Pharmacologic aspects of new classes of anti-cancer agents: inhibitors of topoisomerase I or tubulin

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Farmacologische aspecten van nieuwe groepen antikanker middelen: remmers van topoisomerase I of tubuline

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Voor Dop

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Chapter 1

Introduction to the thesis

INTRODUCTION

Many processes involved in unregulated proliferation of cells are subject to antitumor therapy, inhibition of the nucleair enzyme topoisomerase I and protein tubulin being two of them. Important (pre-)clinical observations, such as synergism with other cytotoxic agents, the benefices of oral therapy as a means of prolonged exposure and the crucial role of the solubilization agent Cremophor EL, are involved in their further clinical development and in refinement of existing therapies. Given the narrow therapeutic window, means to improve the individual dosing precision need to be studied. Clinical pharmacological studies, as described in this thesis, are intended to serve as a guide to better chemotherapy schedules for the individual cancer patient.

The first inhibitors of topoisomerase I and tubulin, camptothecin and paclitaxel respectively, both originated from the National Cancer Institute's screening program of natural products in the 1960s. Camptothecin is a plant alkaloid extract from the *Camptotheca acuminata*, an oriental tree that is cultivated through Asia, and paclitaxel is the active ingredient of the crude extract of the bark of the Western yew tree, *Taxus brevifolia*. Both extracts were found to be active against several murine tumors (1,2). Clinical development of both compounds was initially hindered, postponing their registration for clinical use to the 1990s. Initial clinical studies with camptothecin were complicated by severe and unpredictable toxicity, but development of less toxic analogues of camptothecin led to registration of topotecan and irinotecan, while other analogues are in different stages of development. Because of problems in drug solubilization, paclitaxel's development also was initially suspended. A formulation using 50% ethanol and 50% Cremophor EL (produced by the reaction of castor oil, obtained from the seeds of *Ricinus communis*, with ethylene oxide) was finally selected for further development. However, the important clinical consequences of Cremophor EL have been underestimated thus far. In the first part of this thesis pharmacological and phase I studies of new oral camptothecin analogues are described, whereas pharmacological studies of paclitaxel with a focus on the clinical relevance of Cremophor EL is the subject of the second part.

Part I starts with a review on oral topoisomerase I inhibitors. This group of anticancer agents shows significant activity in human tumor xenografts especially with prolonged duration of exposure (3). Since oral drug delivery is a convenient method for prolonged drug exposure and preferred by patients (4), further development of oral formulations seems warranted. For this development, efforts to improve absorption and to reduce the inter- and intrapatient variation in systemic exposure are as important as the search for optimal schedules and synergistic combinations with other anticancer agents. The next chapter describes a phase I and pharmacological study of the oral homocamptothecin diflomotecan and defines a recommended dose for further studies. Homocamptothecins, more potent than other topoisomerase I inhibitors *in vitro*, exert greater stability of the active lactone form in plasma, which may be advantageous for antitumor activity (5). Our studies show a high bioavailability of oral diflomotecan of 67%, which is another advantage over the other oral topoisomerase I inhibitors. Three different phase I and pharmacological studies with combinations of oral topotecan and intravenous cisplatin are described in part I. These

combinations are attractive given the observed pre-clinical synergism (6). This synergism may be based on the combination of DNA-damage by cisplatin and interference with DNA-repair by the topoisomerase I inhibitor topotecan. This concept may also explain the observed sequence dependent pharmacodynamic interaction, in the absence of any pharmacokinetic interaction. Related to these 3 phase I studies 3 pharmacological studies originated: one addressing the influence of pleural- and ascitic fluid on the pharmacokinetics of topotecan, another study on the gender-dependent differences in oral topotecan pharmacokinetics and finally one on the (non)sense of body-surface area dosing of oral topotecan. The 'bridge' to the second part of the thesis involves a study on the influence of Cremophor EL on the bone marrow toxicity caused by cisplatin, administered in combination with topotecan.

Part II of the thesis also starts with a review. The important biological and pharmacological, and therefore clinical, role of Cremophor EL, is outlined. It will be essential to find alternative ways to solubilize the drugs currently formulated in CrEL and, in some cases, to explore potential positive effects of this castor oil derivative. An example of such a positive effect is the observation that Cremophor EL is responsible for high concentrations of paclitaxel after intraperitoneal chemotherapy with paclitaxel, possibly by entrapment in Cremophor micelles (7). Since Cremophor El clearance increases with extending the infusion duration, we hypothesized that exposure to unbound paclitaxel was also schedule dependent, as would be paclitaxel-related side effects. Part II was completed with a pharmacokinetic study using [G^3H]paclitaxel in a patient with severely impaired renal function, to determine paclitaxel's complete metabolic fate in such a case.

This thesis describes some classical phase I studies with oral topoisomerase I inhibitors, but the main part consists of extensive pharmacologic evaluation of patients receiving inhibitors of topoisomerase I and paclitaxel: solving as many clinical questions as raising them, but hopefully serving the ultimate goal of better treatment for cancer patients.

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PART I

INHIBITORS OF TOPOISOMERASE I

Chapter 2

Oral topoisomerase I inhibitors in adult patients: present and future

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SUMMARY

The renewed interest in topoisomerase I inhibitors, based on new insights on the mechanism of action and the development of semi-synthetic derivates of camptothecin with a more favourable toxicity profile, has led to extensive preclinical and clinical research. Significant levels of anti-tumor activity in human tumor xenografts were seen especially with prolonged duration of exposure. Since oral drug delivery is a more convenient method for prolonged drug administration, and preferred by patients, further development of oral formulations seems attractive.

Common concerns in the development of oral formulations are their sometimes low oral bioavailability and the frequently large intra- and interpatient variation in systemic exposure. Efforts to improve absorption and minimize intestinal metabolism/efflux of the oral chemotherapeutic agent using new formulas might lead to better bioavailability. Pharmacokinetic and pharmacodynamic evaluations have enabled guidance in recommendations of schedules. Given the interpatient variation in exposure it is interesting to note that flat dosing of topotecan resulted in the same systemic exposure compared with the more complex dosing per body surface area. In order to diminish the interpatient variation in exposure to 9-AC a limited sampling model for oral 9-AC was developed, enabling prediction of the systemic exposure for 9-AC and optimizing treatment for any given patient.

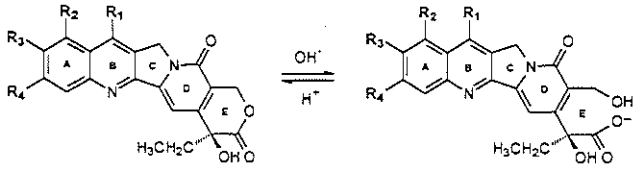
Drug sequencing plays a key role in the combination topotecan/cisplatin and might be important for combination with other classes of drugs. Therefore, forthcoming phase I trials on combination therapy with oral topoisomerase I inhibitors should include studies on sequence dependence and pharmacokinetic analyses to evaluate any mutual interaction.

INTRODUCTION

In the 1960's camptothecin, a plant alkaloid extract from the Camptotheca acuminata, an oriental tree which is cultivated throughout Asia, was found to be active against L1210 murine leukemia in the National Cancer Institute's screening program of natural products [1]. Meanwhile, we have learned that camptothecin analogues are a class of anticancer agents acting through reversible inhibition of DNA topoisomerase I [2,3]. Topoisomerase I is a nuclear enzyme present in all eukaryotic cells [4], that induces single strand breaks allowing the hypercoiled, double stranded DNA to uncoil, thereby enabling replication and transcription and subsequent strand religation in DNA. Topoisomerase I inhibitors bind to the topoisomerase I DNA complex, stabilizing the enzyme on DNA and preventing topoisomerase I from resealing the DNA strand prior to division and replication. Topoisomerase I inhibitors exert their cytotoxic effect by the S-phase specific arrest of replication at the single strand level causing irreversible double strand breaks and cell death [5-7].

In the early 1970's, the parent compound camptothecin underwent clinical testing. However further clinical development was precluded due to severe and unpredictable toxicities including myelosuppression, diarrhea and hemorrhagic cystitis [8-11]. In the 1980's,

topoisomerase I was identified as the major target for the antitumor effect of camptothecin [12,1] and overexpression of topoisomerase I levels were found in colon and ovarian cancer compared with normal tissue [13,14]. These findings led to renewed interest in this class of agents resulting in the development of better water soluble semi-synthetic analogues of camptothecin (see figure 1) that were to be less toxic through their better solubility, whereas toxicity was also hoped to be better predictable. This development has recently led to the approval of topotecan for use in advanced ovarian cancer in patients who failed one platinum-based regimen [15] and of irinotecan for use in the second line treatment of recurrent metastatic colorectal cancer after failure on 5FU-based therapy [16].



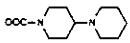
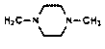
Compound	R_1	R_2	R_3	R_4
topotecan	H	H_2C-N-$(CH_2)_2$	H	H
CPT-11	CH_2CH_3	H		H
SN-38	H	H	OH	H
9-AC	H	NH_2	H	H
9-NC	H	NO_2	H	H
camptothecin	H	H	H	H
lurtotecan		H	O−CH_2-CH_2-O	

Figure 1 Chemical structures and pH-dependent interconversion of the lactone and carboxylate forms of topoisomerase-I inhibitors

Assessment of the efficacy of topotecan and irinotecan in other tumor types, the combination with other cytotoxic agents, and the development of other new topoisomerase I inhibitors are subject to many preclinical and clinical studies.

In in vitro studies topoisomerase I inhibitors showed more pronounced antitumor efficacy with protracted exposure at low concentration. Also in animal models, low dose prolonged exposure resulted in less toxicity [17-26]. It should be stated though, that most animal

models are poor models for toxicity with the camptothecin analogues since they are relatively resistant to the myelosuppressive effects. In order to simulate these prolonged exposures, various phase I and II studies have focused on low dose continuous infusion of topoisomerase I inhibitors in cancer patients [27-36]. Most of the studies showed that continous i.v. administration is feasible. Whether it is also more effective is yet unknown. Since oral administration is a more convenient and more cost effective method for prolonged drug administration further development of oral formulations of topoisomerase I inhibitors was given priority, also in view of data indicating that intragastric administration was effective in animal models [25]. Since most of the oral topoisomerase I inhibitors have relatively short half-lives the use of protracted oral dosing is not always the same as continuous intravenous administration, although if the concept of time over threshold concentration is a valid indication of toxicity and efficacy, oral dosing can mimic continuous infusion [37].
This review focuses on the early clinical studies related to the development of the oral topoisomerase I inhibitors.

