzerland).

Evaluation and safety criteria Primary objective is the event free survival within the observation period of 30 weeks, according to the method of Kaplan-Meier and compared by the (stratified) generalized Wilcoxon test [31]. Clinical events were cardiac death, non-fatal myocardial infarction, CABG or bail out procedure, repeat angioplasty or elective stent implantation at previously dilated sites.

The secondary objectives were four-fold: (1) Noting all clinical events occurring within the first 96 h after start of trial medication; (2) taking account of the severity of a patient status graded as cardiac death > non-fatal myocardial infarction > CABG (or bail out procedure) > repeat angioplasty or elective stent implantation > anginal status (Canadian Cardiovascular Society classification [32]) post-PTCA or none of the above at 30 week follow-up; (3) assessing the MLD of the dilated sites measured by quantitative coronary angiography (QCA) at 26-week follow-up. If for any reason angiography is carried out prior to 18 weeks after PTCA, further angiography within the recommended time window is required, unless a primary clinical endpoint, as defined, is reached; (4) noting the change in MLD of dilated sites between the immediate post-PTCA and the 26-week follow-up coronary angiogram.

Safety is evaluated on the basis of bleeding, immuno-allergic and other complications.

The HELVETICA study; the appealing aspects

The Braunwald classification Grading of unstable angina according to the Braunwald classification [28], although clinical, can be related to the underlying disease. Sherman et al [33] described the correlation between the presence of an angioscopic finding of non-occlusive thrombi and recent onset of anginal episodes at rest. With the progression of time, these thrombi undergo lysis and organization, and anti-thrombin therapy might be less effective in PTCA patients when

compared to stable class I angina pectoris. This trial provides detailed information on angina severity, clinical circumstances in which unstable angina pectoris occurs, electrocardiographic changes during chest pain, and the intensity of treatment under which anginal episodes occur.

Dynamic versus static restenosis criteria; MLD at follow-up as a quantitative angiographic end-point. Restenosis following vessel wall injury, as in successful angioplasty, has been shown to be a proliferative reaction influencing virtually all coronary obstructions which have been exposed to therapeutic angioplasty [13,34-37]. Luminal renarrowing of the coronary arteries should be viewed as the tail end of an almost normally distributed phenomenon rather than a unique disease entity, developing in some lesions but not in others [38,39]. Restenosis criteria currently in use can be classified at those which describe the change in lesion severity during follow-up (dynamic criterion) and those which merely describe lesion severity at follow-up (static criterion). Examples of the first category are the loss in lumen diameter of more than 0.72 mm as proposed by Serruys et al. [29,40-42] and a change in percent diameter stenosis. Examples of the second category are the criterion of >50% diameter stenosis at follow-up and a minimal luminal diameter of more than 1.4 mm at follow-up [43].

To reflect current clinical practice, by protocol the use of new interventional tools as directional or rotational atherectomy and excimer laser, are allowed in the HELVETICA study. However, because there are different interventional tools currently in use discrepancies arise which are not reconciled by using dynamic restenosis criteria. Due to the different nature of the interventions applied here (e.g. atherectomy, balloon and excimer laser angioplasty), the immediate procedural outcome is no longer comparable; atherectomy induces a larger gain in minimal luminal diameter than conventional balloon therapy which makes the immediate post-procedural features dissimilar so that the loss during follow-up is no longer a helpful comparison of the long-term benefit. The most valid parameter for the comparison of two interventional devices is the minimal luminal diameter at follow-up because this static parameter, in itself, represents the final luminal improvement at follow-up. Moreover, the minimal luminal diameter at follow-up may have some functional component; in accordance with Danchin et al [43], we [44] found that a minimal luminal diameter of 1.45 mm correlates with recurrence of anginal episodes (sensitivity and specificity of 72%). This information suggests that the absolute value of the minimal luminal diameter at follow-up may prove to be an even more useful parameter than parameters obtained by clinical examination or exercise testing. Therefore a static restenosis parameter which describes lesion severity at follow-up angiography should be used when different interventional techniques are included in a trial.

The use of QCA secondary to clinical endpoints Evidence to support the use of QCA as a surrogate endpoint for clinical coronary events, arises from studies on progression and regression, where initially isolated angiographic evidence of progression has been subsequently followed during 'longer-term' follow-up by higher clinical event rates. This positive predictive value of angiographic end-

points has been demonstrated for both visual assessment [45] and semi-automated QCA [46,47]. Is the coupling of QCA and subsequent clinical events due to a paraphenomenon, or does the measurement of a severe coronary stenosis by QCA directly predict an increased risk of occurrence of a coronary event in this target vessel at the point of the minimal luminal diameter? Little et al. [48] and other investigators have revealed that coronary occlusions which produce a myocardial infarction frequently occur at sites which previously contained minor, 'insignificant' luminal narrowings and that the lesions with the most severe stenosis are often found to be patent in such patients [48-52]. It has been proposed that angiographic evidence of progression or regression in one segment may be a marker of disease activity which may involve other coronary segments but may not be apparent on the coronary angiogram [49]. Whether this holds true because QCA demonstrated severely restenotic lesions (large luminal loss over 6 months) following intervention has yet to be verified. It has been proposed that the endoluminal cap of a restenosed lesion is more stable and less susceptible to fissuring than a primary atherosclerotic plaque of equivalent luminal diameter. Thus it might be expected that the detection of restenosis following intervention by QCA might be more predictive of functional reversible ischaemia, recurrence of angina and subsequent symptomatic need for revascularization, rather than predicting myocardial infarction and death.

Markers for activation of coagulation Prothrombin fragment 1 and 2 is a direct product from prothrombin conversion and is therefore a sensitive marker for activation of coagulation. It can be used to monitor the biological efficacy of experimental anticoagulants. The $F_{1.2}$ levels before treatment provide a clear view of the relationship between severity of unstable angina and the coagulative state. Furthermore we may be able to draw conclusions from the correlate coagulation markers prior to and after angioplasty and the rate of acute complications and long term restenosis. In this we may find support for the rationale of thrombin inhibition and restenosis prevention.

Conclusion

The rationale for use of recombinant hirudin in the HELVETICA trial is based on experimental evidence that demonstrated the strong anti-thrombotic properties of this compound. The mechanism of action of hirudin as a thrombin inhibitor implies that it will prevent both acute occlusion due to thrombin induced thrombus formation and neointimal proliferation following vessel wall injury. As a consequence, trial medication is started before the procedure, i.e. before wall injury takes place. The prospective, randomized, double-blind, heparin controlled strategy, the use of multiple intervention techniques, the sample size calculation and both clinical and angiographic evaluation criteria provide an opportunity to evaluate the influence and impact of coagulation activation and the effect of thrombin inhibition on early and late outcome after PTCA, weighted against the risk of increased bleeding.

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Chapter 9

A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty

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Abstract Background The likelihood of restenosis is a major limitation of coronary angioplasty. We studied whether hirudin, a highly selective inhibitor of thrombin with irreversible effects, would prevent restenosis after angioplasty. We compared two dose regimens of recombinant hirudin with heparin.

Methods We randomly assigned 1141 patients with unstable angina who were scheduled for angioplasty to receive one of the following treatments: (1) a bolus dose of 10,000 IU of heparin followed by an intravenous infusion of heparin for 24 hours and subcutaneous placebo twice daily for three days (382 patients), (2) a bolus dose of 40 mg of hirudin followed by an intravenous infusion of hirudin for 24 hours and subcutaneous placebo twice daily for three days (381 patients) or (3) the same hirudin regimen except that 40 mg of hirudin was given subcutaneously instead of placebo twice daily for three days (378 patients). The primary end point was event free survival at seven months. Other endpoints were early cardiac events (within 96 hours), bleeding and other complications of the study treatment, and angiographic measurements of coronary diameter at six months of follow-up.

Results At seven months, event free survival was 67.3 percent in the group receiving heparin, 63.5 percent in the group receiving intravenous hirudin, and 68.0 percent in the group receiving both intravenous and subcutaneous hirudin (P-value 0.61). However, the administration of hirudin was associated with a significant reduction in early cardiac events, which occurred in 11.0, 7.9, and 5.6 percent of patients in the respective groups (combined relative risk with hirudin, 0.61; 95 percent confidence interval 0.41 to 0.90; P=0.023). The mean minimal luminal diameters in the respective groups on follow-up angiography at 6 months was 1.54, 1.47, and 1.56 mm (P=0.08).

Conclusions Although significantly fewer early cardiac events occurred with hirudin than with heparin, hirudin had no apparent benefit with longer-term follow-up. (New England Journal of Medicine 1995;333:757-763)

Platelet aggregation, the generation of thrombin, and the release of growth factors at the site of angioplasty have all been implicated in the process of restenosis [1, 2]. Consequently, anticoagulants, antiplatelet agents and specific antithrombin agents have been considered for the prevention of restenosis [3]. Thrombin is the most potent platelet activator known, stimulating the production of platelet-derived growth factor and the secretion of prostacyclin, platelet-activating factor, and plasminogen-activator inhibitor. Thrombin has apparent mitogenic effects on lymphocytes and vascular smooth-muscle cells [4, 5].

Hirudin, a 65-amino-acid compound originally extracted from the salivary gland of the leech, is a specific inhibitor of thrombin. The advantage of hirudin over other serine protease inhibitors is its potency in irreversibly blocking thrombin at multiple sites without the need for circulating antithrombin III [6]. Because of the small size of the hirudin molecule, this substance can inhibit clot-bound thrombin and restrict the further formation of thrombus [7].

Hirudin has reduced the deposition of platelets after vascular injury in pigs [8]. and lowered the rate of restenosis in hypercholesterolemic rabbits [9], providing a rationale for its use in patients undergoing angioplasty. In this trial we evaluated whether the inhibition of thrombin with hirudin improved event-free survival in patients undergoing coronary angioplasty.

Methods

Study population Patients with unstable angina and one or more clinically important new or restenotic coronary narrowings suitable for treatment with percutaneous transluminal coronary angioplasty were eligible for the study. From September 1992 through May 1993, 1154 patients from various institutions (listed in the Appendix) were randomized. All had unstable angina, as defined by the new onset of angina pectoris or the worsening of angina (i.e., their condition changed by two or more classes according to the classification of the Canadian Cardiovascular Society[10] or they needed additional antianginal medication), angina at rest, or both in the preceeding 3 months[11]. The criteria for exclusion from the study were stable angina, a planned multistage procedure or stent implantation, myocardial infarction occurring within the preceding two weeks, hypertension, diabetic retinopathy and body weight over 100 kg.

The study was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments and with the laws and regulations of the countries in where the trial took place. Before randomization, each patient gave written informed consent.

Antithrombin regimens The patients were randomly assigned in a double blind fashion to receive recombinant hirudin (™REVASC, Ciba-Geigy, Basel, Switzerland [12]) in one of two dose regimens or to receive unfractionated sodium heparin. The randomization was stratified according to whether heparin had been administered in the preceding 24 hours. Heparin had to be discontinued at least 30 minutes before the start of treatment with the study medication.

The patients received one of the following three treatments: heparin (an intravenous bolus injection of 10,000 IU of heparin followed by a continuous intravenous infusion of 15 IU of heparin per kilogram of bodyweight per hour for 24 hours, with placebo given subcutaneously twice daily for three consecutive days, intravenous hirudin (an intravenous bolus injection of 40 mg of hirudin followed by a continuous intravenous infusion of 0.2 mg of hirudin per hour for 24 hours, with placebo given subcutaneously twice daily for three consecutive days), or intravenous and subcutaneous hirudin (an intravenous bolus injection of 40 mg of hirudin followed by a continuous intravenous infusion of 0.2 mg of hirudin per hour for 24 hours, with 40 mg of hirudin given subcutaneously twice daily for three consecutive days. If the angioplasty lasted more than one hour, an additional bolus dose of 5,000 IU of heparin could be administered at the option of the physician to the patients in the heparin group, or an equivalent amount of placebo could be given to the patients in the hirudin groups. The operators remained blinded to the results of clotting studies, and no adjustment of the rate of infusion of the study medication was allowed. A concomitant dose of aspirin (100 to 500 mg once daily) was given on the day of angioplasty, and this treatment was continued for at least 14 days.

Criteria for Evaluation *Efficacy* The primary endpoint was event-free survival 30 weeks after angioplasty -that is, the absence of death, non-fatal myocardial infarction, coronary-artery bypass grafting or the use of a 'bailout' procedure (e.g., stenting), or second angioplasty at previously dilated sites. Myocardial infarction was diagnosed on the basis of new Q-waves [according to the Minnesota Code13] or an increase in the serum creatinine kinase level to more than twice the upper limit of the normal range, with a concomitant increase in the MB fraction. If a stent was implanted electively after the initial angioplasty (i.e. not as a part of a bail out procedure), the implantation was considered equivalent to a second angioplasty. Second angioplasty or bypass surgery needed to be preceded by typical anginal symptoms or, if there were atypical anginal symptoms, by electrocardiographic evidence of myocardial ischemia at rest or during exercise and an angiographically determined stenosis greater than 50 percent by visual inspection.

Secondary endpoints were as follows: any of a ranked series of clinical events that incuded death from cardiac causes, non-fatal myocardial infarction, coronary-artery bypass grafting (or the use of a bailout procedure), second angioplasty, and anginal status [according to classification system of the Canadian Cardiovascular Society10] at the 30-week follow-up evaluation; the occurrence of any of these events within 96 hours after the start of the study medication; the minimal luminal diameter of the dilated sites as measured by quantitative coronary angiography at the 26-week follow-up evaluation; and any change in the minimal luminal diameter of dilated sites from immediately after angioplasty to follow-up angiography at 26 weeks.

Safety Safety was evaluated with regard to bleeding and other complications. Bleeding was classified as major if it was overt and led to a decrease in the hemoglobin level by at least 2 g per decilitre; if it necessitated the transfusion of two or more units of whole blood or packed cells; or if it occurred intracranially, retroperitoneally, or at the site of a major joint [14]. Minor bleeding was defined as overt bleeding that did not meet these criteria.

Angiography and assessment of coagulation For each patient, coronary angiograms were obtained in a standardized fashion immediately before and immediately after angioplasty, and at the six-months follow-up evaluation. The angiograms were analyzed in a core laboratory with the Cardiovascular Angiography Analysis System [15,16].

Blood samples for the measurement of coagulation were obtained at regular intervals before and after angioplasty. Blood samples for the determination of activated partial-thromboplastin times and levels of prothrombin fragment one and two (a measure of the generation of thrombin) were obtained separately by atraumatic venipuncture and were analyzed in a central laboratory.

Statistical analysis Outcomes were compared in an intention-to-treat analysis, which included all randomized patients in whom coronary angioplasty was attempted. Patients in whom no angioplasty was attempted (i.e. those whose indication for angioplasty changed or disappeared) were excluded from the analysis. A successful procedure was defined as one in which the stenosis was reduced by more than half; in the case of a failed recanalization of a total occlusion, the second lesion treated was considered to be the first site of angioplasty.

The distribution of event-free survival at 30 weeks was calculated according to the method of Kaplan and Meier, and distributions were compared by the Kruskal-Wallis test [17], for patients with multiple events, the first event was considered. Event rates and rates of bleeding and other complications were compared by the Chi-square test.

A linear logistic-regression analysis for ordered categories was performed for the ranked clinical outcomes, with pretreatment with heparin used as a covariate. The most severe event in each patient was considered in the analysis.

The minimal luminal diameters at the dilated sites 26 weeks after the angioplasty follow-up (the minimal luminal diameter was taken as the mean were compared by analysis of variance, with the mean value for all sites used in cases of angioplasty at multiple

Table I
Baseline Characteristics of the Study Patients According to Group Assignment (N and %).

	Heparin (N = 382)		Intravenous Hirudin (N = 381)		Intravenous and Subcutaneous Hirudin (N = 378)	
Male sex	299	78.3	300	78.7	297	78.6
Age (yr)	58.2±8.7		58.7±9.1		58.8±8.9	
Weight (kg)	76.0±10.9		75.9±11.1		76.7±10.7	
Smoker	104	27.2	90	23.6	84	22.2
Diabetes	44	11.5	42	11.0	40	10.6
Previous MI	148	38.7	152	39.9	154	40.7
Previous CABG	10	2.6	8	2.1	20	5.6
Previous angioplasty	69	18.1	69	18.1	64	16.9
Braunwald class						
I	131	34.3	146	38.3	139	36.8
II	163	42.7	160	42.0	166	43.9
III	88	23.0	75	19.7	73	19.3
Exertional angina						
CCS class 1	7	1.8	10	2.6	6	1.6
CCS class 2	82	21.5	69	18.1	66	17.5
CCS class 3	144	37.7	163	42.8	171	45.2
CCS class 4	102	26.7	96	25.2	89	23.5
IV heparin used at screening	115	30.1	110	28.9	109	28.8
Lesions						
Total no.	482		483		462	
Mean no. per patient	1.26		1.27		1.22	
Location before angioplasty						
RCA	148	30.7	136	28.2	139	30.1
LAD	230	47.7	238	49.2	201	54.5
LCX	104	21.6	109	22.6	121	26.2
LM	0		0		1	0.2

Plus minus values are means ±SD. Except as noted, all other values are numbers of patients followed in parentheses by the percentage of the group.
MI denotes myocardial infarction, CABG denotes coronary-artery bypass graft surgery, CCS Canadian Cardiovascular Society, IV intravenous, RCA right coronary artery, LAD left anterior descending coronary artery, LCX left circumflex coronary artery, and LM left main coronary artery.

sites. All reported P values are two-tailed. Whenever possible, estimates of the magnitude of the treatment effect are provided, with corresponding 95 percent confidence intervals. Relative risks are presented for the combined hirudin groups as compared with the heparin group.