TOPOTECAN

Topotecan, 9-dimethylaminomethyl-10 hydroxycamptothecin, is a water soluble semisynthetic analogue of camptothecin [38]. The active lactone structure undergoes pH dependent reversible hydrolysis to the carboxylate form. At physiological pH most topotecan is in the inactive carboxylate form, whereas in acidic environment the ratio is opposite. Lactone to carboxylate ratios were comparable after oral and intravenous administration [39,40]. The bioavailability of the intravenous formulation when given orally is 32-44% with moderate intrapatient variability. After oral administration topotecan is rapidly absorbed with peak plasma concentrations reached at 0.6 - 0.78 hours after intake. No relationship was found between bioavailability and age, gender, performance score and the presence of liver metastasis. Topotecan is presently supplied in gelatin capsules and is administered at least 10 minutes before a meal, although combination with a high fat meal only led to a small decrease in the rate of absorption but not in the extent of absorption [41]. An overview of all schedules with oral topotecan is presented in table 1.

Table 1 Schedules with oral topotecan: Dose Limiting Toxicity (DLT) and Maximum Tolerated Dose (MTD).

Schedule	DLT	MTD (mg/m^2)	ref.
21 days q 4 weeks	diarrhea	0.5 b.i.d.	42
10 days q 3 weeks	thrombocytopenia and diarrhea	b.i.d. 1.4 o.d.	44
5 days q 3 weeks	neutropenia	2.3 o.d.	56
5 days q 3 weeks with:			
CDDP 75 mg/m2 day 1 or:	neutropenia and thrombocytopenia	1.25 o.d.	71
CDDP 75 mg/m2 day 5		2.0 o.d.	71

Single agent oral topotecan

Given the relatively short half life of topotecan (average 2.4 hrs), and in order to mimic as closely as possible the continuous infusion schedule, the first phase I trial in adults on oral topotecan studied a twice daily administration for 21 days in a 28-day cycle [42]. The hematologic toxicity was mild and mainly consisted of granulocytopenia occurring in 11 of 31 patients. Neutropenic fever was only seen once. Nausea and vomiting common toxicity criteria (CTC) grade 1-2 occurred in respectively 32%, and 12% of patients (without routinely prescribed anti-emetics), diarrhea occurred in 55% of patients, and alopecia in 6%. The dose limiting toxicity (DLT) was reached at a dose of 0.6 mg/m^2 twice daily and consisted of diarrhea, requiring hospitalization for parenteral fluid and electrolyte therapy, with a median day of onset on day 15 (range 12-20) and resolving after a median of 8 days (range 7-16). Vigorous administration of loperamide had no effect on the diarrhea.

In an effort to circumvent the diarrhea, and making use of the report that topoisomerase I inhibition might be optimal after 2 weeks of continuous topotecan administration [43], the next study was performed with a shorter schedule of administration. Patients were treated with oral topotecan once or twice daily for 10 days, cycles repeated every 3 weeks [44]. In the once daily topotecan schedule, DLT was reached at 1.6 mg/m^2/day and consisted of thrombocytopenia and diarrhea. The median duration of diarrhea $\geq$ CTC grade 2 (4 days) was shorter than with the 21 days schedule, with a median onset on day 9, occurring in 7 out of 19 patients. Granulocytopenia was observed in only 4% of courses, occurring at day 8-12 and lasting 8-12 days. One patient had neutropenic fever. In the twice daily regimen, DLT also consisted of thrombocytopenia and diarrhea at 0.8 mg/m^2 b.i.d. Granulocytopenia again was observed in a minority of courses (5%). The recommended phase 2 doses are 0.7 mg/m^2/day b.i.d. and 1.4 mg/m^2/day o.d.

The third phase I trial performed on single agent oral topotecan was based on the widely accepted schedule of intravenous administration of topotecan given daily times 5 every 3 weeks [15,45-55]. Similar to the experience with the intravenous formulation the dose limiting toxicity with this daily times 5 oral topotecan schedule was granulocytopenia. The median duration of granulocytopenia was 6 days (range 2-12) with the nadir found between day 8 and 15. Non-hematologic toxicities were mild. Diarrhea $\geq$ CTC grade 2 occurred in 21% of the patients and was always self-limiting. The recommended dose for further studies was 2.3 mg/m^2/day [56]. Assuming an average body surface area in patients of 1.75 m^2 2.3 mg/m^2/day equals a fixed dose of 4 mg/day. Pharmacokinetics and toxicity were studied at this fixed dose of 4 mg/day in order to see whether dosing on a mg/m^2 basis offered any advantage over the easier fixed dose. Pharmacokinetics at the recommended dose of 2.3 mg/m^2/day (see figure 2A for an example of the plasma concentration versus time curve) were similar to those at the fixed dose of 4 mg/day [56]. The observed non-hematologic toxicities were also comparable. Thus the easier fixed, or flat, dosing of oral topotecan is feasible. The various schedules oral topotecan administration are summarized in table 1.

In general, hematologic toxicity is more pronounced but still mostly mild and non-cumulative with the shorter schedules, whereas diarrhea is an important sometimes unpredictable, severe and uncontrollable side effect of prolonged daily administration. In an

analysis of the pharmacokinetic/pharmacodynamic relationships, it appeared that the total AUC per course did not differ between the various schedules [57]. When analysing the time over a threshold concentration of 1 mg/ml, it appeared that the daily times 5 schedule provided best systemic exposure and toxicity profile [57]. Although the daily times 5 oral topotecan administration, compared with the same i.v. schedule, resulted in a lower systemic exposure, significant sigmoidal relationships were established between the area under the plasma drug concentration-time curve (AUC) of topotecan lactone and the percentage of decrease of leucocytes, granulocytes and platelets. The question still remained whether the systemic exposure resulting from oral administration was sufficient for an antitumor effect. Phase III studies were designed to answer this question.

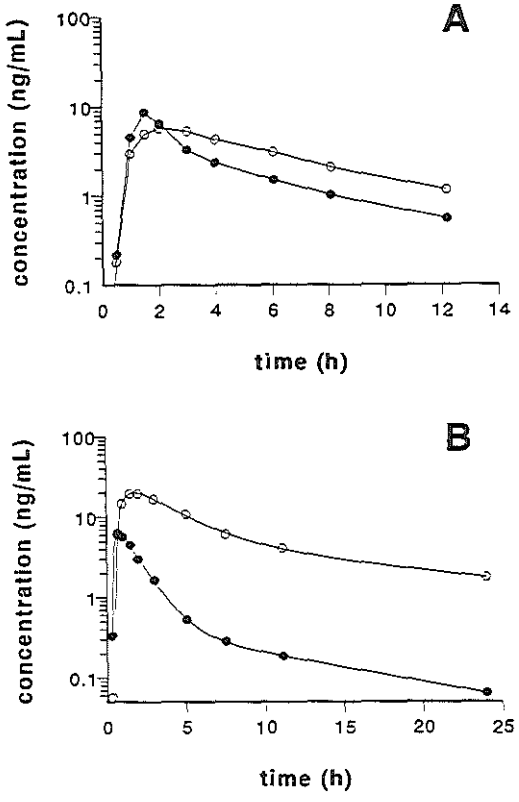


Figure 2 Representative plasma concentration versus time profiles of topotecan (panel A) and 9-amino-camptothecin (panel B) in the lactone (·) and carboxylate forms (°). Data were obtained from 2 single patients receiving topotecan at a dose of 2.3 mg/m^2 and 9-aminocamptothecin at a dose of 0.84 mg/m2 (obtained from Gerrits et al [44] and De Jonge et al [37], respectively). Note the different abcissa scales used for topotecan and 9-aminocamptothecin.

The daily times 5 schedule was used in a study of oral topotecan versus i.v. topotecan for second line therapy in sensitive (defined as recurrence ≥ 3 months after cessation of initial chemotherapy) patients with small-cell lung cancer [58]. One hundred and six patients were randomized: 52 oral and 54 i.v. In the oral arm, the response rate was 23,1%, the median time to progression 14.9 weeks and the median survival 31.4 weeks: in the i.v. arm respectively: 14,8%, 13.1 weeks and 25.7 weeks. Grade 3-4 neutropenia was less frequent with the oral formulation (oral: grade 3-25%, grade 4-13% and i.v.: grade 3-39%, grade 4-34%), other toxicity was comparable. These preliminary data suggest that the oral formulation of topotecan has similar efficacy to the i.v. form whilst associated with less neutropenia. Comparative results were obtained in a similar study in patients with advanced epithelial ovarian carcinoma who had received one platinum-based regimen [59].

In conclusion: the preliminary reports of these phase II and III studies comparing the daily x 5 every 3 weeks schedule of oral topotecan versus i.v., resulted in similar efficacy and less hematological toxicity. And thus render oral use of topotecan very attractive for continual development.

Combination therapy with oral topotecan

Since topoisomerase I inhibitors are known to interfere in processes involved in DNA repair, combining these agents with DNA damaging drugs would seem advantageous. The combination of topotecan and cisplatin showed synergism in several cancer cell lines: ovarian cancer, teratocarcinoma, glioma and (non-)small cell lung cancer [60-63]. The mechanism of interaction was studied by Zeghari and Goldwasser [64,65]. The topoisomerase I inhibitor delayed the reversal of cisplatin induced DNA interstrand cross links (ISC's) without modifying the formation of ISC's. Simultaneous treatment also prolonged the DNA and RNA synthesis inhibition induced by either drug alone. Ma et al [66] observed a strong schedule dependent in vitro effect, with synergy increasing when topotecan (or irinotecan) was preceded by a platinum derivative. The different toxicity profiles of topoisomerase I inhibitors and platinum derivatives further support the use of these agents in combination. In clinical trials, several schedules combining i.v. topotecan with cisplatin were studied [63,67-70]. These studies showed that considerable dose reduction of topotecan was required as compared to the single agent dose, and that cisplatin given before topotecan produced a greater incidence of severe neutropenia. Congruent to the phase I study on i.v. topotecan by Rowinsky et al [63], a phase I study was performed on the combination of oral topotecan given daily times 5 every 3 weeks and cisplatin 75 mg/m^2 administered i.v. either on day 1 or day 5 in a cross-over design [71]. Neutropenia and thrombocytopenia were dose limiting. The recommended dose of oral topotecan for phase II studies in the sequence of cisplatin followed by topotecan was 1.25 mg/m^2/day, day 1-5. In the reversed sequence topotecan 2.0 mg/m^2/day could be combined with cisplatin on day 5. Again, the sequence that permitted the highest dose intensity was topotecan followed by cisplatin. In contrast to the results of Rowinsky et al [63], a pharmacokinetic interaction that could explain the difference in side effects was not observed [72]. Also, the repair of cisplatin induced DNA damage measured as platinum-DNA adduct formation in white blood cells was not different

in the two schedules. Whether the drug sequence is also relevant for the anti-tumor activity is yet unknown. Phase II/III studies in patients with tumors that are sensitive to cisplatin and topotecan will be required to answer this question.

In an effort to enhance the dose intensity of the cisplatin, a phase I study is being performed in patients with recurrent or progressive ovarian cancer combining weekly cisplatin at a dose of 70 mg/m^2 with various schedules of oral topotecan. This study is based on experience obtained with cisplatin given weekly in combination with either oral VP16 or with i.v. paclitaxel, yielding high response rates in patients with recurrent or progressive ovarian cancer after prior platin based regimens [73,74].

IRINOTECAN

Irinotecan (CPT-11), 7-ethyl-10 [4-(pipiridino)-1-piperidino] carboxyloxy-camptothecin is a water-soluble analogue of camptothecin. CPT-11 is a pro-drug that is converted in the liver by carboxylesterase and/or butyrylcholinesterase [75,76] to SN-38 (7-ethyl-10-hydroxycamptothecin), a metabolite that is 1000-fold more potent in vitro than the parent drug [77,78]. In animals, peripheral conversion of CPT-11 to SN-38 also has been found in serum [79], small intestine [80] and possibly even within the tumor [81,82]. SN-38 undergoes conjugation to an inactive β-glucuronide derivate SN-38 G [83]. Other known inactive CPT-11 metabolites are APC:(7-ethyl-10-[4-N-(5-aminopentatanoicacid)-1-piperidino] carbonyloxy-camptothecin) and NPC:(7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecin) resulting from a cytochrome P-450 3A-mediated pathway [84.85]. A study to determine the complete metabolic fate and disposition of irinotecan in plasma, urine and feces was only able to account for half of the administered dose in urine and feces, indicating the possible existence of further unknown metabolites [86-88]. The terminal disposition half-life ($t_{1/2}$) of irinotecan is approximately 17 hours which is much longer than the $t_{1/2}$ of topotecan. Therefore, irinotecan has been studied in schedules different from topotecan. However, the optimal administration schedule remains uncertain. A phase III study comparing the weekly versus 3-weekly administration of irinotecan in patients with colon cancer is ongoing. Apart from the antitumor activity in colorectal carcinoma, interesting response rates were observed in patients with non-small cell lung cancer, cervical cancer, epithelial ovarian cancer as well as in small-cell lung cancer [89-91]. Combination therapy with platinum-derivatives seems to be highly active in several other tumor types as well [92-103]. No pharmacokinetic interaction or sequence dependency between cisplatin and irinotecan was found thus far. Dose limiting toxicities in most studies were neutropenia and diarrhea. In single agent regimens, diarrhea is the most important side effect of irinotecan. The early onset diarrhea with abdominal cramping, flushes and transpiration, suggestive of a release of vaso-active compounds, responds well to treatment with atropine. The late onset secretory diarrhea, which seems to be correlated with the biliary excretion of SN-38 in the intestinal lumen [104] and with a high fecal SN-38 to SN-38G ratio is thought to be related to structural and functional injuries to the intestinal tract by SN-38.

Oral irinotecan

Presystemic conversion in the intestine of irinotecan to the active form SN-38 as demonstrated in tumor bearing mice constitutes one of the rationales for further studies on oral irinotecan [80]. The efficacy of CPT-11 in tumor bearing mice is similar for the oral and i.v. routes of administration and the oral bioavailability in mice is 12-20% [105]. A phase I study with oral irinotecan administered daily times 5 every 3 weeks has been completed [106]. The i.v. formulation of irinotecan was administered orally, diluted in Cran-Grape® juice, after 4 hours of fasting to 28 patients (currently a gelatin capsule is in development). The acidic gastric pH could favor the absorption of the active lactone forms of irinotecan and SN-38. Delayed diarrhea, with a median onset on day 5, was the principal DLT encountered at the 80 mg/m^2/d dosage in patients younger than 65 years of age, and at 66 mg/m^2/d in patients $\geq$ 65 years of age. Grade 4 neutropenia was observed in one patient < 65 years of age treated at the 80 mg/m^2/d dosage. Median time to neutrophil nadir was 9 days, and the duration was only 1-2 days. All other toxicities were mild. The recommended phase 2 dose for oral CPT-11 administered daily for 5 days every 3 weeks is 66 mg/m^2/d in patients younger than 65 years of age and 50 mg/m^2/d in patients 65 or older (table 2). Interestingly, pharmacokinetic data reveal a mean relative ratio of total SN-38 concentration to total CPT-11 AUC of 0.15 and 0.11 on days 1 and 5, respectively. This compares with values that generally have been between 0.02 and 0.07 after i.v. administration [107] suggesting that oral dosing results in presystemic conversion of CPT-11 to the active SN-38. Although CPT-11 lactone AUC's achieved orally were 5 times lower than those reported after i.v. administration, an average of 72 % of SN-38 existed in the active lactone form during the first 24 hours after drug administration which compared with 45-64% reported in trials involving i.v. CPT-11 [108]. SN-38 also persisted for a longer period in the lactone form compared to the i.v. route [106].

Table 2 Schedules with other oral topoisomerase I inhibitors: Dose Limiting Toxicity (DLT) and Maximum Tolerated Dose (MTD)

Schedule	**DLT**	**MTD (mg/m^2/d)**	**Ref.**
Irinotecan day 1-5	diarrhea	50 ($\geq$ 65 years)	106
q 3 weeks		66 (<65 years)	
9-AC day 1-14	thrombocytopenia, neutropenia	0.84	37
q 3 weeks	and diarrhea		
9-NC day 1-5	anemia, neutropenia and	1.5	124
q 1 week	thrombocytopenia		
Camptothecin day 1-21	diarrhea	6.5	124
q 4 weeks			
Camptothecin day 1-14	neutropenia	6	129
and etoposide day 20 q?		500	

These data, implicating a higher therapeutic index for the oral formulation compared to the i.v. administration of CPT-11, favour the further development of an oral formulation. Other studies with oral irinotecan are ongoing and no official reports have been published yet.

9-AMINO-20(S)-CAMPTOTHECIN (9-AC)

9-AC is a synthetic derivate of camptothecin, that does not induce the haemorrhagic cystitis associated with the parent compound. As for other camptothecins, in biological systems 9-AC exist in equilibrium between the active lactone and inactive hydrolised (open-ring) form. Following an i.v. infusion, only 9% of the drug is in the active lactone form. Pre-clinical studies indicated that a prolonged exposure above a threshold concentration of $\geq$ 10 nmol/L was necessary to maximise drug efficacy [109,110]. Therefore, clinical development focused on studies with various schedules of prolonged i.v. administration [111-115] and subsequent studies with prolonged oral exposure. Unlike other camptothecin analogues, 9-AC is poorly water soluble and requires a lipophilic formulation. It can be administered orally as a colloid dispersion (CD) or as gelatine capsules in polyethylene glycol (PEG)1000.

Oral 9-AC

The oral CD formulation was studied in a daily times five schedule with cycles repeated every 2 weeks [116], based upon results obtained in a 5-day oral study in dogs [117]. The formulation was administered in 20 ml orange juice to a group of 18 cancer patients at doses of 0.2 - 0.6 mg/m^2 daily. Grade 1-2 nausea was common. Wide interpatient variability in the 9-AC AUC and maximal drug concentration were observed. Likewise, there was a poor correlation of these parameters with the dose administered. The investigators concluded that the oral CD formulation was not suitable for further clinical development.

The bioavailability of an oral [PEG-1000] formulation was investigated in a phase I cross-over study [118]. Twelve patients were randomised to receive either 1.5 mg/m^2 9-AC orally on day 1 and 1.0 mg/m^2 i.v. on day 8 or vice versa. Except from one patient who experienced nausea, no other toxicities were seen. The overall oral bioavailability averaged 48.6 $\pm$ 17.6%, indicating significant systemic exposure to the drug, which may enable oral treatment. Twenty-seven patients were treated in the phase I and pharmacokinetic study on oral [PEG-1000] 9-AC given once a day for 7 or 14 days repeated every 21 days [27]. DLT of oral 9-AC given for 14 days every 3 weeks consisted of a combination of thrombocytopenia, febrile neutropenia and diarrhea. Although the hematological toxicity was relatively mild, treatment had to be delayed in 23% of the cycles due to prolonged myelosuppression. Diarrhea was usually also mild with a median day of onset on day 12, and a median duration of 3 days. The recommended dose for phase II studies is 0.84 mg/m^2/day. Pharmacokinetic evaluation showed that concentrations of 9-AC lactone $\geq$ 3.93 ng/ml (10 nmol/L) were achieved on every treatment day (day 1-14) at the recommended dose level (see figure 2B for an example of one treatment day). In previous studies of the intravenous administration of 9-AC only in the schedule studying the 24-hour infusion of 9-AC once weekly for 4 weeks every 5

weeks, the steady state concentration of 9-AC reached this threshold value [119]. This might be a therapeutical advantage for the oral [PEG-1000] formulation. Although there was a high degree of interpatient pharmacokinetic variability, the intrapatient variability was fairly limited and oral delivery was not associated with increased interpatient differences in systemic exposure compared. A limited sampling model to predict systemic exposure to oral 9-AC was developed with the use of the pharmacokinetic data of the phase I study with oral [PEG-1000] 9-AC [120]. The reliable prediction of the systemic exposure to oral 9-AC using one time point determination permits treatment optimization for patients on the basis of individual pharmacokinetic characteristics. Development of intravenous 9-AC has been discontinued because of its limited activity. Since systemic exposure achieved with the intravenous schedules was considerably lower than with the 14 days every 3 week schedule of the oral [PEG-1000] formulation of 9-AC, the oral formulation still warrants further study using an individualized dosing strategy.