Results

Study population A total of 1154 patients were randomized. Of 5686 patients screened, 21 percent were ineligible because they had stable angina, 16 percent for reasons involving logistics, and 11 percent because they had had myocardial infarctions during the previous two weeks. The remaining screened patients (32 percent) were excluded for a wide variety of reasons. Thirteen patients were not included in the intention to treat analysis because no angioplasty was attempted. Among the remaining 1141 patients in whom angioplasty was attempted, 382 were randomly assigned to heparin, 381 to intravenous hirudin, and 378 to intravenous and subcutaneous hirudin. Angioplasty was successful in 91.7 percent, and the results of angiographic follow-up were available for 86.4 percent. Clinical follow-up was complete for all but one patient.

The clinical and angiographic characteristics of the patients at base line are shown in table I. The characteristics of the three groups were similar. Almost one third of the patients received intravenous heparin before randomization because of the severity of their unstable angina.

Efficacy Among the study patients, 125 patients assigned to heparin, 139 assigned to intravenous hirudin, and 121 assigned to intravenous and subcutaneous hirudin reached a primary endpoint. The distribution of patients free of events is shown in figure 1. No significant differences were observed among the treatment groups (P=0.61 by the Kruskal-Wallis test), even after stratification according to pretreatment with heparin.

The incidence of clinical events and angina at 30 weeks is shown in table II, with no significant differences among the three groups (P=0.61). An analysis of subgroups according to whether patients were pretreated with heparin yielded similar results.

The incidence of early events (those occurring in the first 96 hours after angioplasty) is also shown in table II. Forty-two patients assigned to heparin, 30 patients assigned to intravenous hirudin, and 21 patients assigned to intravenous and subcutaneous hirudin had such events (relative risk in the combined hirudin groups, 0.61; 95 percent confidence interval, 0.41 to 0.90; P=0.023). Among the patients pretreated with heparin, there were 20, 7, and 7 events, respectively (combined relative risk with hirudin, 0.37; 95 percent confidence interval, 0.19 to 0.70; P=0.007). Because these results suggested a particular benefit of hirudin in the most unstable patients (those with Braunwald class III angina), an additional analysis was performed of the 236 patients who had angina at rest during the 48 hours before randomization. The event rate among these patients was 21.6 percent in the heparin group, as compared with 5.3 percent among patients receiving intravenous hirudin and 12.3 percent among patients receiving intravenous and subcutaneous hirudin (combined relative risk with hirudin 0.41; 95 percent confidence interval, 0.21 to 0.78; P=0.006).

Figure 1
Kaplan-Meier Distribution of Patients without Events in the Intention to Treat Analysis (N=1141). The groups were compared by the Kruskal-Wallis test.

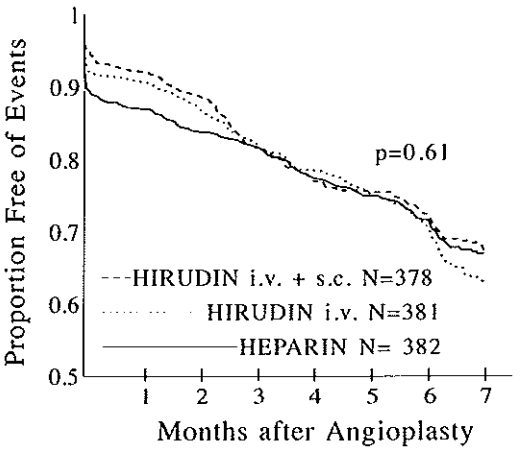
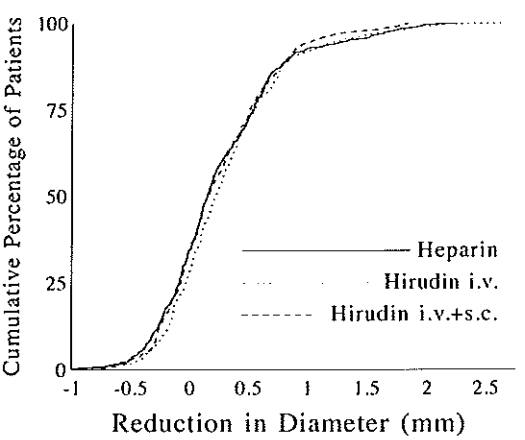


Figure 2
Cumulative Distribution of the Reduction in Minimal Luminal Diameter from Immediately after Angioplasty to Follow-up at seven months.



The imbalance in the number of deaths (table II) calls for a description of their exact causes. In the heparin group, three myocardial infarctions and one non-hemorrhagic cerebrovascular accident resulted in death. In the group receiving intravenous hirudin, there was one sudden death. In the group receiving intravenous and subcutaneous hirudin, five patients had fatal myocardial infarctions. In this group there were also two cerebrovascular accidents (one of which haemorrhagic), one episode of cardiac tamponade, and one sudden death; one patient died of respiratory insufficiency, and one of wound infection and sepsis after bypass surgery.

Linear logistic regression analysis for ordered categorical data revealed that pretreatment with heparin was significantly associated with worse clinical outcomes at 7 months (P=0.03). The type of study medication did not influence outcome in this model.

Base-line angiographic measurements and gains in luminal diameter achieved by angioplasty were similar in the three groups (table III). The changes in minimal luminal diameter from immediately after angioplasty to follow-up were also similar (figure 2).

Safety The incidence of bleeding complications is shown in table IV. No differences with respect to major or minor bleeding were observed among groups. There were three cerebro vascular accidents. One patient receiving intravenous subcutaneous hirudin was readmitted to the hospital with hemiplegia 14 hours after the final subcutaneous injection; despite surgical evacuation of the intracerebral hematoma causing the condition, the patient died six days after the start of the study treatment. Two intra-cerebral thrombotic events were observed. One patient (receiving intravenous and subcutaneous hirudin), who presented with symptoms of neurologic deficit one day after discharge from the hospital and who had multiple brain infarctions on computed axial tomography, died five days after the start of the study treatment. Another patient (in the heparin group) presented with massive pulmonary embolism. Paradoxical embolization through a patent foramen ovale caused an extensive, expanding cerebral infarction and led to the patient's death eight days after the start of the study medication.

Anticoagulant effects Levels of prothrombin fragment one and two are shown in figure 3. The median levels peaked in both hirudin groups at the end of the procedure (from 1.1 nmol per liter at the time of screening to 1.4 nmol per liter in the group receiving intravenous hirudin, and from 1.0 to 1.3 nmol per liter in the group receiving intravenous and subcutaneous hirudin, whereas in the heparin group the levels were slightly reduced (to 0.9 nmol per liter) as compared with those at the time of screening (1.0 nmol per liter). Levels of prothrombin fragment one and two measured at 24, 96, 98 hours subsequently returned to the base-line values in all three groups.

Measurements of activated partial-thromboplastin time (figure 3) were higher at the end of the procedure in the subjects receiving heparin than in those receiving hirudin, an effect that disappeared after 24 hours. The infusion of hirudin resulted in a more stable effect. Slightly prolonged activated partial-

Table II
Clinical Events in the First 96 Hours and the First 30 Weeks in the Intention-to-Treat Analysis.*

Event	Heparin (N = 382)		Intravenous Hirudin (N = 381)		Intravenous and Subcutaneous Hirudin (N = 378)	
	96 hr	30 wk	96 hr	30 wk	96 hr	30 wk
Death	2(0.5)	4(1.0)	0(-)	1(0.3)	0(-)	11(2.9)
Myocardial Infarction	16(4.2)	20(5.2)	13(3.4)	19(5.0)	9(2.4)	23(6.1)
Coronary bypass surgery	9(2.4)	21(5.5)	6(1.6)	21(5.5)	3(0.8)	25(6.6)
Bail-out procedure	18(4.7)	18(4.7)	12(3.1)	12(3.1)	8(2.1)	8(2.1)
Second angioplasty	13(3.4)	91(23.8)	7(1.8)	109(28.6)	5(1.3)	93(24.6)
Any event	42(11.0)	125(32.7)	30(7.9)	139(36.5)	21(5.6)	121(32.0)
Any exertional angina	-	55(14.4)	-	71(18.6)	-	71(18.8)
No event or symptoms	-	202(52.9)	-	171(44.9)	-	186(49.2)

* At 96 hours, the combined relative risk in the hirudin groups as compared with the heparin group was 0.61 (95 percent confidence interval, 0.41 to 0.90; P=0.023).

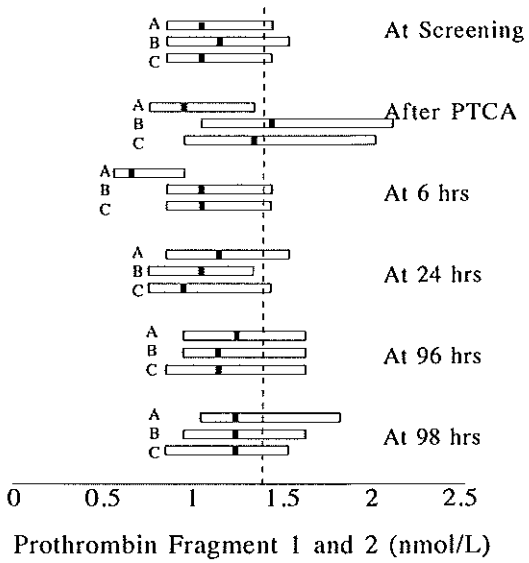
Table III
Mean (±SD) Angiographic Measurements in the Intention-to-Treat Analysis of Patients for Whom Follow-up Data Were Available.

Variable	Heparin (N = 330)	Intravenous Hirudin (N = 341)	Intravenous and Subcutaneous Hirudin (N = 315)
Reference luminal diameter *	2.69 ±0.51	2.67 ±0.51	2.70 ±0.51
Minimal luminal diameter			
Before angioplasty	0.99 ±0.38	0.97 ±0.39	1.03 ±0.35
After angioplasty	1.80 ±0.37	1.78 ±0.36	1.82 ±0.37
At follow-up	1.54 ±0.59	1.47 ±0.56	1.56 ±0.50
Gain	0.81 ±0.41	0.82 ±0.44	0.79 ±0.41
Loss	0.26 ±0.52	0.32 ±0.50	0.26 ±0.45

* As estimated by computer techniques on the basis of the diameters of segments proximal and distal to the site of the stenosis

Table IV
Bleeding Complications

Complications	Heparin (N = 382)		Intravenous Hirudin (N = 381)		Intravenous and Subcutaneous Hirudin (N = 378)	
	number (percent) of patients					
Major bleeding						
Overt, with decrease in Hb by ≥ 2 g/dl	24	6.2	18	4.7	28	7.4
Overt, requiring transfusion of ≥ 2 units whole blood or packed cells	0	-	3	0.8	0	-
Intracranial	0	-	0	-	1	0.3
Retroperitoneal or in a major joint	0	-	0	-	0	-
All	24	6.2	21	5.5	29	7.7
Minor bleeding	43	11.3	50	13.1	57	15.0



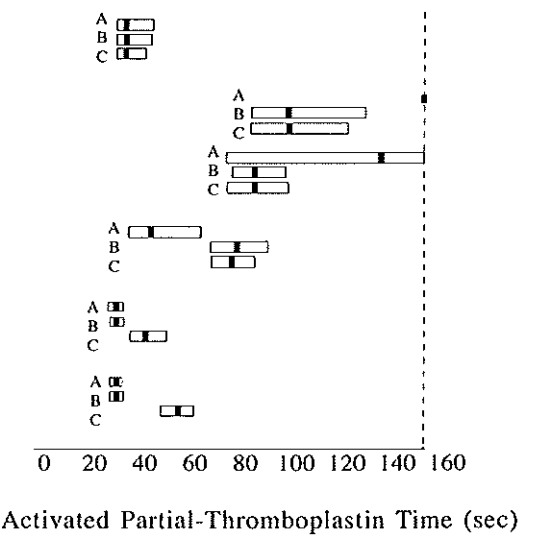


Figure 3

Levels of Prothrombin Fragment 1 and 2 and Activated Partial-Thromboplastin Times in the Three Study Groups at Various Times before and after Angioplasty.

A *denotes the group receiving heparin,*
B *the group receiving intravenous hirudin, and*
C *the group receiving intravenous and subcutaneous hirudin. The solid area inside each box indicates the median value, and the left and right margins of the box indicate the upper limits of the first and third quartiles, respectively.*
In the upper panel, the dotted vertical line indicates the upper limit of the normal level of prothrombin fragment 1 and 2 (1.4 nmol per liter). Heparin tended to control the generation of thrombin better than hirudin both immediately after angioplasty and six hours after the start of infusion.
In the lower panel, the activated partial-thromboplastin time was measured up to a maximum of 150 seconds. Over the first 24 hours this value was more than double the base-line value in the hirudin-treated groups, whereas in the heparin-treated group it returned almost to the base-line level.

thromboplastin time were observed at 96 hours after angioplasty in the group receiving intravenous and subcutaneous hirudin.

Discussion

Although hirudin was associated with impressive reductions in the rate of major cardiac events in the first 96 hours after angioplasty as compared with heparin, the primary goal of this trial, a reduction in the rate of cardiac events at seven months, was not accomplished. Event-free survival at seven months did not differ among the treatment groups.

At least three other trials using specific antiplatelet drugs have demonstrated beneficial effects on the acute complications of coronary angioplasty without favorably influencing long-term clinical outcomes [18-20]. These findings differ from the results of the Evaluation of c7E3 for the Prevention of Ischemic Complications (EPIC) trial [21, 22], in which the glycoprotein IIb/IIIa receptor was presumed to have been blocked completely and which showed a reduction in early cardiac events that was maintained with longer-term follow-up.

The dosage and duration of treatment in the present trial were chosen as a compromise among safety issues, logistic considerations and the scientific evidence available when the trial was designed. Primarily, the dosage was based on safety data obtained in healthy volunteers, stable patients undergoing angioplasty, and patients undergoing orthopedic surgery [23-25]. However, the results of assays of prothrombin fragment one and two immediately after angioplasty suggest that the generation of thrombin was not satisfactorily inhibited in either hirudin group whereas the dosage of heparin we used resulted in an appropriate decrease in levels of prothrombin fragment one and two at six hours. It can be inferred from these data that the adjustment in the infusion rate -from 0.16 mg per kilogram per hour in the pilot study of patients with stable angina [25] to 0.20 mg per kilogram per hour in the current trial of patients with unstable angina and presumably higher levels of thrombin generation- was too cautious a change in dosage. Zoldhelyi et al. [26] recently reported failing to block the generation of thrombin in their patients despite the presence of a 10,000-fold molar excess of free hirudin over the amount bound in complexes with thrombin. Infusion rates of hirudin in experiments with animals were as much as five times higher than those currently used, a finding that may explain the lack of a long-term effect in the present study [8,9].

When hirudin was administered subcutaneously in healthy volunteers at a dose of 0.5 mg per kilogram twice daily, the activated partial-thromboplastin time 12 hours after the first injection was subtherapeutic [23], and it might be inferred that the inhibition of the conversion of pro-thrombin was also inadequate in the first three days of the trial. A putative explanation for the apparent paradox by which the early outcome is improved although there is less appropriate control of thrombin may be that the dosage used was not sufficient to produce an adequate level of anticoagulation, but was sufficient to limit the thrombin-mediated aggregation and activation of platelets, causing effects similar to those observed over the short term in the EPIC trial [8,21,22].

The optimal duration of treatment is unknown, even in animal models.

Conflicting findings about the time course of thrombogenecity in the injured vessel wall have been reported [27-30]. In this study we decided to maintain our patients at effective levels of antithrombin activity as long as possible. Since ethical considerations necessitated monitoring the patient's safety in the hospital during the subcutaneous injections of hirudin, a reasonable compromise between the duration of hirudin administration and logistic considerations of the trial was presumably achieved by administering the drug intravenously for 24 hours and subcutaneously for three consecutive days.