9-NITROCAMPTOTHECIN (9-NC)

9-NC is a prodrug of 9-AC [121,122]. The anti-tumor effect of 9-NC has been documented in pre-clinical studies and again the prolonged exposure schedule seems to be the most effective [123]. 9-NC is chemically more stable than 9-AC and easier to produce. Like 9-AC, 9-NC is also water insoluble. For oral use the drug is encapsulated in gelatin capsules. Only one phase 1 study evaluating oral 9-NC has been published [124]. Twenty-eight patients with advanced cancer refractory to conventional chemotherapy were treated for 5 days every week for 4 weeks. The DLT consisted of anemia, neutropenia and thrombocytopenia and the MTD has been estimated at 1.5 mg/m^2/day for 5 consecutive days weekly. Non-hematological side effects consisted of diarrhea (14% of all patients), nausea and vomiting (18%) and haemorrhagic cystitis (14%). In two patients, haemorrhagic cystitis led to treatment discontinuation. One third of patients treated at the next dose level of 2.0 mg/m^2/day experienced dose limiting toxicity, thus indicating a very narrow therapeutic index. An important drawback of the study was the lack of information on bioavailability. A phase II study at the recommended dose and schedule was performed in patients with refractory epithelial ovarian, tubal or peritoneal cancer [125]. In 29 evaluable patients, 2 partial remissions and 10 stabilizations of disease were observed. The median survival in this heavily pretreated group was 35 weeks. Gastrointestinal and urine bladder toxicity were the most prominent side effects, apart from myelosuppression, as reported for the parent compound, camptothecin, in earlier studies.

CAMPTOTHECIN

20-(S)-Camptothecin (CPT), the parent compound of all topoisomerase I inhibitors, has recently been reintroduced into clinical studies as an oral formulation in gelatin capsules. The initial clinical trials in the 1970's did not make use of native camptothecin, but of its water soluble salt. As has been established, conversion to the sodium salt opens the lactone ring of

camptothecin, effectively neutralizing its antitumor activity. Oral camptothecin given daily for 21 days every 4 weeks was studied in a phase I study in 52 patients [126]. In 12 patients, treatment could be continued for 6-12 months, in 5 patients for more than one year without progression of disease. Diarrhea was dose limiting at 15.4 mg/m^2/day. The recommended dose in this schedule was 6.5 mg/m^2/d. The difference between DLT and recommended dose is remarkable. An unacceptable number of patients (20%) experienced chemical cystitis. Pharmacokinetic data were too scarse for calculation of the oral bioavailability of the drug. A second phase I study investigated the combination of escalating doses of intravenous etoposide administered on day 20 and preteatment with oral camptothecin (6 mg/m^2/daily day 1-14). This study was based on preclinical studies indicating synergistic- and sequence dependent cytotoxicity of the combination of topoisomerase I and II inhibitors. The expression of topoisomerase II is enhanced in topoisomerase I deficient cells [127] and cell lines with deficient topoisomerase I activity appear to be more sensitive to treatment with topoisomerase II inhibitors [128]. Dose limiting toxicity proved to be neutropenia lasting for more than 7 days in 2 out of 6 patients treated at an etoposide dose of 500 mg/m^2. Chemical cystitis was observed in 3 out of 23 treated patients and resolved completely after discontinuation of the drug. No other toxicities were seen [129]. Only 4.8% of the total drug exposure was found to be in the active plasma lactone form. Substantial interpatient variability in bioavailability in the rate and extent of absorption was observed which has been shown by others not to be pH dependent [130]. The unpredictable toxicity and absorption render this formulation of the agent unattractive for further studies.

LURTOTECAN (GI 147211, GG211)

GI 147211, (7-(methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin, is a water soluble semisynthetic camptothecin analogue. Again the best effect on tumor growth in pre-clinical studies was achieved by prolonged dosing [131]. A daily times 5 every 3 weeks i.v. schedule induced myelosuppression as DLT [132]. Another phase I study using a 72-hour continuous infusion led to frequent flebitis [133]. Therefore, the use of an oral formulation was explored in an oral bioavailability study in 19 patients with GI 147221 [134]. GI147221 was formulated as a clear solution containing a mixture of 0.5 mg GI147211 and 100 mg dextrose. The pH was adjusted to 3.5 with sodium hydroxide or hydrochloric acid. GI147211 for oral intake was mixed with 50 ml 5% dextrose in a plastic container. Unfortunately, the bioavailability (which was not affected by food co-administration) of oral GI 147221 was low (11.3 $\pm$ 5.2%) and the interpatient variation relatively high, resulting in a poorly predictable level of individual drug exposure. In view of these data the development of this oral formulation appeared unattractive, and was abandoned.

CONCLUSION AND FUTURE PERSPECTIVES

The renewed interest in topoisomerase I inhibitors, based on new insights in the mechanism of action and the development of semi-synthetic derivatives of camptothecin with

a more favourable toxicity profile, has led to extensive preclinical and clinical research. Apart from the drugs reviewed, various other oral formulations of topoisomerase I inhibitors are presently in development, e.g. BN-80915 [135]. This group of anticancer agents shows significant activity in human tumor xenografts especially with prolonged duration of exposure. Therefore, various clinical studies focused on low dose continuous infusion or frequently fractionated dosing schedules, given the short plasma half life of most topoisomerase I inhibitors. Since oral drug delivery is a more convenient method for prolonged drug administration and preferred by patients [136], further development of oral formulations seems warranted. However, for oral chemotherapy to be effective, patient's compliance is of utmost importance. It is known that patient's compliance can be tremendously improved with the use of patient education, psychologic support and exercises in pill taking [136,137]. Another concern in the development of oral formulations is their sometimes low oral bioavailability and large interpatient and intrapatient variation in systemic exposure. However, in the daily times 5 schedule of oral and i.v. topotecan the inter- and intrapatient variation for the AUC was comparable [138,56]. Efforts to improve the absorption of oral chemotherapeutic agents using new formulations will lead to better bioavailability. Pharmacokinetic and pharmacodynamic evaluations have enabled guidance in recommendation of schedules. Given the interpatient variation in exposure it is interesting to note that flat dosing of topotecan resulted in the same systemic exposure compared with the more complex dosing per body surface area. A limited sampling model was developed to predict the systemic exposure of oral 9-AC and thus diminish the intrapatient variability in exposure to 9-AC and optimize the treatment for the individual patient [120]. Future development will include the evaluation of combination therapy with the oral topoisomerase I inhibitors. Drug sequencing plays a key role in the combination of topotecan/cisplatin and might also be important for combination with other classes of drugs. Therefore, forthcoming phase I combination trials with oral topoisomerase I inhibitors should include studies on sequence dependence of the drug administration. In previous studies, the bioavailability of the oral formulation was tested in fasted patients. This situation does not, however, reflect the true clinical setting. Evaluation of the interaction of food co-administration on the rate and extent of absorption is important for the development of all oral cytotoxic agents. Future phase I studies should incorporate the investigation of the effect of food co-administration on the pharmacokinetics.

The position of oral topoisomerase I inhibitors in our armament will be defined in the next years. If the ongoing phase III trials in ovarian and small-cell lung cancer confirm the efficacy of oral topotecan, then oral topotecan might be incorporated in first line regimens, as single agent or in combination. Especially combination therapy of oral topoisomerase I inhibitors and other cytotoxic drugs seems attractive.

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Chapter 3

Phase I, pharmacological and bioavailability study of oral diflomotecan, a novel E-ring modified camptothecin analogue, in adult patients with solid tumors

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ABSTRACT

Purpose Diflomotecan (BN80915) is an E-ring modified camptothecin analog topoisomerase I inhibitor which confers greater lactone stability in plasma, a potential advantage for anti-tumor activity. As with other camptothecins oral administration has pharmacological and clinical advantages, and fixed dosing is as accurate as dosing per square meter given the interpatient pharmacokinetic and pharmacodynamic variability. This phase I study was performed to assess the feasibility of the administration of oral diflomotecan, to determine the bioavailability and maximum-tolerated dosage (MTD) and to explore the pharmacokinetics.

Patients and Methods Fourteen days after an initial i.v. bolus to assess the bioavailability, diflomotecan was administered orally (p.o.) once daily for 5 days every 3 weeks to adult patients with solid malignant tumors.

Results Twenty-four patients were entered onto the study. Four flat oral dose levels of 0.10, 0.20, 0.27 and 0.35 mg were explored. A total of 57 cycles of oral diflomotecan were administered. The main toxicity was hematological, with some patients experiencing alopecia, mild gastrointestinal toxicity and fatigue. At the 0.35 mg dose 2/4 patients experienced dose limiting toxicity (DLT) with grade 3 thrombocytopenia with epistaxis and febrile neutropenia. Toxicity was acceptable at the 0.27 mg dose: DLTs were observed in 3/12 patients (grade 4 neutropenia > 7 days, without signs of infection). After 2 cycles 5 patients had disease stabilization, which was maintained in 4 patients after 4 cycles or more. Pharmacokinetics of diflomotecan was linear over the dose range studied, showed a moderate degree of interpatient variability and systemic exposure was correlated to the decrease in white blood cells. The bioavailability of 67.1% was much higher than for other oral topoisomerase I inhibitors.

Conclusion The recommended dose for phase II studies is diflomotecan 0.27 mg/d p.o. x 5 every 3 weeks is convenient, generally well tolerated and exerts a favorable pharmacokinetic profile.

INTRODUCTION

The fluorinated homocamptothecin diflomotecan (5-ethyl 9,10-difluoro-4,5,dihydroxy-5hydroxy-1H-oxepino[3',4':6,7]indolazino [1,2-b]quinolone-3,15[13H]dione; Beaufour Ipsen, Paris, France) is a recently developed water insoluble topoisomerase I inhibitor [1,2]. Homocamptothecins are camptothecin analogues bearing a 7-membered β-hydroxylactone ring with enhanced lactone stability, which is the active form, instead of the naturally occurring 6-membered α-hydroxylactone. Since a 1-carbon ring expansion is chemically termed a homoligation, these new lactone- or E-ring modified compounds were named homocamptothecins. Because of the enhanced lactone stability, homocamptothecins were expected to exert better topoisomerase I-inhibition and antitumor efficacy than the currently used analogues such as irinotecan and topotecan.

This was confirmed in *in vivo* studies, after intraperitoneal and oral administration, using murine leukemia, human coloncarcinoma, melanoma, pancreatic and ovarian cancer, brain glioblastoma, prostate and (non-)small cell lung tumor mice models [3-6], and *ex vivo* in human colon cancers [7].

Since topoisomerase I inhibitors *in vitro* show more pronounced antitumor activity with protracted exposure, which can be achieved by oral administration and since oral administration is a more convenient and less expensive method for drug administration [8], oral administration of diflomotecan was selected for this study. In preclinical studies absolute oral bioavailability of the compound ranged from 30 % in rats [9] to 60 % in dogs [10]. Radioactivity studies in rats showed that BN80915 and its lactone open ring analogue BN80942 were mainly recovered in feces (69-74%), and approximately 16% in urine. The elimination mainly occurs through bio-transformation to a non-active metabolite [11]. In animal studies the main side effects were gastrointestinal toxicity and myelosuppression, no evidence of neurotoxicity was observed.

We conducted a phase I and pharmacologic study of diflomotecan administered orally (p.o.) once daily for 5 days every 3 weeks to adult patients with solid malignant tumors. Since for other topoisomerase I inhibitors fixed dosing is as accurate as dosing per square meter, given the interpatient pharmacokinetic and pharmacodynamic variability after oral administration [12], it was decided to use the more convenient flat dosing in this study as well. This phase I study was performed to assess the feasibility of the administration of oral diflomotecan to humans, to determine the maximum-tolerated dosage (MTD) and bioavailability and to explore the pharmacokinetics and pharmacodynamic correlations.

PATIENTS AND METHODS

Patient selection

Eligible patients had histological or cytological proof of a solid malignant tumor that was resistant to standard forms of therapy or for whom no active standard therapy was available. Other inclusion criteria included the following: age at least 18 years old; World Health Organization (WHO) performance status $\leq$ 2; no recent previous chemotherapy and/or radiotherapy, adequate hematopoietic function [absolute neutrophil count (ANC) $\geq$ 1.5 x 10^9/L and platelet count (PLT) $\geq$ 100 x 10^9/L], renal function [creatinine clearance > 60 mL/min], and hepatic function [total serum bilirubin level $\leq$ 1.25 x the upper limit of normal (ULN) and amino aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels $\leq$ 2.5 X ULN, in case of liver metastasis ASAT and ALAT were allowed $\leq$ 5 x ULN]. A specific exclusion criterion was: any condition precluding oral intake or oral absorption. The local medical ethical committees of the participating institutions in Rotterdam and Glasgow approved the study protocol and all patients gave written informed consent before entering the study.

Dosage and dose escalation

Two weeks after a 20 minute i.v. infusion of diflomotecan, escalating doses of oral diflomotecan were administered for 5 days every 3 weeks. The starting i.v. and oral dose was 0.1 mg/d, giving a total oral dose of 0.5 mg per oral cycle. This dose is less than 10% of the estimated LD_{10} in rats. Dose escalation depended on prior dose level toxicities. At least three patients were treated at each dose level. If 1 of 3 patients experienced DLT, three additional patients were entered at that dose level. MTD was defined as the dose level below the dose that induced DLT in two of three to six patients. Additional patients were included at MTD up to a total of 12 patients to assess pharmacokinetics and pharmacodynamics. DLT was defined as grade 4 neutropenia (National Cancer Institute Common Toxicity Criteria, NCI-CTC, version 2.0) lasting 8 days or more, grade 3 or 4 neutropenia complicated by fever, grade 4 thrombocytopenia or grade 3 complicated with hemorrhage, and/or grade $\geq$ 3 non-hematologic toxicity, excluding vomiting without appropriate treatment. If a patient experienced DLT, the dose of diflomotecan was decreased one dose level at the time of retreatment. The treatment was resumed when the ANC had recovered to $\geq 1.5 \times 10^9$/L and PLT to $\geq 100 \times 10^9$/L. As long as there was no disease progression and in case of acceptable toxicity the treatment was continued in best interest of the patient.

Drug administration

Diflomotecan for both i.v. and oral administrations was supplied by Ipsen Biotech (Paris, France) in the form of brown-glass vials containing 20 mg of the compound, with 3.3 mL dimethyl acetamide (DMA) and a glass bottle of solvent, containing 3.5 g Montanox VGDF80, 0.4 g sodium chloride and water for injection to make up 200 mL of aqueous, sterile isotonic solution. For reconstituting the product, the 3.3 mL vial of diflomotecan was diluted in the solvent, protected from light and stored at between 2 and 8 °C. In aqueous solution the stability of diflomotecan at room temperature is adequate for the expected duration of the i.v. infusion and oral administrations, but the reconstituted solution should be protected from light by wrapping the bottle in aluminum foil. The i.v. administration consisted of a 20 minute infusion. For oral administration the appropriate volume of the product was prepared in syringes and was taken by the patient for 5 days every 3 weeks. The solution was prescribed on an empty stomach in the morning. The complete dose was directly swallowed, followed immediately by up to 150 mL of water. When the patient complained of bad taste of the product, he/she was allowed to drink Coca-Cola® instead of water. Diflomotecan was found to be stable in this beverage in drug stability studies [13]. No standard premedication was prescribed. In case of nausea or vomiting a prophylactic 20 mg of metoclopramide or domperidon was prescribed for the subsequent administrations.

Treatment assessment

Before therapy a complete medical history was taken and a physical examination was performed. A complete blood cell count (CBC) including white blood cell (WBC) differential, and serum biochemistry, which involved sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, ASAT, ALAT, γ-

glutamyl transferase, lactate dehydrogenase (LDH) and glucose, were performed, as was measurement of creatinine clearance and urine analysis. Weekly evaluations included history, physical examination, toxicity assessment according to the NCI-CTC version 2.0, and serum chemistry. CBC was determined twice weekly. Tumor evaluation according to the WHO criteria for response was performed at baseline and every 2 oral courses during therapy. Duration of response was calculated from the start of treatment.

Sample collection for pharmacokinetic analysis

Blood samples for pharmacokinetic analysis, collected in 4.5 mL glass tubes containing lithium heparinate as anticoagulant, were obtained in all patients at the following time points: before dosing and at 10, 20, 30, 45 minutes and 1.5, 3, 4.5, 6, 8, 10 and 24 hours after the first i.v. administration and first and fifth oral administration of diflomotecan. Additional samples were taken before oral administration on day 3 and 4. Immediately after sampling, tubes were placed on ice and centrifuged at 2000 *g* for 15 minutes at 4 °C. From each sample, two 1.2 mL aliquots of plasma were placed in polypropylene tubes and frozen at –80 °C until the day of analysis.

Pharmacokinetic assays

The concentration of BN80915 was determined by the LC-MS/MS method using ^{13}C-labelled analogue of diflomotecan as standard (BN81011). The limit of quantification of this method was 0.05 ng/mL plasma. Accuracy and precision values expressed as relative error in percentage are 1.5 and 4.2% respectively for BN80915, at the limit of quantification level.

Pharmacokinetic data analysis

The pharmacokinetic analysis of BN80915 and BN80942 plasma concentration-time data set following i.v. and oral administrations were performed by a non-compartmental approach using the WinNonLin pharmacokinetic program (Version 1.1. Scientific Consulting Inc. 1995). The following pharmacokinetic parameters were estimated: the oral bioavailability F, the empirical peak serum level C_{max}, the empirical time of peak serum level t_{max}, the apparent elimination rate constant (λ_z), estimated by linear regression of the terminal phase of the semilogarithmic serum levels curve, when it was clearly defined. The elimination half-life ($t_{1/2z}$) was defined as $ln2/\lambda_z$. The area under the concentration-time curve (AUC) was estimated by the linear-trapezoidal rule.

After oral administration the amount of unchanged drug excreted in urine and feces was determined and expressed as % of unchanged excretion. Pharmacokinetic data are reported as mean $\pm$ SD.

RESULTS

A total of 24 patients entered this study between March 1999 and December 2000. Patient characteristics are listed in Table 1. All patients were eligible and 22 patients were

evaluable: 2 patients only received the first i.v. administration and went off study due to early signs of disease progression.

The majority of patients had mild symptoms of disease. Nine patients were female and 15 male. Twenty-one patients had received prior chemotherapy, the median number of regimens was 2 and 1 patient even had previously received stem cell transplantation after myeloablative chemotherapy. Eleven patients had received prior radiotherapy. The most common tumor types were colorectal, adenocarcinoma of unknown primary (ACUP) and sarcoma. Dose levels of diflomotecan studied were 0,10 i.v/0.10 p.o, 0.10 i.v./0.20 p.o, 0.20 i.v./0.27 p.o. and 0.27 i.v./0.35 p.o., all mg/d. The total number of assessable oral courses for evaluable patients was 57. The median number of courses per patient was two (range one to eight).

Myelosuppression was the principal DLT of this regimen. Four patients required dose reductions after experiencing DLT. Once dose reduction had taken place, the courses administered to these patients were evaluated for toxicity at the lower dose level.