A clear beneficial effect of hirudin on platelet aggregation and thrombus formation was indicated by the prevention of acute ischemic events early after angioplasty. The failure of hirudin in this trial to alter the longer-term outcomes indicates either that thrombin generation and thrombus formation in the period immediately after angioplasty may be less important in the process of restenosis than previously believed or that complete reversal of the thrombogenecity of the injured vessel wall was not achieved or requires moretime. Whether the large decrease in major events observed with hirudin early after the infusion can be translated to improved long term outcome with prolonged subcutaneous administration of hirudin deserves further study.

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Appendix

The following institutions and investigators participated in the HELVETICA (Hirudin in a European Trial versus Heparin in the Prevention of Restenosis after PTCA) trial. The number of patients enrolled at each centre is given in parentheses, followed by an asterisk when all patients in the cardiac catheterization laboratory at a center were screened and the results entered in a logbook.

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Chapter 10

Clinical outcome in patients undergoing coronary angioplasty for unstable angina pectoris

Predictors of adverse in-hospital and 30-weeks clinical outcome following percutaneous transluminal coronary angioplasty in 1141 patients receiving either desirudin or heparin therapy: A multivariable analysis

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Abstract Objectives Restenosis and related clinical events following percutaneous transluminal coronary angioplasty (PTCA) hamper the early and long term result of this therapeutic technique. It is believed to be a multi factorial response to vessel wall injury, induced by the mechanical enlargement of the lumen, that might be modified by the administration of antithrombotic drugs. To identify predictors of early and late occurring major adverse cardiac events following coronary angioplasty, we analyzed qualitative and quantitative clinical, biological and angiographic data obtained in 1141 patients in a multivariable fashion. Knowledge of these predictors, could improve selection of patients at risk for clinical events and luminal renarrowing on an objective and scientific basis.
Methods Clinical and angiographic follow-up were obtained in an international, multicenter, double blind, prospective and heparin controlled restenosis prevention study, investigating the safety and efficacy of desirudin in patients undergoing percutaneous transluminal coronary angioplasty for unstable angina pectoris. In this trial 1141 patients were randomized and treated for 1426 lesions. Patients had an angiographic follow-up at 6 months, and a clinical follow-up during 7 months. Over 200 qualitative and quantitative variables were evaluated in a multivariable model, with major adverse cardiac events occurring within 4 days or 7 months as dependent variable.
Results Clinical and angiographic follow-up data were obtained in 100% and 86.4% respectively. Adverse independent risk factors of major adverse cardiac events occurring within the first four days following angioplasty were the presence of Braunwald class III, age ≥60 years, dissection and thrombus post-PTCA. Presence of lesion type A, use of long acting nitrates at the time of randomization and randomization to hirudin treatment were independent protective factors. Predictability of this model is 0.80. Adverse independent predictors of major adverse cardiac events occurring within 7 months following angioplasty were the presence of Braunwald class II and III, age ≥60 years and a high level of prothrombin fragment 1 and 2 post-PTCA. Lesion length <10 mm, history of myocardial infarction and minimal lumen diameter post-PTCA were independent protective factors. Predictability of this model is 0.65.
Conclusions Using the multivariable approach, predictability of the occurrence of major adverse cardiac events within 4 days following PTCA is substantial, dependent from hirudin treatment and is mainly dependent

from pre-procedural variables. Predictability of the occurrence of major adverse cardiac events 7 months following PTCA is modest. (Submitted to the European Heart Journal)

Since Andreas Grüntzig performed the first coronary balloon dilatation on September the 16th 1977 [1] as an alternative to coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA) has emerged as a widely accepted safe and effective treatment modality in selected patients with obstructive coronary artery disease. Despite expanded indication, including patients with unstable angina, multivessel disease, or totally occluded coronary arteries, the initial successrate of this technique is high, and the complication rate low [2, 3]. However, acute vessel closure and late restenosis are inherent to PTCA and continue to compromise its efficacy.

An abundance of clinical and angiographic studies have been carried out over the last two decades, in an attempt to identify predictors for angioplasty success and the occurrence of major adverse cardiac events (MACE) during and after PTCA. Although the exact pathophysiology and factors responsible for restenosis are largely unknown, some clinical and angiographic variables have been identified as potential risk factors [4-22]. Due to differences in patient selection, method of analysis and definitions of endpoints, the study results are sometimes conflicting [23, 24]. Knowledge of specific risk factors for cardiac events could be useful in selection of therapy tailored to the individual patient or, to the very extreme might indicate PTCA as a contraindicated therapy because of multiple cumulative risk factors. Besides, selection of patients at risk for luminal renarrowing, enables constitution of a riskful target population for pharmacological and device studies aiming at the prevention of restenosis.

This study was performed to analyze the relationship between clinical, biological, procedural and angiographic characteristics to the in-hospital (4 days) or late (7 months) development of cardiac death, non-fatal myocardial infarction, CABG, bail-out stenting or repeat-PTCA in 1141 angioplasty patients suffering exclusively unstable angina pectoris.

Methods

Patient identification The study cohort was constituted of 1141 patients in whom PTCA was attempted in the period between September 1992 and May 1993. These patients were prospectively enroled in a European restenosis prevention study (HELVETICA: Hirudin in an European restenosis prevention triaL Versus hEparin Treatment In ptCA patients) comparing a two dose regimen of recombinant hirudin (CGP 39 393/™REVASC) to heparin treatment [25]. All patients were treated for unstable angina pectoris, due to either de novo or restenotic lesions in native vessels, without having had a myocardial infarction in the two weeks prior to angioplasty therapy. Selection of angioplasty device (balloon, rotational or directional atherectomy, excimer laser) was left to the discretion of the operator. Elective stent implantation or multistage angioplasty was an exclusion criterium for the study.

Patients in the HELVETICA study received one of the following treatments: unfractionated heparin (an i.v. bolus injection of 10,000 IU of heparin followed by a continuous i.v. infusion of 15 IU/kg/hr of heparin for 24 hours, with placebo given s.c. b.i.d. for three consecutive days); intravenous hirudin (an i.v. bolus injection of 40 mg of

hirudin followed by a continuous intravenous infusion of 0.2 mg/kg/hr hirudin for 24 hours, with placebo given s.c. b.i.d. for three consecutive days) or intravenous and subcutaneous hirudin (an i.v. bolus injection of 40 mg of hirudin followed by a continuous i.v. infusion of 0.2 mg/kg/hr of hirudin for 24 hours, with 40 mg of hirudin given s.c. b.i.d. for three consecutive days). A concomitant dose of aspirin (100-500 mg once daily) was given on the day of angioplasty and continued for at least 14 days.

Hirudin treatment was associated with significant reductions in the rate of MACE in the first 4 days after angioplasty as compared with heparin (relative risk of desirudin versus heparin 0.61, 95% CI 0.41-0.90, P= 0.023) [25]. At seven months however, a reduction in the rate of cardiac events by hirudin, was not accomplished. The distribution of MACE is outlined in table I and the distribution of clinical baseline characteristics are shown in table II.

Angiography and coagulation assessment For each patient coronary angiograms were obtained for quantitative and qualitative evaluation, in a standardized fashion immediately before and after angioplasty, and at six-month follow-up. A successful angioplasty was defined as an angioplasty which reduces the diameter of the stenosis at the first lesion to less than 50% on visual inspection immediately after the angioplasty. All 35 mm cinefilms were analyzed at a core laboratory (Cardialysis B.V. Rotterdam, The Netherlands) using the Coronary Angiography Analysis System (CAAS) [26, 27]. The distribution of baseline lesion characteristics is summarized in table III.

Blood samples for the determination of coagulation parameters, including levels of activated partial thrombin times and prothrombin fragment 1 and 2, were obtained at regular intervals before and after angioplasty by atraumatic venipunctures and were analyzed in a central laboratory.

Clinical endpoint Clinical endpoints were recorded during 7 months following angioplasty and included death, non-fatal myocardial infarction, coronary artery bypass graft, bail out stenting and repeat angioplasty at previously dilated sites. Death was considered cardiac unless proof of the contrary. Myocardial infarction was diagnosed either on the basis of new Q-waves (Minnesota Code [28]) or on creatinine kinase increase of more than twice the normal upper limit with concomitant increase in myocardial bound fraction. A bail out stent procedure was considered equivalent to coronary artery bypass graft if patency of the target vessel could not be maintained otherwise. Repeat angioplasty was defined as re-insertion of a guiding catheter followed by balloon angioplasty or stent implantation at the same site. Repeat angioplasty or bypass surgery needed to be preceded by typical anginal symptoms or, in case of atypical anginal symptoms, electrocardiographic evidence of myocardial ischaemia at rest or during exercise and an angiographic diameter stenosis of >50% by visual inspection.

Statistical analysis Categorical variables are presented as absolute numbers and as percentages of the entire patient population. Differences in these variables between subgroups of patients are evaluated by Chi^2-tests. Continuous variables are expressed as mean ± 1 Standard Deviation (SD); differences are studied by unpaired Student's t-tests. Data were evaluated by per patient analysis. If multiple lesions were treated per patient, lesion-specific (angiographic) data were converted as follows: observations of continuous variables were averaged; all possible outcomes of categorical variables were defined as new variables with a yes/no outcome and counted subsequently.

Univariable logistic regression analysis was performed to determine clinical, procedural as well as angiographic variables predictive for Major Adverse Cardiac Events (MACE; death, myocardial infarction, coronary bypass surgery and repeat PTCA) at short (4 days) and long (7 months) term follow-up. The relevance of several variables was evaluated by Chi^2-analysis. Odds ratio's (OR) and their 95% Confidence Intervals (CI) were reported.

Multivariable logistic regression analysis was performed to evaluate the indepen-

dence of univariable significant predictors. A multivariate regression model was constructed by stepwise procedures (forward addition and backward elimination). Receiver Operating Characteristic (ROC) analysis was performed to describe the predictive power of the model [29]. The area under the ROC-curve is reported.

Statistical significance of all tests was stated at the 0.05 probability level. The SAS software package was used for statistical calculations [30].

Results

Clinical follow-up was complete for all but one patient. Angioplasty was successful in 91.7%, and the results of angiographic follow-up were available for 86.4%. In total 121 early MACE were encountered in 93 patients (1.30 event per patient). In total 476 late MACE were encountered in 385 patients (1.24 event per patient). Table I demonstrates the distribution of types of events occurring within 4 days or 7 months.

Baseline clinical and angiographic variables are listed in tables II and III, respectively. There was a similar distribution of characteristics over the three antithrombin regimens [25]. Patients were mainly male and non-smoker (78.5 and 75.5% respectively). The mean (median) age was 58.6± (58.8±) years. The Braunwald unstable angina classification was class I in 36.5% of the patients, class II in 42.8%, and class III in 20.6%. At screening 29.3% of the patients received heparin for their unstable syndrome (table II). Seventy eight of the 1141 patients had a total occlusion (three patients had two total occlusions, table III).

The univariate relation of clinical and angiographic continuous and categorical variables to MACE within 4 days and 7 months following PTCA is described in table II-V and table VI-IX respectively.

Multivariable analysis of clinical, procedural and angiographic related variables revealed that Braunwald III, age ≥60 years, thrombus and dissection post-PTCA were independent risk factors for adverse in-hospital clinical outcome, and desirudin treatment, use of long acting nitrates at the time of randomization and lesion type A were independent protective factors (table X). The area under the ROC-curve is 80%.

Concerning 7-months follow-up, Braunwald class II and III, age ≥60 years and higher levels of prothrombin fragment 1 and 2 appeared to be independent risk factors for adverse clinical outcome. A history of myocardial infarction, lesion length <10 mm and a larger minimal lumen diameter post-PTCA were independent protective factors. The ROC area of this 7 months model is 65%. Outcomes of the multivariate analyses are summarized in table X and XI.

Discussion

Clinical risk factors Prognosis and therapy of patients with unstable angina present a wide heterogenecity. To enable critical evaluation of diagnostic and therapeutic strategies, and to facilitate clinical decision making and intercollegial discussion [31], Braunwald defined clinically relevant and practically applicable subgroups of patients with unstable angina pectoris [32]. This classification including all subgroup categories (severity of disease, clinical circumstances, ECG changes and intensity of treatment) was prospectively investigated in 417 consecutive (non-angioplasty) patients who were admitted for ischemic chest

Table I
Major Adverse Clinical Events (N(%)) occurring within 4 days and 7 months.

N = 1141	MACE within 4 days		MACE within 7 months	
	N	%	N	%
Death	2	0.2	16	1.4
Myocardial Infarction	38	3.3	62	5.4
CABG	18	1.6	67	5.9
Bail-out	38	3.3	38	3.3
Re-PTCA	25	2.2	293	25.7
Patient with any event	93	8.2	385	33.7

MACE = Major Adverse Clinical Events,
CABG= Coronary artery bypass graft surgery,
Re-PTCA = repeat percutaneous transluminal coronary angioplasty,
N = number of patients.

Table II
Relationship of categorical patient characteristics to Major Adverse Clinical Events occurring within 4 days following PTCA.

Characteristic	N	% MACE	Odds ratio	P univariate
Treatment group				
Hirudin	759	6.7	0.58 (0.38-0.89)	0.013
Heparin	382	11.0	1.00	
Anginal class				
Braunwald I	416	5.8	1.00	
Braunwald II	489	7.6	1.34 (0.79-2.27)	0.284
Braunwald III	236	13.6	2.56 (1.47-4.47)	0.001
CCS class 1	23	8.7	1.43 (0.32-6.51)	0.641
CCS class 2	217	5.5	1.00	
CCS class 3	478	6.5	1.04 (0.59-1.84)	0.883
CCS class 4	287	13.2	2.30 (1.32-3.98)	0.003
Current smoker				
No	862	9.1	1.00	
Yes	278	5.4	0.57 (0.32-1.01)	0.05
Gender				
Male	896	8.2	1.00	
Female	245	8.2	1.00 (0.60-1.68)	0.99
Age < 60 years	625	5.9	1.00	
Age ≥ 60 years	516	10.9	1.94 (1.26-2.98)	0.003
Number of lesions				
One	899	8.6	1.00	
Two	198	6.1	0.69 (0.37-1.29)	0.245
Three	44	9.1	1.07 (0.37-3.06)	0.903
Medical history				
Previous CABG				
No	1103	8.1	1.00	
Yes	38	10.5	1.34 (0.47-3.86)	0.59
Previous MI				
No	687	8.9	1.00	
Yes	454	7.1	0.78 (0.50-1.22)	0.27
Previous PTCA				
No	940	8.3	1.00	
Yes	201	7.5	0.89 (0.50-1.58)	0.70

Table II continued
Relationship of categorical patient characteristics to Major Adverse Clinical Events occurring within 4 days following PTCA.

Characteristic	N	% MACE	Odds ratio	P univariate
NIDDM				
No	1055	8.0	1.00	
Yes	86	10.5	1.35 (0.65-2.79)	0.42
IDDM				
No	1101	8.3	1.00	
Yes	40	5.0	0.58 (0.14-2.46)	0.46
Hypercholesterolemia				
No	706	8.8	1.00	
Yes	435	7.1	0.80 (0.51-1.25)	0.32
Hypertension				
No	661	8.0	1.00	
Yes	480	8.3	1.04 (0.68-1.60)	0.85
History of stroke				
No	1129	8.2	1.00	
Yes	12	8.3	1.03 (0.13-8.03)	0.98
History of TIA				
No	1124	8.0	1.00	
Yes	17	17.7	2.46 (0.70-8.73)	0.15
Medication at screening				
Heparin i.v. at screening				
No	807	7.3	1.00	
Yes	334	10.2	1.44 (0.92-2.24)	0.11
Long acting nitrates				
No	512	10.2	1.00	
Yes	629	6.5	0.62 (0.40-0.95)	0.03
Short acting nitrates				
No	676	6.8	1.00	
Yes	465	10.1	1.54 (1.01-2.36)	0.05
Cholesterol lowering drugs				
No	982	8.7	1.00	
Yes	159	5.0	0.56 (0.27-1.18)	0.12

Table III
Relationship of categorical morphologic variables to Major Adverse Clinical Events occurring within 4 days following PTCA.