Table 1. Patient characteristics

Characteristic		No. of patients
Patients entered		24
Patients assessable for toxicity		22
Age, years		
Median	56	
Range	33-71	
Sex		
Female		9
Male		15
Performance status		
Median	1	
Range	0-2	
Tumor type		
Anal and Colorectal		6
Sarcoma		4
ACUP		4
Melanoma*		2
Ovarian cancer		2
Other**		6
Previous therapy		
Chemotherapy		21
No. of chemotherapy regimens		
Median	2	
Range	0-3	
Radiotherapy and Chemotherapy		11

* 1 patient also had Renal cell carcinoma

** Mesothelioma, Non-Small Cell Lung-, Head and Neck-, Cervical -, Breast- and Prostate Cancer.

Table 2. Worst hematological NCI-CTC grade toxicity and dose limiting toxicities (DLTs) of oral diflomotecan during all cycles

Dose mg	No. of pts/ No.of cycles	Leukocytopenia (grade)		Neutropenia (grade)		Thrombocytopenia (grade)	
		3	4	3	4	3	4
0.10	3/6	0	0	0	0	0	0
0.20	3/5	1	0	1	1	0	0
0.20[1]	3/5	3	0	1	1	1	0
0.27	12/29	4	3	3	4[2]	1	0
0.27[1]	2/2	1	1	1	1	1	0
0.35	4/10	2	2	0	2[3]	2[4]	0

[1] Dose level after dose reduction

[2] 3 DLTs: 1 patient had febrile neutropenia and 2 patients grade 4 neutropenia > 7 days

[3] 1 DLT: 1 patient had febrile neutropenia (also grade 4 neutropenia > 7 days)

[4] 1 DLT: 1 patient had grade 3 thrombocytopenia with bleeding, requiring platelet transfusion

Hematologic toxicity

The severity of the observed hematological toxicity after oral administration was clearly dependent on the dose level explored (Table 2). At the oral diflomotecan dose level of 0.27 mg one of the initial six patients treated had DLT, consisting of grade 4 neutropenia for 12 days, permitting dose escalation. However, at the next dose level of 0.35 mg two out of four patients had DLT: one patient had febrile neutropenia and grade 4 neutropenia for 9 days and another patient had thrombocytopenia grade 3 with recurrent epistaxis requiring a platelet transfusion. Because two of four patients experienced DLT, no further dose escalation was attempted. Six additional patients were included at the dose level directly below that level in order to further detail the pharmacokinetic parameters and pharmacodynamic relationships at this dose level recommended for further studies. Of these additional six patients, treated at 0.27 mg, two more patients experienced DLT: one patient had febrile neutropenia and another grade 4 neutropenia for more than 7 days. Both of these patients continued treatment at the lower dose level of 0.2 mg for 1 to 3 cycles until progressive disease occurred. Hence, a total of three of twelve patients experienced DLT at the 0.27 mg dose level, which was defined MTD according to the protocol. Only 2 of 31 courses at the recommended dose level were delayed because of prolonged neutropenia. A marked inhibition of hematopoiesis was observed. The percentage of patients requiring blood transfusions was 22%; transfusions were needed in 7 of 57 cycles.

Overall, hematological toxicity was the main toxicity of diflomotecan administered orally. Although all DLTs were hematological, toxicity at the recommended dose level was easily manageable.

Table 3. Worst NCI-CTC grade non-hematological toxicity of oral diflomotecan during all cycles

Dose mg	No. of pts/ No.of cycles	Nausea (grade)		Vomiting (grade)		Diarrhea (grade)		Fatigue (grade)	
		2	3	2	3	2	3	2	3
0.10	3/6	0	0	0	0	0	0	1[1]	0
0.20	6[2]/10	1	0	0	0	1	0	0	0
0.27	14[3]/31	7	1	2	0	1	0	6[4]	3[5]
0.35	4/10	0	0	0	0	1	0	0	0

[1.] the patient had grade 1 at baseline, [2.] 3 patients after dose reduction, [3.] 2 patients after dose reduction, [4.] 4 patients with grade 1 at baseline, [5.] 2 patients with grade 2 at baseline and 1 with grade 1; fatigue was considered related to disease progression

Table 4. BN80915 pharmacokinetics after first and fifth day oral administration of diflomotecan

Dose (mg/d)	No. Of pts.	AUC (ng.h/mL)	C_{max} (ng/mL)	t_{max} (h)
0.1				
day 1	3	0.89±0.99	0.50±0.21	0.53±0.21
day 5	3	1.62±0.58	1.07±0.86	1.27±0.40
0.2				
day 1	2	0.97±0.83	0.76±0.25	0.51±0.01
day 5	2	1.85±1.99	0.70±0.26	1.63±1.95
0.27				
day 1	10	12.20±7.69	2.70±1.31	0.66±0.46
day 5	10	14.47±10.46	3.27±3.13	0.71±0.37
0.35				
day 1	3	18.03±11.89	4.22±0.77	0.67±0.15
day 5	3	11.26±6.15	3.02±1.42	0.81±0.06

NOTE: Data are expressed as mean±SD

Abbreviations: AUC, area under the plasma concentration-time curve; C_{max}, the empirical peak serum level, t_{max}, the empirical time of peak serum level, $t_{1/2z}$, the elimination half-life.

Nonhematologic toxicity

Gastrointestinal toxicity was mild (Table 3) and was mainly caused by the bad taste of the product. After a protocol amendment allowing patients to drink Coca Cola® at administration of the drug and the routinely use of metaclopramide or domperidon prophylaxis, the incidence of nausea and vomiting was even further reduced. Diarrhea hardly ever occurred and only 1 patient experienced grade 2 mucositis in 1 cycle.

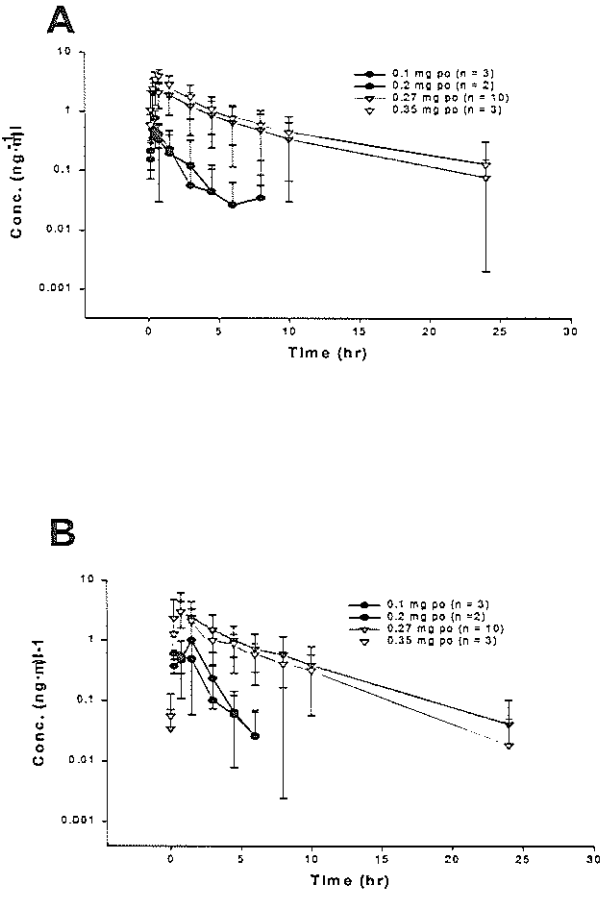

Figure 1. Plasma concentration-time curves (mean levels) of BN80915 after oral administration on the first (A) and fifth day (B).

Fatigue was observed rather frequently and was often related to disease progression or anemia. A total of 3 patients, who had baseline grade 1 or 2 fatigue, experienced grade 3 fatigue related to disease progression and therefore this was not considered a DLT. A relationship with the study drug could not be ruled out in several other cases of grade 1 or 2 fatigue. Development of alopecia was seen in 5 out of 22 patients and another 4 patients had alopecia at baseline. One patient experienced grade 3 vertigo, which was not observed on rechallenge with the study medication, and was therefore not considered related. Nephrotoxicity grade 1 was observed in 1 patient and resolved at the subsequent cycle. One patient developed oligospermia. No grade 4 non-hematologic toxicity was observed.

Antitumor activity

Five patients had disease stabilization after 2 cycles. One of these patients with pulmonary metastasis of an anal carcinoma withdrew consent to further treatment after 2 cycles, and remained free of disease progression for more than 1 year. Another patient with colon carcinoma completed 8 cycles and is still 11 months after start of the treatment without signs of disease progression. The other 3 patients, diagnosed with desmoplastic round cell sarcoma, breast cancer or clear cell sarcoma, developed progressive disease after 2 to 4 subsequent cycles.

Table 5. BN80915 pharmacokinetics after i.v. administration of diflomotecan

Parameters	Units	dose 0.1 mg	dose 0.2 mg	dose 0.27 mg
n	-	3	8	4
C_{max}	ng/mL	1.21±0.41	6.36±6.66	5.74±2.02
T_{max}	h	0.36±0.15	0.29±0.08	0.27±0.12
AUC	ng.h/mL	1.52±0.47	16.70±17.90	19.51±12.57
$T_{1/2z}$	h	1.76±0.85	3.89±2.07	3.47±1.23
Cl	L/m^2.h	38.93±11.54	13.23±9.38	11.08±7.39
Vd_{ss}	l/m^2	70.90±27.24	42.31±16.40	42.68±12.46
MRT	h	2.08±1.37	4.45±2.13	4.78±1.88

NOTE: Data are expressed as mean±SD

Abbreviations: AUC, area under the plasma concentration-time curve; C_{max}, the empirical peak serum level, t_{max}, the empirical time of peak serum level, $t_{1/2z}$, the elimination half-life, Cl; the plasma clearance, Vd_{ss}, volume of distribution, MRT; mean residence time

Pharmacokinetics and pharmacodynamics

Fifteen patients receiving i.v. diflomotecan and eighteen patients, receiving oral diflomotecan, were pharmacokinetically evaluable: due to logistical problems 1 patient at dose level 0.2 and 0.35 mg/d and 2 patients at the 0.27 mg/d dose level were not evaluable. The results are presented in Table 4 and 5. Plasma concentration-time curves of BN80915 after oral administration on the first and fifth day are shown in figure 1A and B.