Pre-procedural characteristics	N patients	N lesions	% MACE	Odds ratio (95% CI)	P univariate
Vessel dilated					
LAD	587	699	8.0	0.96 (0.63-1.47)	0.855
LCX	288	334	5.9	0.64 (0.37-1.11)	0.109
RCA	363	423	9.1	1.20 (0.77-1.87)	0.428
Concentric	432	485	4.4	1.00	
Eccentric	601	674	10.1	2.06 (1.25-3.39)	0.004
Multiple irregularities, tandem lesion or total occlusion	239	267	9.9	1.93 (1.11-3.36)	0.020
Patent vessel	1063	345	8.1	1.00	
Total occlusion	781	81	9.0	1.12 (0.50-2.51)	0.074
Non-ostial	1071	1356	8.3	1.00	
Ostial location	70	70	5.7	0.67 (0.24-1.88)	0.444
Branchpoint absent	846	1100	7.7	1.00	
Branchpoint in stenosis	295	326	9.5	1.26 (0.79-2.00)	0.335
Non-angulated lesion	1036	1318	7.4	1.00	
Location in bend	105	108	15.2	2.24 (1.25-4.00)	0.007
Thrombus absent	1077	1360	7.7	1.00	
Thrombus pre-PTCA	64	66	15.6	2.22 (1.09-4.51)	0.028
Non-calcified lesion	962	1216	8.0	1.00	
Calcification	179	210	8.9	1.13 (0.64-1.98)	0.678
Lesion length (visual)					
< 10 mm	802	956	6.9	1.00	
10-20 mm	291	312	10.0	1.44 (0.90-2.32)	0.131
>20 mm	69	73	13.0	1.90 (0.93-4.16)	0.079
ABC lesion type					
type A	213	234	2.8	1.00	
type B1	518	584	6.4	2.11 (0.80-5.57)	0.131
type B2	430	483	11.4	4.03 (1.57-10.3)	0.004
type C	114	123	11.4	4.07 (1.41-11.8)	0.010
Type A	213	234	2.8	1.00	
Type B1/B2/C	977	1190	8.9	3.13 (1.25-7.70)	0.015

Table III continued
Relationship of categorical morphologic variables to Major Adverse Clinical Events occurring within 4 days following PTCA.

Post-procedural characteristics	N patients	N lesions	% MACE	Odds ratio (95% CI)	P univariate
Dissection absent	639	857	2.9	1.00	
Dissection type A	172	181	8.7	1.39 0.75-2.55	0.290
Dissection type B	221	236	7.2	1.10 0.61-1.98	0.760
Dissection C,D,E or F	128	130	32.0	3.62 1.79-7.30	0.000
Dissection absent	639	857	2.9	1.00	
Dissection present	502	547	14.1	5.54 (3.25-9.42)	0.001
No tortuosity	1031	268	7.7	1.00	
Moderate tortuosity	1101	158	11.6	1.57 (0.88-2.83)	0.129
No thrombus post-PTCA	1097	1379	7.2	1.00	
Thrombus post-PTCA	44	47	25.0	4.29 (2.09-8.81)	0.001

Table IV
Relationship of continuous clinical variables to Major Adverse Clinical Events occurring within 4 days following PTCA.

Variable	Eventfree	Event	P uni-variate	OR (95% CI)
Age (years)	58.4±9.0	60.6±8.2	0.023	1.03 (1.00-1.06)
Weight (kilogram)	76.4±11.0	73.8±10.0	0.030	0.98 (0.96-1.00)
Platelets ($*10^9$/L)	243±67	237±59	0.361	1.00 (0.95-1.01)
Haemoglobin (gram/L)	141±13.7	139±12	0.061	0.99 (0.97-1.00)
Haematocrit (%)	41.5±3.9	40.4±3.5	0.011	0.93 (0.88-0.98)
Leucocytes ($*10^9$/L)	7.18±1.9	7.36±2.1	0.406	1.05 (0.94-1.17)
$F_{1.2}$ (screening nmol/L)	1.54±2.5	1.39±0.86	0.248	0.97 (0.86-1.10)
$F_{1.2}$ (post-PTCA nmol/L)	2.24±3.84	2.30±3.40	0.912	1.00 (0.94-1.07)
aPTT (post-PTCA sec)	136.6±56.8	148±58.1	0.116	1.00 (1.00-1.01)

Table V
Relationship of continuous angiographic variables to Major Adverse Clinical Events occurring within 4 days following PTCA.

Variable	Eventfree	Event	P uni-variate	OR (95% CI)
Reference diam. pre	2.68±0.51	2.74±0.50	0.308	1.24 (0.82-1.88)
Obstruction diam. pre	0.98±0.39	0.98±0.39	0.963	0.99 (0.57-1.70)
Obstruction diam. post	1.80±0.37	1.76±0.40	0.416	0.70 (0.29-1.67)
Absolute gain	0.81±0.42	0.85±0.47	0.501	1.27 (0.63-2.58)
Relative gain	0.30±0.15	0.31±0.16	0.591	1.73 (0.24-12.7)
Lesion length pre-PTCA	6.84±2.24	7.57±2.58	0.005	1.14 (1.05-1.24)
Area-plaque pre-PTCA	7.52±3.56	9.17±5.49	0.008	1.09 (1.04-1.15)

Table VI
Relationship of categorical patient characteristics to Major Adverse Clinical Events occurring within 7 months following PTCA.

Characteristic	N	% MACE	Odds ratio	P univariate
Treatment group				
Hirudin	759	34.3	1.07 (0.83-1.39)	0.605
Heparin	382	32.7	1.00	
Anginal class				
Braunwald I	416	26.2	1.00	
Braunwald II	489	37.8	1.71 (1.29-2.28)	0.000
Braunwald III	236	38.5	1.77 (1.26-2.49)	0.001
CCS class 1	23	43.5	1.77 (0.75-4.16)	0.191
CCS class 2	217	29.5	1.00	
CCS class 3	478	29.3	0.95 (0.71-1.29)	0.750
CCS class 4	287	44.6	1.85 (1.34-2.56)	0.000
Current smoker				
No	862	35.7	1.00	
Yes	278	27.7	0.69 (0.51-0.93)	0.014
Gender				
Male	896	33.7	1.00	
Female	245	33.9	1.01 (0.75-1.36)	0.960
Age < 60 years	625	28.3	1.00	
Age ≥ 60 years	516	40.3	1.71 (1.34-2.19)	0.000
Number of lesions				
One	899	32.4	1.00	
Two	198	36.9	1.22 (0.89-1.68)	0.224
Three	44	47.8	1.91 (1.04-3.50)	0.037
Medical history				
Previous CABG				
No	1103	33.5	1.00	
Yes	38	42.1	1.45 (0.75-2.79)	0.270
Previous MI				
No	37.1	8.9	1.00	
Yes	28.6	7.1	0.68 (0.53-0.88)	0.003
Previous PTCA				
No	940	33.5	1.00	
Yes	201	34.8	1.06 (0.77-1.46)	0.720

Table VI continued
Relationship of categorical patient characteristics to Major Adverse Clinical Events occurring within 7 months following PTCA.

Characteristic	N	% MACE	Odds ratio	P univariate
NIDDM				
No	1055	33.4	1.00	
Yes	86	38.4	1.24 (0.79-1.96)	0.346
IDDM				
No	1101	34.1	1.00	
Yes	40	25.0	0.65 (0.31-1.33)	0.237
Hypercholesterolemia				
No	706	36.4	1.00	
Yes	435	29.4	0.73 (0.56-0.94)	0.016
Hypertension				
No	661	32.2	1.00	
Yes	480	35.8	1.18 (0.92-1.51)	0.203
History of stroke				
No	1129	33.5	1.00	
Yes	12	58.3	2.78 (0.88-8.82)	0.082
History of TIA				
No	1124	33.7	1.00	
Yes	17	35.3	1.07 (0.39-2.92)	0.892
Medication at screening				
Heparin i.v. at screening				
No	807	31.7	1.00	
Yes	334	38.6	1.35 (1.04-1.77)	0.025
Long acting nitrates				
No	512	37.5	1.00	
Yes	629	30.7	0.74 (0.58-0.94)	0.016
Short acting nitrates				
No	676	31.7	1.00	
Yes	465	36.8	1.26 (0.98-1.61)	0.073
Cholesterol lowering drugs				
No	982	34.3	1.00	
Yes	159	30.2	0.83 (0.58-1.19)	0.307

Table VII
Relationship of categorical morphologic variables to Major Adverse Clinical Events occurring within 7 months following PTCA.

Pre-procedural characteristics	N patients	N lesions	% MACE	Odds ratio (95% CI)	P univariate
Vessel dilated					
LAD	587	699	36.5	1.29 (1.00-1.64)	0.046
LCX	288	334	31.6	0.88 (0.66-1.17)	0.373
RCA	363	423	31.4	0.86 (0.66-1.12)	0.254
Concentric	432	485	33.1	1.00	
Eccentric	601	674	35.7	1.24 (0.95-1.63)	0.111
Multiple irregularities, tandem lesion or total occlusion	239	267	38.2	1.26 (0.91-1.74)	0.167
Patent vessel	1063	345	34.0	1.00	
Total occlusion	781	81	30.8	0.86 (0.53-1.42)	0.562
Non-ostial	1071	1356	33.0	1.00	
Ostial location	70	70	45.7	1.71 1.05-2.78)	0.031
Branchpoint absent	846	1100	31.5	1.00	
Branchpoint in stenosis	295	326	40.3	1.47 (1.12-1.93)	0.006
Non-angulated lesion	1036	1318	32.7	1.00	
Location in bend	105	108	44.7	1.67 (1.11-2.51)	0.013
Thrombus absent	1077	1360	33.5	1.00	
Thrombus pre-PTCA	64	66	39.1	1.28 (0.76-2.14)	0.358
Non-calcified lesion	962	1216	33.3	1.00	
Calcification	179	210	36.3	1.14 (0.82-1.59)	0.434
Lesion length (visual)					
< 10 mm	802	956	31.7	1.00	
10-20 mm	291	312	41.6	1.59 (1.20-2.11)	0.001
>20 mm	69	73	40.6	1.55 (0.94-2.57)	0.087
ABC lesion type					
type A	213	234	28.6	1.00	
type B1	518	584	33.2	1.45 (0.96-2.18)	0.077
type B2	430	483	40.0	2.00 (1.33-3.01)	0.001
type C	114	123	33.3	1.54 (0.91-2.61)	0.110

Table VII continued
Relationship of categorical morphologic variables to Major Adverse Clinical Events occurring within 7 months following PTCA.

Post-procedural characteristics	N patients	N lesions	% MACE	Odds ratio (95% CI)	P univariate
Dissection absent	639	857	30.8	1.00	
Dissection type A	172	181	33.7	1.08 (0.76-1.55)	0.660
Dissection type B	221	236	31.7	0.91 (0.65-1.26)	0.558
Dissection C,D,E or F	128	130	50.0	1.41 (0.82-2.44)	0.216
Dissection absent	639	857	30.8	1.00	
Dissection present	502	547	37.1	1.32 (1.03-1.69)	0.028
No tortuosity	1031	268	32.4	1.00	
Moderate tortuosity	1101	158	44.2	1.65 (1.14-2.39)	0.008
No thrombus post-PTCA	1097	1379	32.8	1.00	
Thrombus post-PTCA	44	47	54.5	2.46 (1.34-4.52)	0.004

Table VIII
Relationship of continuous clinical variables to Major Adverse Clinical Events occurring within 7 months following PTCA.

Variable	Eventfree	Event	P uni-variate	OR (95% CI)
Age (years)	57.9±8.7	59.8±9.3	0.001	1.02 (1.01-1.04)
Weight (kilogram)	76.8±11.0	75.1±10.7	0.016	0.99 (0.97-0.99)
Platelets ($*10^9$/L)	245±70	239±57	0.13	1.00 (1.00-1.00)
Haemoglobin (gram/L)	142±14	141±13	0.32	0.99 (0.99-1.00)
Haematocrit (%)	41.5±3.9	41.3±3.9	0.43	0.99 (0.96-1.02)
Leucocytes ($*10^9$/L)	7.2±2.0	7.2±1.8	0.93	1.00 (0.94-1.06)
$F_{1.2}$ (screening nmol/L)	1.5±2.5	1.5±2.2	0.87	1.00 (0.95-1.06)
$F_{1.2}$ (post-PTCA nmol/L)	2.1±3.5	2.6±4.4	0.053	1.03 (1.00-1.07)
aPTT (post-PTCA sec)	138±57	136±58	0.50	1.00 (1.00-1.00)

Table IX
Relationship of continuous angiographic variables to Major Adverse Clinical Events occurring within 7 months following PTCA.

Variable	Eventfree	Event	P uni-variate	OR (95% CI)
Reference diam. pre	2.71±0.51	2.65±0.51	0.074	0.80 (0.62-1.02)
Obstruction diam. pre	0.98±0.40	0.96±0.36	0.430	0.88 (0.64-1.12)
Obstruction diam. post	1.84±0.35	1.72±0.38	0.000	0.42 (0.29-0.61)
Obstruction diam. f-up	1.68±0.51	1.18±0.50	*	
Absolute gain	0.83±0.43	0.76±0.45	0.013	0.66 (0.48-0.92)
Absolute loss	0.16±0.43	0.54±0.52	*	
Relative gain	0.31±0.15	0.28±0.15	0.010	0.30 (0.12-0.75)
Relative loss	0.06±0.16	0.20±0.19	*	
Lesion length pre-PTCA	6.84±2.25	6.98±2.34	0.350	1.03 (0.97-1.08)
Area-plaque pre-PTCA	7.56±3.60	7.58±4.10	0.247	1.02 (0.99-1.05)

* Not all p-values are entered in to the table since their relation to the occurrence of clinical events is evidently inherent.

Table X
Independent predictors for the occurrence of Major Adverse Clinical Events at 4 days.

Variable	P multi-variate	Odds ratio	95% CI
Hirudin treatment	0.036	0.61	0.38 – 0.97
Long acting nitrates	0.018	0.57	0.36 – 0.91
Lesion type A	0.001	0.37	0.21 – 0.65
Braunwald class III	0.003	2.19	1.32 – 3.63
Age ≥ 60 years	0.004	1.97	1.24 – 3.15
Dissection	0.000	5.48	3.18 – 9.44
Thrombus post-PTCA	0.001	3.73	1.71 – 8.13

Table XI
Independent predictors for the occurrence of Major Adverse Clinical Events at 7 months.

Variable	P multi-variate	Odds ratio	95% CI
Braunwald class II	0.002	1.64	1.19-2.24
Braunwald class III	0.014	1.61	1.10-2.36
Age ≥ 60 years	0.001	1.61	1.22-2.11
F1.2 post-PTCA nmol/L	0.035	1.04	1.00-1.07
History of MI	0.012	0.69	0.52-0.92
Lesion length < 10mm	0.006	0.67	0.50-0.88
MLD post-PTCA (mm)	0.000	0.46	0.31-0.68

pain [33]. This study demonstrated the classification to be appropriate for risk stratification in clinical practice.

The Braunwald classification may be especially relevant to the dynamic changes within the coronary arteries. The occurrence of anginal pain episodes over time may parallel repeated formation and recovery of an unstable plaque, resulting in a layered thrombus [34]. Furthermore, the clinical decrease in frequency of symptoms over time corresponds to the decrease of angiographic presence of intracoronary thrombus over time [6].

Our data demonstrate a gradual increase in risk from Braunwald class I to class III for the occurrence of cardiac events. This observation matches the gradual increased incidence of present smoking status (21.4% vs. 24.8% vs. 28.8% in respectively class I, II and III, P=0.033), female gender (19.2% vs. 21.1% vs. 26.2%, P=0.044), previous PTCA (16.6% vs. 15.5% vs. 23.7%, P=0.049) and presence of a type C lesion (7.7% vs. 10.4% vs. 13.2%, P=0.022). Where coronary angiography considerably underestimates the presence of intracoronary thrombus [35], Braunwald class was not related to the angiographic evidence of pre-procedural presence of intracoronary thrombus (6.0% vs. 5.7% vs. 4.7% in respectively class I, II and III, P=0.504). Braunwald class was surprisingly also not related to the pre-procedural levels of prothrombin fragment 1 and 2 (2.22, 2.03, and 2.70 nmol/L., P=0.093) [36].

Risk factors for adverse procedural outcome are related to the presence of a large myocardium at risk, while taking into account the extent of pre-existing non-viable myocardium. The protective effect of a previous myocardial infarction in our analysis may be due to the lesser amount of myocardium at risk in those patients, especially when the dilatation is in the vessel supplying the infarct related area. However, we could not reconstruct this hypothesis, because of restrictions in the available data and the retrospective nature of this investigation.

In our study, increased age is an independent risk factor for the occurrence of MACE. The ≥60 years cut off point was based on the median age of 58.8 years and includes 45.2% of the studied population. Despite the plausible relationship between higher age and the occurrence of MACE [7, 8, 9], we must not underestimate the fact that long term event free survival and life-expectancy after PTCA in patients under 35 years of age are seriously and detrimentally altered as well [10].

The subgroup of patients over 60 years of age significantly overrepresented females. Thirty percent of the patients over 60 years, and 14.4% in the younger population were females (P=0.001). Because of their higher age, female patients suffered significantly more frequent diabetes mellitus (15.5% of the females versus 9.8% of the male population, P=0.012) and hypertension (55.1% of the females is hypertensive versus 38.5% of the males P=0.001). Despite this, the incidence of early and late events is exactly similar in man and women, suggesting that women's cardiovascular system is less extensive diseased [8, 37]. There is a trend towards a lower incidence of multiple lesion angioplasty in women (19.4%) compared to men (24.4%).

A significantly lower age is observed in the smoking subgroup (54.4 ± 8.7 versus 59.9 ± 8.6 years, p = 0.0001), who in addition have a lower incidence of

hypertension (32.7% vs. 45.1%, p=0.001) and diabetes mellitus (7.6% vs. 12.2%, p=0.032). When multivariable correction for baseline characteristics is made, smoking does not prevent the occurrence of clinical events and appears merely dependent from age.

$F_{1.2}$ is a biological marker of prothrombin conversion, and the elevated levels post-PTCA might reflect the local coagulation activation by the disruptive action of successful angioplasty [36, 38]. In this study, samples for $F_{1.2}$ were taken from separate and peripheral venapunctures. Despite the fact that the measurement is from a diluted and systemic sample, an increased level of $F_{1.2}$ post-PTCA remains a significantly independent predictor for the overall occurrence of MACE 7 months after PTCA. This generates the question whether direct sampling from the great cardiac vein would underscore this relationship and possibly would disclose a threshold by which restenosis might occur. At least these data support the concept of thrombin inhibition and restenosis as scientific basis of the HELVETICA trial.

Qualitative angiographic risk factors All lesion characteristics that induce an unequal distribution of the dilating forces or transferred energy, increase the risk of brutal vessel wall disruption. Radial forces in case of an eccentric lesion will be mainly directed in compression of the protruding tissue. In 1985, Ambrose [39] recognised the relationship between lesion morphology and both unstable angina and myocardial infarction. Also the negative role of coronary artery thrombus formation, most likely secondary to plaque rupture, inducing unstable angina pectoris is suggested by several investigators [4, 5, 6, 40]. Early angiographic assessment in patients who had rest pain within 24 hours demonstrated the presence of intracoronary thrombus in 50% of the patients [6]. This figure was only 21% in those with late elective angiography.

In an NHLBI study (1500 PTCA patients), the complication rate was 26% in unstable patients, versus 17% in the stable group ($p<0.001$) [41]. It was recently demonstrated that culprit lesions in unstable syndromes show a focal vasoconstrictive response [42]. The presence of a vulnerable fissured plaque, and latent or active coagulant conditions is reflected by a significant contribution of,thrombus post-PTCA in the multivariate model for early outcome, and of $F_{1.2}$ in the long term multivariate model. The two studies suggest a link between lesion morphology in unstable patients and the occurrence of clinical complications. The predominance of eccentric lesions in our study population reflects the acute process of ruptured plaque or partially lysed thrombus which is present in unstable syndromes [39].

Although dissection increases the propensity for thrombus post-PTCA ($p=0.049$), it does not translate into increased levels of prothrombin fragment 1 and 2.

Location in a bend and branchpoint involvement increase the predisposition for development of a dissection, as apparently do more severe lesions (minimal lumen diameter pre-PTCA 1.01 ± 0.38 mm in vessels without dissection, 0.94 ± 0.39 mm in vessels with dissection, $p=0.0006$) and procedures in which a larger gain is achieved (0.84 ± 0.45 mm in vessels with dissection vs. 0.79

±0.40 mm in vessels without dissection P=0.05). Also visually estimated lesion length >10 mm, as independent predictor of adverse long term clinical outcome, significantly contributed to the dissection of coronaries (p=0.001) and to the formation of post-procedural thrombus (p=0.043).

Quantitative parameters: Minimal lumen diameter post-angioplasty A larger minimal lumen diameter post angioplasty is an independent protective factor for late clinical outcome. This variable correlates significantly to minimal lumen diameter pre-angioplasty, absolute gain and vessel size. Previously our group reported the positive independent influences (p<0.0001) of vessel size, luminal gain and MLD before PTCA on loss and MLD at follow up [11]. This is confirmed in our study where MLD post-PTCA is an independent predictor of late clinical outcome.

Administration of long acting nitrates and Hirudin treatment The administration of long acting nitrates at the time of randomization was a protective variable in the multivariate model for early clinical outcome. This relationship is not previously reported. This effect might conceivably be due to the protracted metabolization of nitrates to nitric oxide [43]. This results in both smooth muscle cell relaxation and inhibition of platelet aggregation and platelet activation through the stimulation of guanylate cyclase. Due to the retrospective nature of this trial however, we do not have any biological or haemostatic data supporting this assumption. At least there was no relationship between the use of long acting nitrates and loss in MLD.

Hirudin administration was independently associated with an impressive reduction of MACE in the first 4 days after angioplasty. The primary goal of the HELVETICA trial however, the reduction of MACE at 7 months was not achieved. The event free survival at 7 months did not differ among the treatment groups. A putative explanation for the apparent paradox might be that the dosage used was insufficient to reach an adequate level of anticoagulation, but sufficient to limit thrombin mediated platelet aggregation and activation, thereby exhibiting effects similar to those observed acutely in the EPIC trial [31, 44, 45].

Relevance and predictability of the multivariate model Predictability of the 4 day model is 80%. At the time of design of the trial, non-elective stent implantation was considered to be a bail-out procedure, equivalent to emergency CABG. To date however, bail-out stenting is routinely used in the standard armamentarium of the interventionalists and no longer viewed as a clinical event unless it causes untoward clinical effects. Therefore it might be argued that the importance of stenting in terms of clinical endpoints at the time of coronary angioplasty in this trial is overstated. On the other hand, up till now, no randomized trial has proven the merits of stenting or bail-out stenting in exclusively unstable patients. When bail-out stenting is not considered as a clinical endpoint in the analysis of the multivariate model, the predictability of the analysis remains high (79%). Remarkably hirudin treatment then loses significant contribution to the model.

A pre-procedural risk assessment would eliminate peri-procedural and

post-PTCA variables from the model, therefore reducing it's prognostic value. Accordingly, when thrombus post-PTCA and dissection are excluded from the model, predictability lowers to 70%, and hirudin treatment remains as a favourable factor in the model. When in this model bail-out stenting is not considered, predictability is less affected and only partially reduced to a level of 73%, while hirudin treatment loses it's significant contribution to the model.

Long term predictability of the model is 66%. Contribution of bail-out stenting to the long term predictability is low so that predictability is 65% if bail-out is not considered as a clinical endpoint. When post-procedural MLD and $F_{1,2}$ are not considered, and the model is limited to only pre-procedural characteristics, predictability of the model falls to 64%.

Recently the activation status of circulating phagocytes just prior to PTCA was found to be predictive for late lumen loss [12]. The expression of the membrane antigen CD66 by granulocytes was inversely associated with the relative loss at 6 months after PTCA, while the production of IL-1ß by stimulated monocytes was positively associated with the relative loss. Multivariable linear regression analysis showed that luminal renarrowing could be predicted reliably (R^2=0.65; P<0.0001) based on these two biological risk factors.

Another multivariable model identified predictors for luminal narrowing at 6 months following PTCA by visually assessed lesion length, pre-procedural minimal lumen diameter, diabetes mellitus, duration of angina, relative gain in lumen diameter, and thrombus post-angioplasty [23]. To evaluate the effectiveness to predict the amount of luminal narrowing at follow-up, the percentage of correct classified lesions was calculated for 5 intervals of predicted change in lumen diameter. Correct prediction of the model was poor. Only 10% of lesions in the middle 3 categories were correctly classified by the model.

Conclusion

In the HELVETICA trial, randomization to hirudin therapy was associated with impressive reductions in the rate of MACE in the first 4 days after angioplasty as compared with heparin. However, a reduction in the rate of cardiac events at seven months by hirudin, was not accomplished. In this ancillary study we demonstrate that the advanced multivariable model to predict in-hospital clinical outcome is fairly accurate (80%). The multivariable model for 7 months clinical outcome is somewhat less predictive (66%). The contribution of seven independent variables however, creates a complex model. An ideal model contains only a very limited number of variables in order to simplify risk stratification. Finally, we should not forget that the NHLBI registry observed the significant relationship between major complication rate and cardiologist's experience [41].

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Chapter 11

The current role of antithrombins in interventional cardiology

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Introduction Despite developments in technology and increased knowledge of mechanism of action and pathophysiology of intracoronary angioplasty procedures, the high initial success rates of percutaneous transluminal coronary angioplasties (PTCAs) remain hampered by early and late reocclusion [1]. The introduction of adjunctive devices in interventional cardiology has augmented indications for angioplasty in patients, which are expected to result in a higher complication rate. Several randomized pharmacologic multicenter trials have been designed to diminish the rate of reocclusion, but until now no benefit is detected from the variety of drugs tested.

It is evident that thrombin plays an unequivocal detrimental role in the vascular wall injury resulting from angioplasty procedures. Pharmacologic research currently concentrates on inhibition of prothrombin conversion and thrombus formation. Animal experimental work in this field is encouraging, and results of clinical trials will elucidate important questions about safety, efficacy and immunoallergic potential of modern antithrombins. (Current Review of Interventional Cardiology (edn3). Current Medicine-1996;chapter 18)

Thrombin

Thrombin is the most potent known platelet activator and interacts with a variety of immunomodulatory cells and hematologic factors. Its action includes a stimulation in the endothelial layer of platelet-derived growth factor production, prostacyclin secretion, platelet-activating factor, and plasminogen activator-inhibitor. In vivo, thrombin receptor expression was demonstrated by endothelial cells in intact vessel walls, macrophages, and vascular smooth muscle cells in atherosclerotic lesions and in endarterectomy specimens [2]. Selectin formation at the endothelial cell surface is stimulated by thrombin and plays a role in the incursion of monocytes and neutrophils to an injured vessel wall. In addition to this, thrombin has a direct chemotactic effect on monocytes [3], and has apparent mitogenic effects on lymphocytes [4] and vascular smooth muscle cells [5] (Figure 1).

The transmembrane thrombin receptor exposes an amino terminal extension that bares two thrombin binding sites: the thrombin cleavage site and a hirudinlike domain [6]. The identification of the thrombin receptor has enabled sophisticated designs of molecules that fit exactly into those sites.

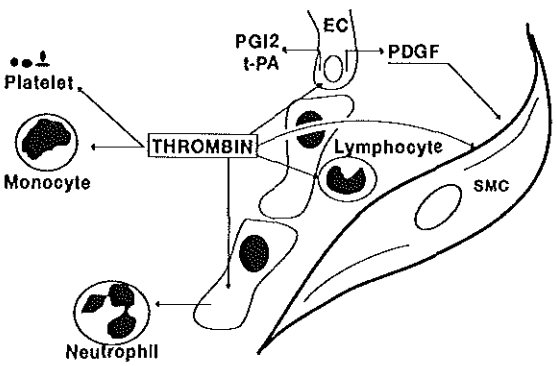
In case of deep endothelial injury (as with a successful angioplasty), blood is exposed to collagen and other substances of the subintima, which are potent stimuli for platelet aggregation mediated by the release of adenosine diphosphate (ADP); serotonin; thromboxane A_2 (TXA_2); and the adhesive molecules fibrinogen, fibronectin, thrombospondin and von Willebrand Factor. These substances, including thrombin, activate neighbouring platelets by way of different metabolic pathways (TXA_2, ADP and a platelet-activating factor) and promote intramural thrombus formation, which could cause restenosis [7]. In addition,

Figure 1

The central role of thrombin in platelet aggregation and activation, chemotaxis, and mitogenesis for immunomodulatory and mesenchymal cells, including vascular endothelial cells.

EC=endothelial cell
PGI2=prostaglandin I2
PDGF=platelet derived growth factor
SMC=smooth muscle cell
tPA=tissue plasminogen activator

Modified from Coughlin SR et al. J Clin Invest 1992;89:351-5. With permission.



several growth factors, important ingredients of the platelet granulae are released during activation. Receptors for these factors are expressed in mesenchymal cells such as the smooth muscle cells and play a prominent role in late restenosis.

Limitations of available antithrombotic drugs

Currently, substantial research is focused on antithrombotic and antithrombin approach. Until now, no major breakthrough has been reported [8, 9], although a clear progression in the understanding and interfering of the mechanism has occurred. The limitations of several antithrombin agents have been determined. Warfarins' action does not block thrombin's extrinsic pathway, but does hinder the action of several commonly used drugs. Another weakness of warfarin is the delay between administration and therapeutic effect, as is the case with ticlopidine.

Aspirin has the ability to inhibit platelet TxA_2 synthetase and subsequent platelet activation by irreversibly blocking the enzyme cyclo-oxygenase that is responsible for the conversion of arachidonic acid to prostaglandin G_2 (PGG_2). This intermediate prostaglandin can be transformed in the metabolic pathway to TxA_2 and thromboxane B_2. Aspirin results in a decreased production of this substance in platelets. It also results in cyclo-oxygenase blockade in the endothelial cell [10], which is responsible for the conversion of arachidonic acid to prostaglandin I_2 (PGI_2). Thromboxane A_2 is a powerful platelet aggregator and vasoconstrictor, but because the biologic actions of TxA_2 and PGI_2 are opposing, acetylsalicylic acid is an antagonist of itself. In addition, it only partially inhibits platelet aggregation induced by ADP, serotonin, epinephrin, collagen, or thrombin.

The effectiveness of heparin is limited in patients having insufficient antithrombin III levels. Another major issue is the limited ability of the large heparin-antithrombin III complex to gain access to clot-bound thrombin or factor Xa in the prothrombinase complex. Furthermore, heparin action is inhibited by fibrin II monomers, thrombospondin, and platelet factor 4, which is present in the platelet-rich thrombus. The dose-effect response is poor and relatively unpredictable.

The action of low-molecular-weight heparin (LMWH) in factor X inhibition is hampered by the limited ability of the antithrombin III-LMWH complex to block phospholipid membrane- bound factor Xa; also LMWH shares the limitations of heparin in gaining access to clot-bound thrombin and in functioning effectively under conditions of insufficient antithrombin levels. As is true of heparin, LMWH is a widely varied mixture of sizes of depolymerized heparin molecules and therefore is subject to variety in effectiveness from lot to lot. Both heparin and LMWH have effects on platelets that may manifest as thrombocytopenia.

The potential advantages of specific antithrombins lie in the fact that they overcome the limitations of conventional treatment. The ideal antithrombin drug is a small-sized molecule, able to penetrate the thrombotic material. It is synthetic and therefore has no natural inhibitors, does not need any co-factor, and does not express any antigenic properties. The dose-response relation is predictable. An important support to the success of such a drug is the development of local drug delivery systems, enabling the interventional cardiologist to block thrombins' action entirely at exactly the right spot, not affecting systemic hemostasis levels.

Animal experimental work and human studies

Heparins Heparin is currently the most widely used anticoagulant drug in the management of acute thrombotic syndromes. It is a heterogeneous mixture of glycosaminoglycans with a molecular weight ranging from 2000 to 40,000 daltons. The antithrombotic capacities are achieved by the reversible binding with the natural circulating anticoagulant antithrombin III. The binding is responsible for a 1000-fold acceleration of antithrombin III-heparin binding to factor IIa. This complex also involves activated coagulation proteins (factors X, IX, XI and XII).

Heparin and its low-molecular-weight derivatives have been shown to reduce smooth muscle cell proliferation [11], inhibit smooth muscle cell migration [11], and decrease the number of cells transformed from a contractile to a synthetic phenotype [12]. The antiproliferative actions of the heparins appear not to be related to anticoagulant properties because particular heparin subfractions retain their antiproliferative effects despite their lack of anticoagulant properties. Smooth muscle cell-bound heparin can be internalized, affecting the cell cycle with a consequent reduction in DNA and RNA synthesis by mechanisms unrelated to oncogene expression [13]. The effect on restenosis of various heparin doses and administration schedules has been investigated using regular heparin samples [14-17]. So far, no beneficial influence on the restenosis rate has been detected. Synthetic heparins and LMWH fractions, lacking the heterogeneous nature, are now the subject of research [18-22]. The clinically used LMWH mixtures diverge in molecular weight from 1 to 10 kD. Where heparin operates as a template to link thrombin and antithrombin III, the heparins with a molecular weight of less than 5400 daltons are too small to bind these proteins synchronously, but nonetheless are able to accomplish the binding of antithrombin to activated factor Xa. Inhibition of this central protease results in reduced thrombin generation. In animal experiments using LMWH, significant effects were detected on intimal hypertrophy or diameter reduction [23-25]. Heras and colleagues [26] analyzed the inhibition of thrombus formation using four doses (100, 200, 400, and 500 U/kg) of LMWH (CY 216) in comparison with unfractionated heparin (100 U/kg) and placebo in deep carotid artery injury in pigs. Examination of the treated vessels after 1 hour, showed platelet deposition of 22, 29, 9, 9, 11, and 42 * $10^6/cm^2$. For fibrinogen deposition, the numbers in the groups were 19, 19, 21, 14, 12 ($p< 0.05$), and 35 molecules *$10^{12}/cm^2$. Thus, the predominantly anti-factor X activity does not clearly inhibit the deposition of thrombotic material after deep arterial injury, when compared to unfractionated heparin.