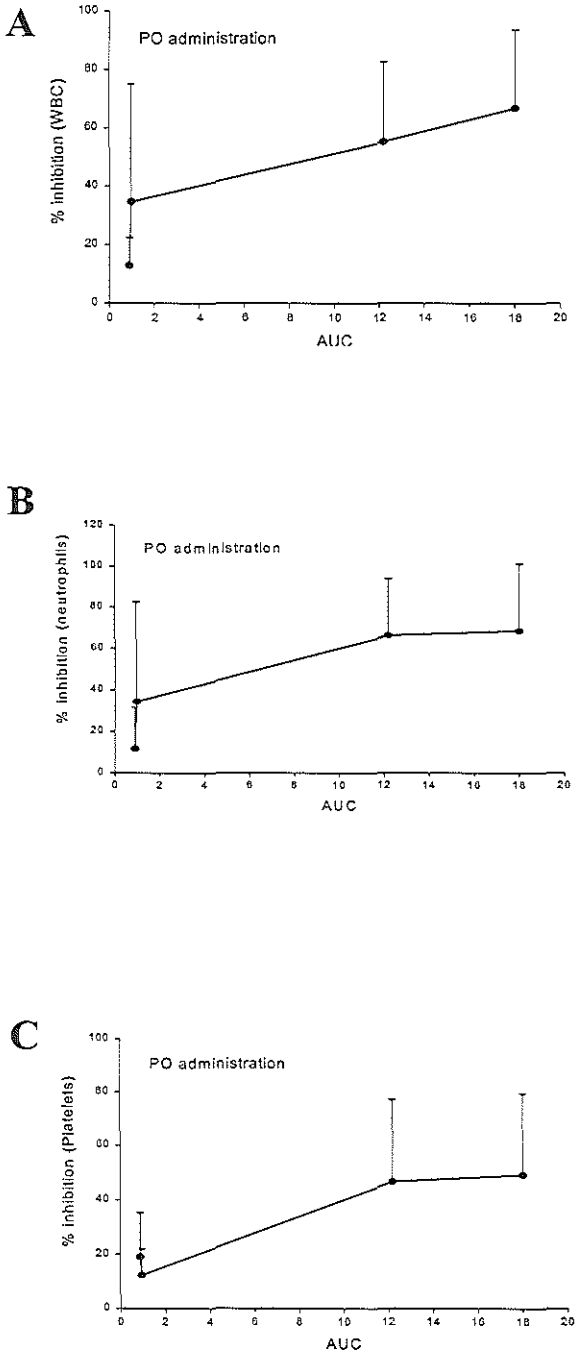

Figure 2. Pharmacodynamic relationship between mean day 1 BN80915 AUC at each oral dose level and % inhibition of white blood cells (A), neutrophils (B) and platelets (C).

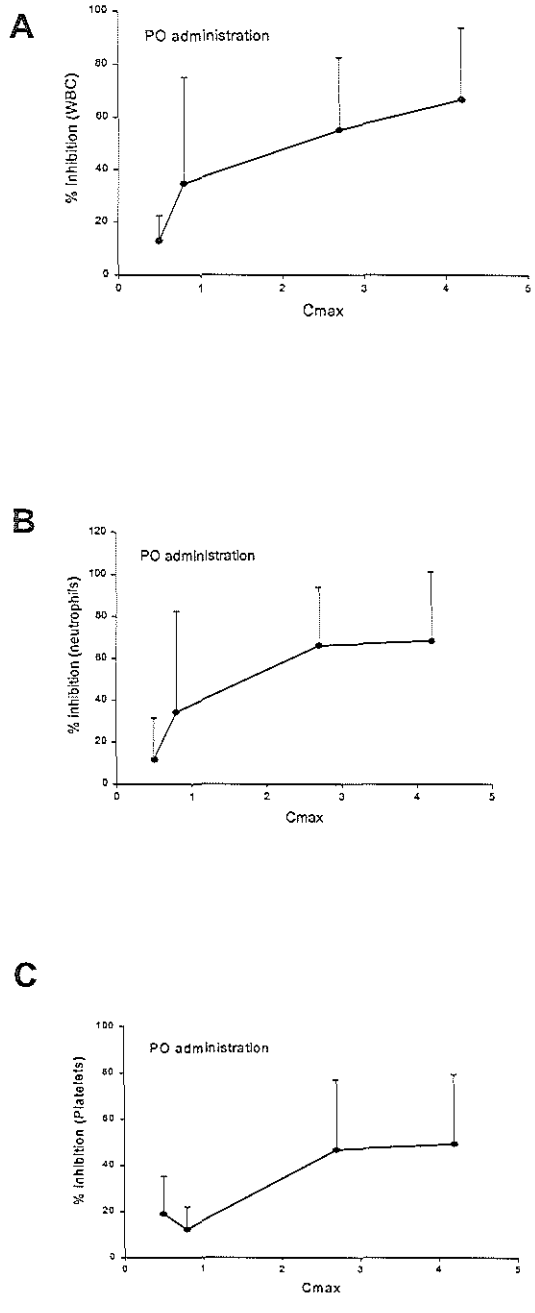

Figure 3. Pharmacodynamic relationship between mean day 1 BN80915 C_{max} at each dose level and % inhibition of white blood cells (A), neutrophils (B) and platelets (C).

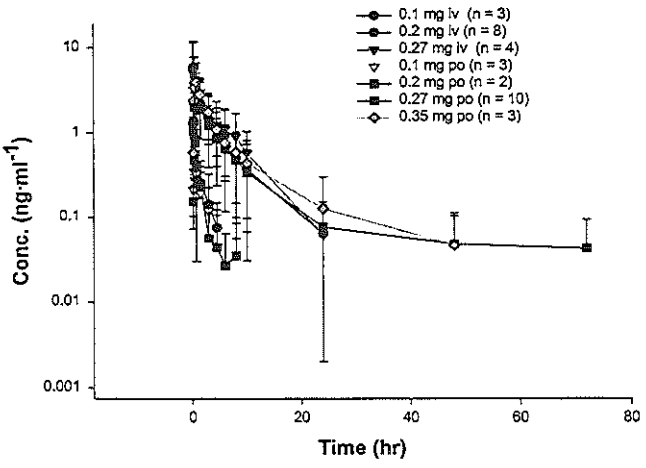

Figure 4. Mean plasma levels after i.v. and oral administration of diflomotecan

Since at several sample time points the concentration of BN80915 was below the level of detection, AUC and C_{max} were underestimated. No relevant changes in parameters were observed between the first and fifth administration at the recommended dose level. The AUC and C_{max} were proportional to dose and the T_{max} was independent of dose, indicating a linear and dose-independent behavior of the compound.

Pharmacodynamic relationships between BN80915 AUC or Cmax and % inhibition of white blood cells, neutrophils and platelets were established (respectively Fig 2A,B,C and 3A,B,C).

DISCUSSION

This study shows that oral administration of diflomotecan is feasible in adult patients with solid tumors. Diflomotecan was well tolerated, with limited gastrointestinal toxicity and fatigue, and few patients experiencing alopecia. As for other oral topoisomerase I inhibitors bone marrow depression was the main toxicity. At the recommended dose level 3 out of 12 patients had neutropenia > 7 days, one of these patients experienced febrile neutropenia without signs of infection or sepsis. Dose delays because of prolonged hematological toxicity were rare. Therefore we conclude that the toxicity was acceptable at the recommended dose level of 0.27 mg/d x 5 every 3 weeks.

The patient population was heavily pretreated and few patients had tumor types that were sensitive on theoretical pre-clinical grounds, such as colon carcinoma. The 2 patients responding to treatment with oral diflomotecan for a prolonged period of time were patients with metastatic colon and anal cancer. These data indicate that oral diflomotecan should be evaluated in a phase II study in colorectal patients.

Oral diflomotecan exerts a linear dose independent pharmacokinetic profile over the higher dose range studied with limited inter- and intrapatient variability. As for many other chemotherapeutic agents. It was also interesting to note that flat dosing resulted in the same variation in AUC as dosing per square meter would have done.

Population pharmacokinetic models might reduce the interpatient variability in the future. As long as these models are not available the more convenient flat dosing of diflomotecan is as accurate as the more complex dosing per body surface area. Pharmacodynamic relationships of oral diflomotecan AUC and C_{max} with percentage decrease in white blood cells, neutrophils and platelets were established. The oral bioavailability at the recommended dose level of 67.1% is much higher than for other oral topoisomerase I inhibitors such as topotecan (F=30-44%) [14,15], lurtotecan (F=12-21%) [16] and 9-AC (F=48.6%) [17]. The high bioavailability and low variation in AUC is a unique merit of diflomotecan.

In conclusion, the recommended dosage for phase II studies involving the oral homocamptothecin diflomotecan as a drinking solution in adult cancer patients, whether heavily pretreated or not, is 0.27 mg/d x 5 every 3 weeks. Oral diflomotecan has a favorable pharmacokinetic profile with high oral bioavailability and pharmacodynamic relationships with hematological toxicity render predictable toxicity. Given the preclinical activity, superior to other topoisomerase I inhibitors and hints of activity in this study, phase II studies in a range of solid tumor types are advisory.

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Chapter 4

Phase I pharmacologic study of oral topotecan and intravenous cisplatin: sequence dependent hematologic side effects

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ABSTRACT

Background and Purpose In in vitro studies synergism and sequence dependent effects were reported for the combination of topotecan and cisplatin. Recently an oral formulation of topotecan became available. This phase I study was performed to assess the feasibility of the combination of oral topotecan and cisplatin, the pharmacokinetic interaction, and sequence dependent effects.

Patients and Methods Topotecan was administered orally daily for five days in escalating doses and cisplatin at a fixed dose of 75 mg/m² i.v. either before topotecan on day 1 (CT) or after topotecan on day 5 (TC) once every 3 weeks. Patients were treated in a randomized cross-over design.

Results Forty-nine patients entered the study; one patient was not eligible. The CT sequence induced significantly more severe myelosuppression than sequence TC, and resulted in MTD at a topotecan dose of 1.25 mg/m²/d×5. In sequence TC, the MTD was topotecan 2.0 mg/m²/d×5. DLT consisted of myelosuppression and diarrhea. Pharmacokinetics of topotecan and cisplatin were linear over the dose range studied, no sequence dependent effects were observed. In addition, topotecan did not influence the protein binding of cisplatin and the platinum-DNA adduct formation in peripheral leukocytes in either sequence.