A group in Tübingen [27] reported the results of 400 anti Xa units/kg/day administration in 55 rabbits who underwent balloon angioplasty of an electrically induced lesion of the carotid artery. The LMWH (LU 47311, mean molecular weight 3.9 kD) was subcutaneously given from day 1 to day 7 after intervention. Observation period was 3, 7, 14, and 28 days. At the end of the study period, intimal wall thickness in the LMWH-treated rabbits increased from 13 (preangioplasty control group) to 20 cell layers in the treated animals ($p<0.05$ versus non-angioplasty control group) and from 13 to 35 cell layers in controls ($p<0.01$ vs

nonangioplasty control group). The proliferative response of smooth muscle cells was evaluated by quantification of cells undergoing DNA synthesis. In controls, this showed an increase in S-phase cells in the intima from 0.7% cells before dilatation to 10.2% and 7.7% 3 and 7 days after angioplasty. A mild increase in DNA synthesizing cells was observed in the treatment group (2.7% at 3 days, $p<0.01$, and 1.9% at 7 days, $p<0.01$). Normalization of proliferation 14 and 28 days after dilatation occurred in both groups.

Other factor Xa inhibitors Antistasin (r-ATS) is a 15- kD peptide isolated from the salivary glands of the Mexican leech [28]. It is a tight-binding and specific long-acting factor Xa inhibitor. It has been studied in a variety of thrombosis models and found superior to heparin in preventing platelet-rich thrombi in dacron-grafted arteriovenous femoral grafts [29] and as an adjunct to alteplase in a canine model of femoral arterial thrombosis [30]. It was found as effective as heparin in suppressing fibrinopeptide A (FPA) levels in a rhesus monkey disseminated intravascular coagulation model [31]. Because of the apparently strong immunogenecity of antistasin, however, further clinical development in its current configuration is unlikely [32].

Tick anticoagulant peptide (TAP), is a short-acting inhibitor of factor Xa, originally isolated from the soft tick Ornithodoros moubata [33]. Both r-ATS and TAP were tested in New Zealand rabbits during balloon angioplasty, followed by 2 hours of infusion [34]. The reduction in luminal diameter as measured at 28-day follow-up angiography was significantly less in the r-ATS and TAP groups compared to the control group, which received heparin (-0.17 ± 0.11 mm, -0.26 ± 0.22 mm, vs 0.66 ± 0.58 mm, $p \leq 0.02$).

Hirudin Hirudin is a specific and direct thrombin blocker. This anticoagulant drug is originally extracted from the salivary gland of the leech (Hirudo medicinalis). It is a 7-kD protein, composed of 65 amino acids. Thrombin has an active site pocket that can be blocked by the N-terminal three amino acids of hirudin, whereas hirudin's carboxyl tail forms several ionic and hydrophobic complexes with the fibrinogen anion-binding site, thus blocking thrombin-catalyzed fibrinogen cleavage. The fortitude of hirudin in comparison to other serine protease inhibitors is in its potency to block thrombin instantaneously and irreversibly at multiple sites [35] without the need for circulating antithrombin III. Hirudin, because of its small volume, is able to inhibit thrombin tied to thrombus, and to restrict further thrombus formation also where the thrombin is clot bound or when thrombolysis takes place [36]. The recombinant configuration differs from the native hirudin by the absence of a sulphate group in the Tyr-63 position [37]. For this reason the recombinant variant is also called desulphatohirudin or desirudin. The terminal half-life of desirudin in healthy, young volunteers is 50 to 65 minutes [38], with a halflife of its effect on the activated partial thromboplastin time (aPTT) of about 2 hours [39, 40]. There is no specific antidote available for reversing the effects of hirudin.

The effects of recombinant hirudin on the restenosing process following balloon-caused arterial injury are intensively studied in animal models. A signifi-

cantly reduced platelet deposition to only one single layer was found using high-dose hirudin (1.0 mg/kg bolus plus intravenous infusion for 1 hour) [41], and a significantly inhibited restenosis response as assessed by quantitative angiography and quantitative histopathology [42] was found after hirudin administration. The group of Buchwald and colleagues [43] observed the significant effect of hirudin on platelet accumulation and fibrin deposition in stented minipigs. The effect of recombinant hirudin was evaluated in patients undergoing angioplasty for stable angina pectoris in a multicenter, double-blind pilot study [44] . A total of 113 patients were randomized in a 2:1 fashion, receiving either 20 mg hirudin before angioplasty followed by a 24-hours infusion of 0.16 mg/kg/hr, or 10,000 IU of heparin followed by a 12 IU/kg/hr infusion for 24 hours. At the end of this infusion period, angiography was performed to assess vessel patency and to obtain quantitative measurements. Coagulation parameters were frequently measured. In the heparin-treated group (39 patients), 4 patients experienced a periprocedural myocardial infarction or emergency coronary artery bypass graft (CABG), compared to only one emergency CABG and one elective CABG 3 weeks after PTCA in the hirudin-allocated group (74 patients). Hemorrhage at the arterial puncture site was seen in four hirudin-treated patients, for which they needed blood transfusion, whereas in the heparin-allocated group one patient experienced visual impairment because of a cerebral infarction. Follow-up angiography at 24 hours revealed a complete perfusion in all the patients from the hirudin-treated group, compared to 92% in the heparin-treated group. Visual assessment furthermore showed one case of intra coronary thrombus in each group. Concentrations of prothrombin fragment one and two ($F_{1.2}$) appeared to be somewhat higher in the hirudin group directly and 6 hours after angioplasty (a median of 1.015 versus 0.795 nmol/L and 0.95 versus 0.8 nmol/L), indicating a slightly diminished thrombin inactivation. It was concluded that safety and tolerance for hirudin is similar to that for heparin [44, 45], and it can be administered in patients undergoing balloon angioplasty for stable angina pectoris. This study surveyed only the short-term effects of thrombin antagonism, but it was followed by a multicenter, randomized, double-blind, heparin-controlled study, aimed at the prevention of restenosis [46].

Recent reports on clinical trials in the setting of acute myocardial infarction (thrombolytics and concomitant aspirin) suggest a narrower safety window with recombinant hirudin than initially anticipated. Extremely high rates of intracranial bleeding were encountered with streptokinase in GUSTO-IIA (Global Utilization of Strategies to Open Occluded Coronary Arteries) (with heparin 2.7% and with hirudin 3.2%) [47], or with alteplase in TIMI 9A (Thrombolysis in Myocardial Infarction) (with heparin 1.9% and with hirudin 1.7%) [48], and HIT-III (r-Hirudin for Improvement of Thrombolysis) (none with heparin and with hirudin 3.4%) [49]. A baseline creatinine of greater than 1.5 mg/dL, older age, lower bodyweight, and higher aPTT levels (100 versus 86 seconds in non-stroke patients), were associated with bleeding in desirudin treated patients.

Hirudin derived drugs The N-terminal three amino acids of hirudin block the thrombin active site pocket, while the C-terminal site form complexes with the

fibrinogen anion-binding site, thus blocking thrombin-catalyzed fibrinogen cleavage. Structure of the latter peptide domain is mimicked in the synthetic peptide hirugen (Figure 2), the potency of which is about 50 times less in comparison with the activity of hirudin. The antithrombin activity of this dodecapeptide is solely due to the blockade of the fibrinogenolytic site and therefore especially valuable in preventing fibrin-dependent thrombosis [50].

X-ray crystallographic analysis enables assembling of highly specific compounds especially designed to inhibit thrombin. Extremely detailed three-dimensional reconstruction of the amino-binding and carboxyl-binding groups has enabled complex technology to link an inhibitor of the active site pocket (D-Phe-Pro-Arg-Pro) to hirugen with optimal positions (at distances of 20 Angstrom) of both of the binding sequences opposite to their target molecules [51]. This drug called hirulog (see Figure 2) has been administrated safely to healthy human volunteers [52]. Hirulog induces a dose-dependent prolongation of all coagulation parameters, with a half-life of 40 minutes [53]. A dose escalating multicenter study [54] evaluated this drug in the setting of intracoronary interventions. In the latter, 291 patients received a bolus dose of 0.15, 0.25, 0.35, 0.45, or 0.55 mg/kg (group 1 to 5 encompassing 54, 53, 44, 74, and 54 patients), followed by a 4-hour infusion of 0.60, 1.00, 1.40, 1.80, or 2.20 mg/kg/hr. All patients were concomitantly pretreated with 325 mg aspirin. A rapid onset and dose-dependent increase in aPTT and activated clotting time (ACT) values at doses of 1.80, and 2.20 mg/kg/hr ensued a sufficient anticoagulant state and reduced abrupt vessel closure within the first 24 hours of the start of the procedure to less than 4% (5 out of 128 patients) versus 11.3% (17 vessels closed in 151 treated patients) in the other three treatment groups. In this study, there was no control group treated with conventional anticoagulant drugs, but the results encouraged a double-blind, randomized clinical trial [55].

Synthetic thrombin inhibitors PPACK is the abbreviation of D-phenylanalyl-L-prolyl-L-arginyl-chloromethyl ketone. This peptide sequence binds to the active site of thrombin. The effect of this substance, in combination and in comparison with 7E3, on platelet deposition was tested at the site of balloon angioplasty in an ex vivo whole artery perfusion model [56]. Perfusion of the arterial segments with PPACK (10^{-5} M) reduced platelet deposition for 47% ($N=4$, $p<0.04$) with the use of this new antithrombin agent compared with the heparin model (2 U/mL).

Argatroban ((2R,4R)-4-methyl-1-[N^2-((3-methyl-1,2,3,4-tetrahydro-8-quinolonyl)sulfonyl)-arginyl]-2-piperidine carboxylic acid) is an arginine derivative, that binds competitively to thrombin (Figure 2) [57]. It is shown in animal experimental work that argatroban can prevent coronary thrombosis more effectively than heparin in models of coronary thrombolysis and vessel wall injury [58, 59]. This highly specific thrombin inhibitor was infused during 4 hours in patients with unstable angina pectoris [60]. Infusion of the drug (0.5 to 5.0 µg/kg/min) increased the aPTT in a dose-related fashion and was effective against recurrence of ischemic episodes, but remarkably 9 of the 43 patients experienced an episode of unstable angina shortly after termination of the drug.

Figure 2
Schematic presentation of thrombin inhibition by hirudin, hirugen, hirulog and argatroban. COOH=carboxyl group; NH2=amino group.

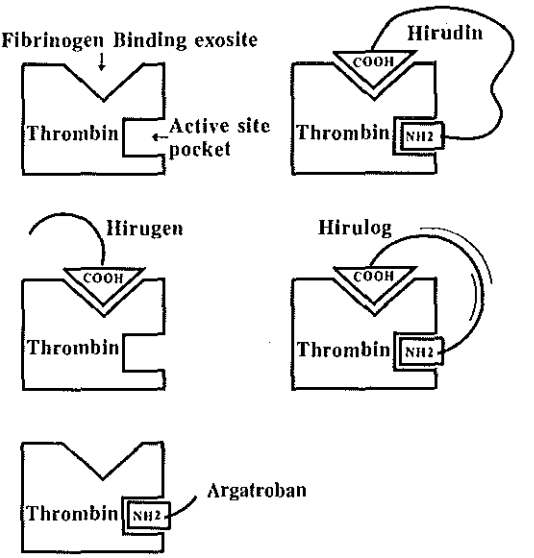
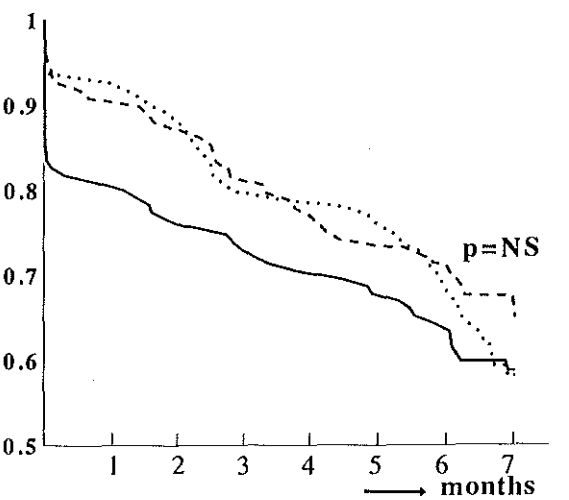


Figure 3
Event free distribution (Kaplan Meier) in the intention to treat population of the patients receiving heparin treatment at the time of randomization (N=324). At four days there is a remarkable beneficial effect in the hirudin treated patients (relative risk of hirudin versus heparin 0.37, 95 % CI 0.19-0.70 p=0.007). Comparison of the treatment groups at 7 months by the Kruskal-Wallis test reveals a nonsignificant effect (P-value 0.61).

Heparin (N=115); ———
Hirudin intravenous (N=110); • • • •
Hirudin intravenous and subcutaneous (N=109); – – – –



These patients in whom angina reoccurred received significantly higher doses of argatroban during infusion (p=0.007) and greater elongation of the aPTT (52±12 seconds in the non-angina group versus 66±15 in the nine patients with angina within 24 hours). The authors describe this phenomenon as a rebound effect.

Fitzgerald (not published) treated 12 angioplasty patients, randomizing them either to a bolus of 30 μg/kg argatroban, followed by an intravenous infusion of 2 μg/kg/min during 2 hours, or to a bolus injection of 10,000 IU of heparin plus a 4-hour infusion of 1000 IU/hr. These results initiated the so called pre-Argaplasty trial, a dose verification study, assessing safety and feasibility in patients undergoing balloon angioplasty for either stable or unstable angina [chapter 6 in this thesis]. A total of 30 patients were included at four dose levels, the highest being an intravenous bolus injection of 250 μg/kg, followed by a 4-hour infusion of 15 μg/kg/min. At 4 hours, the infusion rate was lowered to 3.8 μg/kg/min and continued for 68 hours. At this level, aPTT was approximately two times normal value, and the sheath was removed without infusion adjustment. Hemostatic, clinical, and angiographic data indicate that argatroban infusion in coronary angioplasty patients can be administered safely and results in an adequate, predictable level of anticoagulation [chapter 6 in this thesis].

New antithrombins under development are aptamers (synthetic oligonucleotides that, similar to hirugen, bind only to the exosite of thrombin [61]). Among those low-weight thrombin inhibitors are SDZ-217766 [62] and RWJ-27755, which binds to only the catalytic site of thrombin [63, 64]. The alkylating properties of the latter compound have, however, considerably tempered the enthusiasm for its clinical development [32]. DUP 714 is a boroarginine tripeptide [65]; its anticipated potential for oral administration has not yet resulted in human studies [32].

Other antithrombins still in development are the thrombin receptor antagonist peptides [66] and the hirudisins – hirudin derivates combining IIb/IIIa receptor and thrombin inhibition. In the hirudisins, residues from 32 to 35 of hirudin have been replaced by the integrin motif RGDS and KGDS [67]. In addition to inhibiting GP IIb/IIIa receptor-dependent platelet interactions, the platelet-binding integrin motif is expected to target the antithrombin action of hirudin to platelets, possibly allowing lower and safer doses of hirudin in the treatment of thrombotic disease [68].

Experimental and clinical application of locally delivered antithrombin into the vessel wall

Many reasons for the discrepancy between results from animal models and human trials have been proposed. One explanation has been that doses used in the animal studies were in most instances at least an order of magnitude higher than the highest tolerable doses in humans. It has also been suggested that difficulties in the sustained administration of certain compounds in humans for adequate periods of time to achieve measurable effects may have accounted for the failure of many of the pharmacologic therapies. Finally, the prevention of reste-

nosis judged by histologic measurements in animal models may not translate to angiographic changes in humans [69]. In response to these limitations, the idea of local drug delivery techniques for the administration of pharmacologic compounds at the site of coronary interventions has evolved [70]. Conceptually, local delivery techniques allow for high site-specific concentrations of therapeutic compounds without the complication of systemic side effects.

Administration of heparin using a porous balloon catheter was first accomplished by Wolinsky and Thung [70]. Using catheter-based percutaneous transluminal administration, high concentrations of fluoresceinated heparin within the media could be detected up to 48 hours after infusion. It has been shown that locally delivered heparin can reduce the thrombogenicity of the vessel wall in the acutely injured pig carotid artery model [71]. Site specific administration of PPACK by way of a porous balloon catheter in an ex vivo model of arterial damage has been shown to reduce platelet deposition at the site of balloon injury [72]. In addition, Nunes and associates [73] have demonstrated decreased platelet deposition on dacron grafts after local treatment with PPACK. Dispensed from a hydrogel-coated angioplasty balloon, PPACK showed considerable local effects without causing a prolongation of the coagulation parameters [73]. A similar reduction in platelet deposition was shown after the local administration of the thrombin inhibitor r-hirudin in a porcine carotid artery model of vascular injury [74]. Using a double-balloon perfusion catheter, high local concentrations of r-hirudin were maintained for 60 minutes without the device causing additional vascular injury.

Since the initial reports of the effectiveness of locally administered heparin [75] and LMWH [76, 77] to inhibition of smooth muscle cell proliferation, initial results of long-term studies using site-specific administration of many different drug classes are encouraging. The steady advances made towards the development of clinically applicable atraumatic delivery modalities have allowed the transition from the animal laboratory to human applications.

Preliminary data indicate the technique to be safe for use in human coronary arteries, at diverse anatomic sites [78]. The feasibility of local drug delivery using the Dispatch delivery catheter, a non-dilatational infusion-perfusion catheter, has been evaluated in humans [79]. Successful local delivery of heparin in humans was demonstrated for the first time using this device. A delivery efficacy of 1% to 8% with a retention time up to 14 hours was shown [80]. Preliminary long-term results of the initial patients treated with locally administered heparin at the time of angioplasty using this device are encouraging, showing a reduction in the need for re-intervention and in the degree of angiographically defined restenosis [79, 81-83]. Definitive long-term results are forthcoming.

A group in Tübingen delivered 1500 anti-Xa units LMWH Reviparin locally using a porous balloon, besides 24 hours intravenous and 28 days subcutaneous administration of the compound [84]. No major complication occurred during the 28 days follow-up period in the 15 treated patients. Data on restenosis will be available shortly.

With the increased use of percutaneously introduced stents for the treatment of primary and recurrent coronary stenosis, the possibility of using coated or eluting stents as local drug delivery devices is currently being tested. There

have been two reports of reduction of subacute stent thrombosis in the rabbit carotid [85], and in stented pig coronary arteries [86] using heparin-coated stents. The pilot study of the Benestent II trial has shown that deployment of the Palmaz-Schatz heparin-coated stent was possible with a reduced anticoagulation regimen [87]. Furthermore, the long-term results seem encouraging with an expected restenosis rate lower that those reported in Benestent I (13% in the Benestent II pilot study compared with 20% in the Benestent I) [88, 89]. The dilemma that the inflammatory response of the coating can exceed the beneficial effect of the eluted material is an obstacle that must be overcome [90].

Phase III restenosis prevention studies

The ERA (Enoxaparin Restenosis) trial [91], conducted a study comparing enoxaparin (40 mg q.d. s.c.) to placebo, administered for 28 days, starting within 2 hours of the sheath removal in the prevention of restenosis. A total of 459 patients were enrolled and 86% completed the study protocol, including angiographic follow-up. Treatment comparison resulted in an insignificant difference between the two groups (51% restenosis in the placebo group versus 52% in the treated group, OR 1.07, P=0.63). Analysis of demographic data and the use of other angiographic restenosis definitions could not reveal any difference in the two groups.

The REDUCE (Restenosis prevention after PTCA, Early administration of LMWH (LU 47311), Double blind, Unfractionated heparin and placebo Controlled Evaluation) study is a double-blind, randomized multicenter trial comparing standard therapy (10,000 IU intravenous bolus unfractionated heparin before PTCA, followed by 24,000 IU over 16 ±4 hours and placebo s.c. b.i.d. for a further 4 weeks, beginning at the evening after angioplasty) with a high-dose regimen of reviparin (7000 IU i.v. bolus before PTCA, followed by 10,500 IU over 16 ±4 hours, and 3500 IU s.c. b.i.d. for a further 4 weeks, beginning the evening after angioplasty) in patients undergoing angioplasty for a single de novo lesion. Concomitant aspirin was given. A total of 612 patients were recruited, of whom 306 patients received reviparin. The primary clinical endpoint was the event-free (freedom of death, non-fatal myocardial infarction, and revascularization) survival within 30 weeks following angioplasty. The primary angiographic endpoint was defined in terms of absolute loss in minimal lumen diameter at the dilated site. Restenosis was defined as loss of more than 50% of the initial gain of PTCA, according to the NHLBI IV definition. Long term clinical outcome was similar in both treatment groups (33.3% in the reviparin-treated patients, 32.0% in the control group (RR 1.04, 95% CI 0.83-1.31, P=0.71). The absolute loss in lumen diameter was 0.29 and 0.25 mm (analysis of covariance P=0.59). The incidence of bleeding occurred with a similar frequency in the two treatment groups (2.3% in the reviparin group and 2.6% in the heparin control group RR=0.88, 95% CI 0.32-2.41, P=0.8); however acute events within 24 hours occurred in 3.9% of the reviparin group and in 8.2% of the control group (RR=0.49, 95% CI = 0.26-0.92, P=0.027) (Karsch, unpublished results) [92]. The authors suggest that the lack of effect in this trial may be due to insufficient local drug concentrations which might be resolved by local drug delivery techni-

ques, or that the lack in effect in primates [93] might reflect the presence of a heparin-insensitive pathway of smooth muscle cell activation.

The HELVETICA (Hirudin in a European Trial versus Heparin in the prevention of Restenosis after PTCA) trial is a multicenter, double-blind, randomized heparin-controlled study evaluating the clinical efficacy and safety of two hirudin dose regimens in 1154 angioplasty patients [46]. The primary endpoint was event-free survival 30 weeks after angioplasty. Patients received one of the following treatments: unfractionated heparin (an i.v. bolus injection of 10,000 IU of heparin followed by a continuous i.v. infusion of 15 IU/kg/hr of heparin for 24 hours, with placebo given s.c. twice daily for three consecutive days), intravenous hirudin (an i.v. bolus injection of 40 mg of hirudin followed by a continuous intravenous infusion of 0.2 mg/kg/hr hirudin for 24 hours, with placebo given s.c. b.i.d. for three consecutive days) or intravenous and subcutaneous hirudin (an i.v. bolus injection of 40 mg of hirudin followed by a continuous i.v. infusion of 0.2 mg/kg/hr of hirudin for 24 hours, with 40 mg of hirudin given s.c. b.i.d. for three consecutive days). A concomitant dose of aspirin (100-500 mg once daily) was given on the day of angioplasty and continued for at least 14 days. To provoke a more distinct outcome, this study included only patients suffering unstable angina because this group of patients is known to have higher restenosis rates [94, 95, 96].

The study population was not only limited to balloon angioplasty patients, but also techniques, as laser, directional, and rotational atherectomy were allowed per protocol. Because at the time of design of this trial the anticoagulant drug strategies following stenting were very strict and subject to investigation, elective stent implantation was not allowed, and bail-out stenting resulted in discontinuation of the study drug. The eventfree survival curve did not demonstrate differences among the treatment groups (Kruskal-Wallis test p=0.61). Early events (those in the first 96 hours after angioplasty) occurred in 7.9%, 5.6%, and 11.0% in the hirudin intravenous and subcutaneous, the hirudin intravenous and the heparin group (relative risk of hirudin versus heparin 0.37, 95% CI 0.19-0.70 p=0.007). The incidence of clinical events and angina at 30 weeks was not significantly different in the three groups (p=0.61). An angiographic follow-up in 86.4% of the population was available and demonstrated an equal distribution of angiographic baseline and postangioplasty parameters as well as the loss in lumen diameter at follow-up. In patients receiving heparin pretreatment, early outcome was significantly superior in the hirudin-treated patients (combined relative risk with hirudin: 0.37, 95% CI 0.19 to 0.70, P=0.0016). Because these results suggested a particular benefit of hirudin in the most unstable patients (Braunwald class III), an additional analysis was performed in 236 patients with angina at rest in the 48 hours before randomization. The event rate in this subgroup of patients was 21.6% in the heparin group versus 5.3 and 12.3% in the hirudin intravenous and Hirudin intravenous and subcutaneous groups (combined relative risk with hirudin 0.41, 95% CI 0.21 to 0.78, P=0.006). The eventfree 7-month survival in the heparin-pretreated or Braunwald class III subgroup of patients demonstrated no effect at the long term (Figure 3).

The results of prothrombin fragment one and two ($F_{1.2}$) assays immediately after angioplasty suggest that the thrombin generation in both hirudin groups was unsatisfactorily inhibited, whereas the dosage of heparin used in this trial resulted in an appropriate decrease of $F_{1.2}$ at 6 hours after the intracoronary intervention. The authors suggest that the adjustment in infusion rate from 0.16 mg/kg/hr in the pilot study [44] with stable patients to 0.20 mg/kg/hr in the current trial of unstable patients with presumably higher levels of thrombin generation was a too cautious change in dosage. Zoldhelyi and colleagues [97] reported the failure to block thrombin generation in patients, despite a 10,000-fold molar excess of free hirudin over thrombin-hirudin complex. Infusion rates of hirudin in animal experiments were up to five times higher than those currently used, and this might explain the lack of long-term effect in the present study [41,42].

Another putative explanation for the apparent paradox in improved early outcome despite less appropriate thrombin control might be that the dosage used was insufficient to reach an adequate level of anticoagulation but sufficient to limit thrombin-mediated platelet aggregation and activation, thereby exhibiting effects similar to those observed acutely in the EPIC (Evaluation of c7E3 for the Prevention of Ischemic Complications) trial [98,99].

In the hirulog angioplasty study [55], 4312 patients suffering from unstable or postinfarction angina pectoris and scheduled for angioplasty were assigned to receive a body weight – adjusted dose regimen of either heparin or hirulog and concomitant aspirin (300 to 325 mg). The heparin-allocated patients (N=2039) received a bolus dose of 175 IU/kg followed by an 18- to 24- hour infusion at a rate of 15 IU/kg/hr. Hirulog-treated patients (2059 in total) were given a bolus dose of 1.0 mg/kg, followed by a 4-hour infusion at a rate of 2.5 mg/kg/hr and a 14- to 20-hour infusion at a rate of 0.2 mg/kg. Primary endpoint was the in-hospital occurrence of death; myocardial infarction; abrupt vessel closure; or need for CABG, intra-aortic balloon counterpulsation, or repeat coronary angioplasty. Bivalirudin compared to heparin treatment was not able to lower the incidence of in-hospital cardiac events in the cohort of 4098 treated patients (11.4% versus 12.2% respectively, OR 0.9, 95% CI 0.8-1.1, p=0.44); however, results were positive in the subgroup of patients with post-infarction angina (9.1% versus 14.2%, OR=0.6, 95% CI 0.4-0.9, P=0.04). At 6 months follow-up, the cumulative incidence of cardiac events was similar in the two treatment groups (25.7% versus 26.6%, OR 1.0, 95% CI 0.8-1.1, P=0.54). There was no control angiography included in this study. The level of clinical restenosis, as measured by the incidence of any complication after discharge, was also similar in the two treatment groups (21.0% versus 21.3% OR 1.0, 95% CI not reported, p=0.85). The incidence of bleeding in the bivalirudin-treated patients was significantly lower as compared to the heparin control subjects (3.8% versus 9.8%, OR 0.4, 95% CI 0.3-0.5, P< 0.001). The latter was reflected by the lower levels of systemic anticoagulation in the bivalirudin-treated group, as assessed by the measurement of ACTs. The authors raise the probability that equivalent rates of ischemic complications, may be the result of equivalent degrees of localized thrombin inhibition achieved at lower levels of systemic anticoagulation as reflected by the markedly lower incidence of bleeding. This raised the question whether the preference for

the chosen dosage regimen was maybe too low. Supporting arguments for that were a significantly lower ACT just before the coronary intervention in the hirulog-treated patients, and thus conclusions of the hirulog study are based on non-equivalent anticoagulant doses. However, because of the different properties of bivalirudin, in particular the activity against clot-bound thrombin, absence of natural inhibitors, and more predictable and less variable levels of anticoagulation, it was not a goal to achieve identical ACTs with the two agents. Moreover, a recent publication describes the absence of association between major bleeding and ACTs [100]. The experiences in both the hirulog and the hirudin trial emphasize the vital importance of critical investigation of dose finding studies for each subset of patients and clinical indication. Although the EPIC trial probably benefitted from an initially too potent combination of c7E3 and heparin, the dramatic reduction in clinical events appeared at the cost of bleeding. The reduction in clinical events justified further development and adjustment of the (heparin) dosage regimen to the narrow therapeutic window.

At least four other trials using specific antithrombotic drugs have demonstrated beneficial effects on the acute complications of coronary angioplasty without favourably influencing long-term clinical outcomes [92, 101-103]. This is in contradistinction with the EPIC trial [98, 99], which showed a reduction in early cardiac events that was maintained at long term follow-up. The study was not designed to detect angiographic restenosis; however the evident decrease in revascularization procedures and myocardial infarction may reflect the effect on the restenosis process. In the EPIC study, the event-free survival curve demonstrates a significant initial difference in clinical outcome, which is even more distinct during 6 months' follow-up as shown by the manifest divergence of the survival curves. It has been speculated that interaction of the compound with vitronectin results in an antiproliferative effect, which might explain the latter finding. The significant surplus of major bleeding complications in the EPIC trial (14.0 vs 6.6%, p=0.001) emerged as a major drawback for the monoclonal antibody. To investigate the effect of c7E3 on angiographic restenosis, the EPILOG (Evaluation of PTCA to Improve Long-term Outcome by c7E3 Glycoprotein IIB/IIIA Receptor Blockade) trial was designed testing two c7E3 Fab arms versus placebo in a planned 4800 patients. To control bleeding complications, the heparin regimens in the treatment arms (standard and low-dose heparin) were adjusted for weight. This change resulted in a reduction of bleeding rates to that of the same order as that of placebo. Inclusion was stopped following recruitment of 2792 patients, when interim analysis on 1500 patients revealed that the 30-day incidence of combined death and MI was reduced from 8.1% in the control arm to 2.6% and 3.6% in the low dose and standard heparin dose [104]. Compared with EPIC and because of the low bleeding rates, more patients received their complete c7E3 treatment, and differences in primary endpoint rates were therefore more distinct, despite the effect that in EPILOG patients with less complex lesions and patients suffering stable angina were randomized. Whether these results will translate into a significant reduction in angiographically assessed restenosis awaits adjudication.

Conclusions

The majority of clinical investigations with thrombin inhibitors has been in coronary artery disease. Despite the evident limitations of heparin and aspirin, their role in interventional cardiology is currently not replaced by a specific thrombin inhibitor. Thrombin antagonists represent a promising class of antithrombotic agents, that overcome the pharmacokinetic and pharmacodynamic restrictions of heparin and aspirin and appear safe for clinical application. The lack of specific antidotes with narrow therapeutic windows leaves a risk for overdosing, especially in the context of combined use with thrombolytics and impaired renal clearance, but the anticipated increase in bleeding complications in intracoronary intervention studies is controlled. Notwithstanding all the theoretical advantages this class embodies, including the established significantly superior acute clinical outcome in various restenosis prevention trials, phase III evaluation has not achieved a convincing improvement of the long term outcome. Numerous reasons have been proposed to explain this phenomenon, which is not yet clearly understood. Direct thrombin inhibitors at the currently administered dosage regimens do not block thrombin generation completely, as demonstrated by thrombin-antithrombin complex and $F_{1.2}$ measurements, and enable some thrombin molecules to escape. Local drug delivery devices may be of critical importance in further attempts to restrict thrombin's action. All large-scale and FDA-approved restenosis prevention trials examining antithrombins used only aspirin as concomitant drug, without investigating the potential of (weight-adjusted) concomitant heparin therapy as in the thrombolysis studies. Furthermore, there is much left to be learned from optimal dosing in specific conditions and indications, and finally, the discrepancy between animal experimental work and human surveys may suggest a drug-independent or thrombin-independent pathway of smooth muscle cell activation.

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Overview of the thesis

The two major shortcomings in interventional cardiology are abrupt vessel closure and late restenosis. A number of pharmacological trials have been conducted to address these phenomenons and reduce their clinically related presentation. The data presented and discussed in this thesis focus on the methodological aspects and results of clinical testing of direct thrombin inhibition aimed at the prevention of restenosis and related clinical events.

Several experimental, pathological and clinical studies suggest that critical events in the development of acute clinical events and of restenostic tissue are activation of the coagulation cascade, platelet aggregation and thrombus formation, while several mediators, released from activated platelets, promote proliferation and migration of various cell types. All of these steps give access for a diversity of pharmacological interventions. In chapter 1, the scientific basis of different entries are described and investigations in animals and human subjects carried out till the early nineties are described.

Thrombin itself is identified as the most potent platelet activator, and has a pivotal role in the coagulation system, directly mediating smooth muscle cell proliferation by way of stimulation of thrombin receptors at the smooth muscle cell surface. Thrombus indirectly induces an excessive intimal smooth muscle cell proliferation by means of released mitogens (growth factors), which may contribute to late restenosis. Therefore direct and irreversible thrombin blockade is deemed to be effective in the prevention of restenosis following angioplasty and is the scientific basis of the studies performed and described in this thesis.

We investigated the role of angiographically identifiable mural thrombus before or after angioplasty in the evolution of angiographically defined restenosis (chapter 2). Using categorical and continuous criteria for restenosis, delineated the significant contribution of thrombus to detrimental long term (6 months) results. The higher degree of restenosis in the 160 lesion with thrombus was due primarily to an increased incidence of occlusion at follow-up angiography in this group. This evidence supports the rationale of restenosis prevention by an antithrombotic approach.

The section 'Methodology and Trial Design' focusses at aspects of qualitative and quantitative angiogram reading and the design of phase II and III trials aimed at the prevention of restenosis.

Evaluation of the effect of pharmacological agents at the coronary artery dimensions over time is facilitated by the use of quantitative coronary angiography. At the Thoraxcenter, the computer assisted Cardiovascular Angiography Analysis System (CAAS), using an automated edge detection technique was developed and validated (chapter 3). This method of standardized data acquisition and data analysis enables multicenter investigations and has the advantage of being far more accurate and reproducible in the assessment of lesion severity, than visual or handheld caliper assessments. Besides that, an angiographic core laboratory may help demonstrating the reproducibility of qualitative factors and their role in the occurrence of acute and late complications of PTCA.

We recorded coronary guiding catheters on cinefilm, and compared their

automated quantitative measurements with their true values (assessed by precision micrometer measurement). It is demonstrated that the current generation of guiding catheters shows a variety in radiological quality. Almost all guiding catheters are suitable for calibration of QCA measurements, provided that calibration is done on contrast-empty catheters (chapter 4).

In chapter 5 the validity of quantitative coronary angiographic analysis of video images is assessed and compared to 35mm cinefilm data, acquired from a phantom study and clinical angiograms. Simultaneous recording was performed on 35mm cinefilm and a super-VHS tape with normal images and images with spatial filtering (edge-enhancement). In the phantom series the accuracy and precision of quantitative coronary angiography measurement for cinefilm were -0.10 mm ±0.08 mm, for normal-image videotape -0.11 mm ±0.18 mm and for edge-enhanced videotape -0.10 mm ±0.11 mm. In the clinical series, the differences between measurements from cinefilm and normal-image videotape were 0.14 mm ±0.20 mm and from cinefilm and edge-enhanced videotape were 0.04 mm and ±0.13 mm. In the experimental phantom study, the use of cinefilm resulted in the most precise measurements. In the clinical study, edge-enhanced videotape provided the highest agreement with measurements obtained from cinefilm. These findings suggest that cinefilm is more reliable than video as a recording medium for quantitative coronary analysis in scientific studies; however, for routine practice, videotape with edge enhanced images may provide an acceptable alternative.

We performed repeated visual assessment by two independent observers, to evaluate the reliability of qualitative angiogram readings (chapter 6). Inter-observer variability is expressed as kappa value and the percentage of agreement, and is 0.33 and 67.8% respectively for the ABC lesion classification (AHA/ACC criteria). Kappa and percent agreement for 10 other variables range from 0.29 to 1.00, and from 47.7 to 98.5% respectively. Intra-observer variability demonstrates higher values. The importance of complete and detailed definitions is outlined.

In chapter 7, we report our findings of an open study, where we investigated the safety and feasibility of increasing doses of intravenous argatroban (a selective synthetic thrombin antagonist that binds in a competitive way) in patients undergoing percutaneous transluminal coronary angioplasty. A total of 30 patients was studied in escalating dose regimens. Analysis of the adverse event profile, safety and haemostasis parameters indicate that argatroban in coronary angioplasty patients can be administered safely, and results in an adequate and predictable level of anticoagulation.

The HELVETICA trial is a multicentre, randomized, double blind and heparin controlled study, designed to compare the effects of two dose regimens of desirudin (recombinant desulphato hirudin/™REVASC) to those of heparin on event-free survival, safety and tolerability and luminal renarrowing by quantitative coronary angiography at the latest 26 weeks after the coronary angioplasty procedure (chapter 8). The outcome of this trial is decribed in the 'Results' section of this thesis.

One thousand one hundred and fourty one patients with unstable angina

who were scheduled for angioplasty were randomly assigned to receive one of the three treatments: a) a bolus dose of 10,000 IU of heparin followed by an intravenous infusion of 15 IU/kg/hr for 24 hours and subcutaneous placebo twice daily for 3 days (N=382), b) a bolus dose of 40 mg of desirudin followed by an intravenous infusion of 0.2 mg/kg/hr desirudin for 24 hours and subcutaneous placebo twice daily for 3 days (N=381) or c) the same desirudin regimen except that 40 mg of desirudin was given subcutaneously instead of placebo twice daily for three days (N=378). The primary objective was to compare the effects of treatment on event-free survival up to 7 months. The secondary objectives were to compare the occurrence of in-hospital events (within 96 hours), safety and tolerability of desirudin, and the quantitative cineangiographic measurement of the obstruction diameter at follow-up angiography.
Seven month, event-free survival was 67.3 percent in the group receiving heparin, 63.5 percent in the group receiving desirudin intravenous and 68.0 percent in the group receiving both intravenous and subcutaneous desirudin (P=0.61) (chapter 9). However, administration of desirudin was associated with a significant reduction in in-hospital events, which occurred in 11.0, 7.9, and 5.6 percent of patients in the respective groups (combined relative risk with desirudin: 0.61, 95 percent confidence interval 0.41 to 0.90, P-value 0.023). Minimal luminal diameter in the respective groups at 6 month follow-up angiography was 1.54 mm, 1.47 mm and 1.56 mm (P=0.08). So, although significantly fewer in-hospital major adverse cardiac events were observed in the treated group, administration of desirudin as described relative to heparin was not associated with a significant benefit on event-free survival or minimal luminal diameter at follow-up.

To identify predictors of in-hospital (96 hours) or late (7 months) occurring major adverse cardiac events in the HELVETICA population, we analyzed clinical, procedural and angiographic characteristics in a multivariable fashion (chapter 10). Braunwald anginal class III, age $\geq$ 60 years, presence of thrombus and dissection post-PTCA are independent risk factors for adverse in-hospital clinical outcome, whereas desirudin treatment and lesion type A are independent protective factors. Eighty % of the clinical outcome could be classified correctly using this model. Concerning 7-months outcome, Braunwald anginal class II and III, age $\geq$ 60 years and elevated levels of prothrombin fragment 1 and 2 are independent risk factors, while a history of myocardial infarction, lesion length $<$10 mm and a larger minimal lumen diameter post-PTCA are independent protective factors. Using this model, 66% of the clinical outcome could be classified correctly.

Ultimately, we can conclude that thrombin antagonists represent a very promising class of antithrombotic agents, that overcome particular restrictions of heparin and aspirin, and appear safe for clinical application (chapter 11). Notwithstanding all the theoretical advantages this class embodies, including the established significantly superior acute clinical outcome in various restenosis prevention trials, phase III evaluation has not achieved a convincing improvement of long term outcome. Further prospective and randomized research has to be addressed to dose-optimization and identification of subsets of patients who profit significantly from specific antithrombin therapy.

Samenvatting van de dissertatie

De twee belangrijkste beperkingen in de hedendaagse interventie-cardiologie zijn de acute vaat-afsluiting en de late restenose. Een aantal pharmacologische trials zijn uitgevoerd om deze fenomenen te bestuderen en een vermindering van de klinische presentatie ervan te bewerkstelligen. Het zwaartepunt van de gegevens zoals gepresenteerd en bediscussieerd in dit proefschrift ligt op de methodologische aspecten en resultaten van klinisch onderzoek met directe thrombine inhibitie met als doel de preventie van restenose en de daaraan gerelateerde klinische uiting.

Verschillende experimentele, pathologische en klinische studies suggereren een drietal kritische momenten in de ontwikkeling van acute klinische complicaties en de vorming van restenose weefsel; activatie van de stollings cascade, aggregatie van bloedplaatjes en thrombose vorming, en het aanzetten tot proliferatie en migratie van verschillende cel typen door mediatoren die vrijkomen uit geactiveerde bloedplaatjes. Elk van deze stappen geeft de mogelijkheid voor een aantal pharmacologische interventies. In hoofdstuk 1 wordt de wetenschappelijke achtergrond van deze mogelijkheden belicht en en worden dier-experimentele en humane studies zoals die uitgevoerd zijn tot in de negentiger jaren beschreven.
Thrombine is geïdentificeerd als de meest krachtige plaatjes activator, en speelt een cruciale rol in het stollingssysteem. Het brengt proliferatie teweeg van gladde spiercellen door directe stimulatie van thrombine receptoren aan de oppervlakte van deze cellen. De thrombose zelf induceert groei van gladde spiercellen door het vrijkomen van mitogenen (groei factoren), welke bijdragen aan de late restenose.
Directe en onomkeerbare blokkade van thrombine lijkt daarom effectief in de preventie van restenose na een coronair angioplastiek en vormt de wetenschappelijke basis van de uitgevoerde studies zoals beschreven in dit proefschrift.

We onderzochten de rol van angiografisch geïdentificeerde thrombose vóór of ná angioplastiek in de rol van angiografisch vastgelegde restenose (hoofdstuk 2). Het gebruik van categorische en continue criteria voor restenose maakte de significante bijdrage van thrombose aan de ongunstige lange termijn (6 maanden) resultaten duidelijk. De sterkere mate van restenose in de 160 vernauwingen mét thrombose was primair te wijten aan een toegenomen incidentie van occlusie ten tijde van follow-up angiografie. Deze gegevens ondersteunen het uitgangspunt van een antithrombotische benadering in de preventie van restenose.
Evaluatie van het effect van pharmacologische stoffen op coronaire dimensies in de tijd wordt mogelijk gemaakt door kwantitatieve coronair angiografie (QCA). In het Thoraxcentrum werd het computer geassisteerde 'Cardiovascular Angiography Analysis System (CAAS) ontwikkeld en gevalideerd (hoofdstuk 3). Deze techniek waarbij op een gestandaardizeerde methode data acquisitie en analyse plaatvindt, maakt multicenter onderzoek mogelijk en heeft het voordeel veel accurater en reproduceerbaarder de mate van coronair vernauwing te kunnen meten dan visuele schatting of handmatig afpassen. Daarnaast kan een angiografisch corelab de reproduceerbaarheid van kwalitatieve elementen en hun

rol in het optreden van acute en late complicaties na angioplastiek blootleggen.
De sectie 'Methodology and Trial Design' concentreert zich op aspecten van kwalitatieve en kwantitatieve angiogram beoordeling, de vormgeving en resultaten van fase II en fase III onderzoeken die restenose preventie als doel stellen.

We legden coronaire geleide catheters vast op cinefilm, en vergeleken hun gemeten dimensies (CAAS) met hun reële dimensies (bepaald met een micrometer). De huidige generatie van geleide catheters toont een grote variatie in radiologische kwaliteit. Nagenoeg alle geleide catheters zijn geschikt voor de calibratie van QCA-metingen, vooropgesteld dat deze calibratie plaatsvindt met een catheter tip die niet gevuld is met contrast-medium (hoofdstuk 4).

We verrichtten herhaalde visuele beoordeling door twee onafhankelijke observatoren, om de betrouwbaarheid van kwalitatieve angiogram beoordeling te evalueren (hoofdstuk 5). De 'inter-observer' variabiliteit wordt uitgedrukt in de kappa waarde en als percentage overeenkomst in waarneming. Deze is 0.33 and 67.8% respectievelijk voor de ABC-indeling voor type vernauwing (AHA/ACC criteria). Kappa en percentage overeenkomst voor 10 andere variabelen varieerde van 0.29 tot 1.00, en van 47.7 tot 98.5% respectievelijk. De 'intra-observer' variabiliteit vertoont hogere uitkomsten. Het belang van complete en gedetailleerde definities wordt hier aan de orde gesteld.
In hoofdstuk 6 worden de bevindingen beschreven van een open studie waarin de veiligheid en toepasbaarheid van oplopende doses van intraveneus toegediend Argatroban (een selectieve synthetische thrombine antagonist, die een competitieve binding aangaat) in patienten die een coronair angioplastiek ondergaan, worden geanalyseerd. Een totaal van 30 patienten werd bestudeerd in 4 oplopende doseringsgroepen. Het profiel van ongunstige klinische uitkomst, veiligheid en haemostase parameters werden geanalyseerd en tonen aan dat Argatroban in patienten die een coronaire angioplastiek ondergaan veilig kan worden toegediend, en resulteert in een adequate en voorspelbare omvang van ontstolling.
De HELVETICA studie is een multicentre, gerandomizeerde, dubbel blind opgezette en heparine gecontroleerde studie, opgezet om de effecten van twee verschillende doseringsschema's van desirudine (recombinant desulphato hirudine/™REVASC) te vergelijken met die van heparine op de event-vrije overleving, veiligheid, tolerantie en het optreden van restenose (gemeten met QCA) op 26 weken na de coronaire angioplastiek (hoofdstuk 7). De uitkomsten van deze trial zijn beschreven in het onderdeel 'Results' van dit proefschrift.
Elf honderd en een en veertig patienten lijdend aan onstabiele angina pectoris en wachtend op een coronair angioplastiek werden at random toegewezen in een van de behandelingsgroepen: a) een bolus dosis van 10,000 IU heparine gevolgd door een intraveneuze infusie van 15 IU/kg/hr gedurende 24 uur en subcutaan placebo twee maal gedurende 3 dagen (N=382), b) een bolus dosis van 40 mg desirudine gevolgd door een intraveneuze infusie van 0.2 mg/kg/hr desirudine gedurende 24 uur en subcutaan placebo twee maal daags gedurende 3 dagen (N=381) of c) het zelfde desirudine regime behalve dat 40 mg desirudine subcutaan werd gegeven in de plaats van placebo twee maal daags gedurende drie dagen (N=378). Het

primaire doel was om het eefect te vergelijken van de behandeling op de 7 maanden event-vrije overleving. Het secondaire doel was evaluatie van complicaties tijdens of direct na de angioplastiek binnen 96 uur), veiligheid en tolerantie van desirudine, en de kwantitative cineangiographische meting van de obstructie diameter na 6 maanden.
De 7 maanden event-vrije overleving was 67.3% in deheprine controle groep, 63.5% in de groep die desirudine intraveneus ontvangt en 68.0% in de groep die desirudine zowel intraveneus als subcutaan ontvangt (P=0.61) (hoofdstuk 8). Echter, toediening van desirudine was geassocieerd met een significante reductie in vroege complicaties (binnen 96 uur), welke verschenen in 11.0, 7.9 en 5.6% van de patienten in de respectievelijke groupen (gecombineerd relatief risico met desirudine: 0.61, 95% betrouwbaarheids interval 0.41 tot 0.90, P-waarde 0.023). Minimale luminale diameter in de respectievelijke groepen na 6 maanden angiografie was 1.54 mm, 1.47 mm and 1.56 mm (P=0.08). Hoewel dus significant minder vroege cardiale complicaties werden waargenomen in de met desirudine behandelde groep, was de toediening van desirudine zoals beschreven in vergelijking met heparine niet geassocieerd met een significant voordeel voor de complicatie-vrije overleving of de minimale luminale diameter na 6 maanden.

Om onafhankelijke voorspellers van cardiale complicaties te identificeren, werden klinische, procedurele en angiografische parameters van patienten in de HELVETICA studie op een multivariate wijze geanalyseerd (hoofdstuk 9). Angina pectoris klasse III volgens Braunwald, leeftijd ≥ 60 jaar, aanwezigheid van thrombus en dissectie na de angipolastiek zijn onafhankelijke risico factoren voor ongunstige vroege klinische uitkomst, terwijl behandeling met desirudine en lesie type A (AHA/ACC classificatie) onafhankelijke beschermende factoren zijn. Met behulp van dit model kan in 80% van de patienten de vroege klinische uitkomst correct worden voorspeld. Wat betreft de 7 maanden uitkomst zijn angina klasse II en III volgens de Braunwald classificatie, leeftijd ≥ 60 jaar en verhoogde spiegels van prothrombine fragment 1 en 2 onafhankelijke risico factoren, terwijl een myocard infarct in de voorgeschiedenis, lesie lengte <10 mm en een grotere minimale lumen diameter na de angioplastiek onafhankelijke beschermende factoren zijn. Met gebruik van dit model kan in 66% van de patienten de klinische uitkomst na 7 maanden correct worden geclassificeerd.

Uiteindelijk kunnen we concluderen dat de thrombine antagonisten een veelbelovende klasse van antithrombotische middelen vertegenwoordigd, die de specifieke tekortkomingen van heparine en aspirine overwint, en die veilig toepasbaar zijn voor klinische toepassing (hoofdstuk 10). Echter, afgezien van de theoretische voordelen die deze groep omvat, inclusief de vastgestelde significant superieure acute klinische resultaten in diverse restenose preventie trials, heeft fase III evaluatie met deze pharmaca geen overtuigende verbetering van de lange termijn resultaten kunnen aantonen. Verder prospectief en gerandomizeerd onderzoek, gericht op optimalisering van de gebruikte dosis en identificatie van subgroepen van patienten die significant voordeel behalen van specifieke antithrombine therapie.

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