Conclusion The recommended dose for phase II studies in patients, comparable with the patients studied, is oral topotecan 1.25 mg/m²/day x 5 preceded by cisplatin 75 mg/m² day 1 once every 3 weeks, and topotecan 2.0 mg/m²/day followed by the same dose cisplatin on day 5. No pharmacokinetic interaction could be discerned. The antitumor efficacy of both schedules should be evaluated in a randomized phase II study.

INTRODUCTION

Topotecan (9-dimethylaminomethyl-10-hydroxycamptothecin, Hycamtin®) is a water-soluble topoisomerase I inhibitor (camptothecin). DNA topoisomerase I is a nuclear enzyme, involved in cellular replication and transcription. Topotecan exists in two forms in a pH dependent dynamic balance between the closed lactone ring (active) form and the carboxy acid (inactive) form. By forming a covalent adduct between topoisomerase I and DNA, named the cleavable complex, topoisomerase I inhibitors interfere with the process of DNA breakage and resealing during DNA synthesis. The stabilized cleavable complex blocks the progress of the replication fork resulting in irreversible DNA double-strand breaks leading to cell death [1-3]. Based on their mechanism of action, synergy was suspected for the combination of topoisomerase I inhibitors and DNA damaging agents such as cisplatin. Preclinical studies confirmed this hypothesis. However, the observed interaction seemed to depend on the cell line studied and the schedule of administration of topotecan and cisplatin used [4-17]. When topotecan was preceded by cisplatin, synergy was increased compared to concomitant incubation with both drugs in the IGROV-1 ovarian cancer cell line and the MCF7 cell line [5,6]. Also in the clinical setting drug sequencing seems to be important [17].

To date, topotecan has demonstrated prominent activity in several malignancies, most notable in ovarian [18-23], small cell lung carcinomas [24-26], and hematological malignancies [27-30], in which cisplatin is also highly active. Recently, an oral formulation of topotecan became available, which is a more convenient method of drug administration. The oral formulation has a bioavailability of 32-44% [31-33] with moderate intrapatient variability. The maximally tolerated dose for oral topotecan, administered for five days every 21 days as a gelatin capsule, has been determined as 2.3 mg/m²/day with myelosuppression, in particular neutropenia, as the dose limiting toxicity (DLT) [34]. Non-hematological toxicities were generally mild and not dose limiting, including fatigue, anorexia, nausea, vomiting and diarrhea. In ovarian- [35] and small cell lung cancer [36] randomized studies suggest the oral formulation is equivalent to the intravenous formulation.

Against this background, we initiated a phase I study in which patients were treated in a randomized cross-over design to determine the maximum tolerated dose of oral topotecan given daily for 5 days combined with cisplatin 75 mg/m² i.v. administered either on day 1 or day 5 every 21 days, to describe and quantitate the toxicities of the combination and to determine whether the sequence of topotecan and cisplatin administration has any influence on the observed toxicity or the pharmacokinetic interaction between the drugs.

PATIENTS AND METHODS

Patient selection

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumor resistant to standard forms of therapy were eligible. Other eligibility criteria included the following: age between 18-75 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycin C); no previous therapy with topoisomerase I inhibitors; adequate hematopoietic function (absolute neutrophil count (ANC) ≥ 1.5 x 109/L and platelet count ≥ 100 x 109/L), renal function (creatinine clearance ≥ 60 mL/min) and hepatic (total serum bilirubin ≤ 1.25 x upper normal limit and serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) ≤ 2.0 x upper normal limits, in case of liver metastasis serum ASAT and ALAT ≤ 3.0 x upper normal limits) function. Specific exclusion criteria included the existence of gross ascites and/or any gastrointestinal condition that would alter absorption or motility. All patients gave written informed consent before study entry.

Dosage and dose escalation

Escalating doses of oral topotecan were combined with a fixed dose of cisplatin, 75 mg/m², administered intravenously over 3 hours once every 3 weeks. The starting dose of topotecan was 0.75 mg/m²/day for 5 consecutive days, which is 33% of the recommended dose of oral topotecan, when administered as a single agent. Dose escalation was based on the prior dose level toxicities. At least three patients were treated at each dose level. If one of three patients experienced dose limiting toxicity (DLT), three additional patients were entered at that dose level. The maximum-tolerated dose (MTD) was defined as one dose level below

the dose that induced DLTs in 3 out of 6 patients during the first course in any sequence, which were defined as NCI-CTC grade 4 neutropenia lasting for five days or more, or complicated with fever requiring hospitalization, grade 4 thrombocytopenia and/or non-hematological toxicity ≥ grade 3 (grade 2 for renal toxicity), excluding nausea. Intrapatient dose escalation was not permitted. If a patient encountered DLT, the dose of topotecan was decreased one dose level at re-treatment. The treatment was resumed when the neutrophil count had recovered to ≥ 1.0 x 109/L and the platelet count to ≥ 100 x 109/L. A maximum of six cycles was administered to each individual patient.

Drug administration and sequencing

In the first part of the study, patients were randomly assigned at study entry to one of two treatment groups. Six patients were treated at each dose level.

Group A. In the first treatment course, patients received cisplatin as a 3-hour infusion diluted in 250 mL of hypertonic saline [3% (w/v) sodium chloride] on day 1, immediately followed by the oral administration of topotecan (sequence CT), which was given for five consecutive days on an empty stomach, at least 10 minutes before meals. In the second course, the sequence of administration of topotecan and cisplatin was reversed, starting with topotecan for 5 days and administering cisplatin on day 5, 3-hours after the last oral administration of topotecan at the same doses (sequence TC).

Group B. Patients received the two treatment courses in reversed order.

The third and following courses were administered using the least toxic sequence, with in the third course a 24 hour interval between the administration of cisplatin and topotecan to study the pharmacokinetics of both drugs to rule out the possibility of any pharmacokinetic interaction.

In the second part of the study, after determination of the MTD in the most toxic sequence, further dose escalation of topotecan was pursued in the reversed sequence. Patients were then enrolled to receive that single sequence with a 24-hour interval between the administration of topotecan and cisplatin in the second course only.

In all patients pre-medication consisted of ondansetron (8 mg i.v.) combined with dexamethasone (10 mg i.v.) administered 30 min before the start of the cisplatin infusion. To prevent cisplatin-induced renal damage, the administration of cisplatin was preceded by the infusion of 1000 mL of a mixture of 5% (w/v) dextrose and 0.9% (w/v) sodium chloride over 4 hours, and followed by another 3000 mL with the addition of 20 mM potassium chloride and 2 g/L magnesium sulphate applied over 16 hours. Topotecan capsules containing either 0.25 or 1.00 mg of the active compound were supplied by SmithKline Beecham Pharmaceuticals (Harlow, UK). Cisplatin (Platosin®) was purchased as a powder from Pharmachemie (Haarlem, The Netherlands).

Treatment assessment

Before therapy a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including WBC and differential, and serum biochemistry, which included sodium, potassium, calcium, phosphorus, urea, creatinine, total

protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine transferase (ALAT), γ-glutamyl transferase, glucose and uric acid, were performed, as was creatinine clearance. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria, and serum chemistry. CBC was determined twice weekly. Tumor evaluation was performed after three courses in the first part and after every two courses in the second part of the study according to the World Health Organisation (WHO) criteria for response. Duration of response was calculated from the start of treatment. Patients were treated for at least three cycles of therapy in the first part and two cycles in the second part of the study unless disease progression or unacceptable toxicity was encountered.

Sample collection for pharmacokinetics

Blood samples for pharmacokinetic analysis were obtained during the first 3 treatment courses until the MTD was reached in the most toxic sequence. Hereafter samples were only taken in the first 2 courses. Blood sampling for topotecan pharmacokinetics was performed on the first and fifth day of drug dosing, whereas for cisplatin pharmacokinetics sampling was performed on the day of administration (days 1, 5 or 6, dependent on the schedule). At the doses recommended for further study, additional topotecan pharmacokinetics were performed on day 2 of the first treatment course. Blood was withdrawn from a vein in the arm opposite to that used for drug infusion, and collected in 4.5-mL glass tubes containing lithium heparin as anticoagulant. For analysis of topotecan kinetics, samples were obtained at the following time points: prior to dosing, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after administration of topotecan. Immediately after sampling, tubes were briefly immersed into an ice bath kept at the bedside, and plasma was separated within 10 min by centrifugation at 3000 × *g* for 5 min (4°C). Next, 250-μL aliquots of the plasma supernatant were added to 2.0-mL polypropylene vials (Eppendorf, Hamburg, Germany) containing 750-μL of ice cold (-20°C) methanol. After mixing on a vortex-mixer for 10 s, samples were stored at -80°C until the day of analysis.

Blood samples for measurement of cisplatin concentrations were obtained immediately before infusion; at 1, 2, and 3 hours after start of the infusion; and 0.5, 1, 2, 3, and 18 hours after the end of the infusion. Sample volumes were 4.5 mL each except at predose and 1 and 18 hours after infusion, which were 21 (3×7) mL each. Immediately after sampling plasma was separated by centrifugation at 3000 × *g* for 10 min. Next, 500-μL aliquots of the plasma supernatant were added to 1.0-mL of ice cold (-20°C) ethanol. After mixing on a vortex-mixer for 10 s, samples were stored at -80°C until the day of analysis. Blood samples to determine the cisplatin DNA-adduct levels were obtained immediately before infusion, and 1 and 18 hours after the end of the infusion.

Pharmacokinetic assays

Samples for topotecan kinetics were analyzed by a reversed-phase high-performance liquid chromatographic (HPLC) method, as described [37], that allowed simultaneous determination of the lactone and the hydrolyzed ring-opened carboxylate forms. Prior to drug

analysis, samples were removed from the freezer and centrifuged for 5 min at 23000 × *g* (4°C), A volume of 100 µL was transferred to a clean microtube containing 400 µL phosphate buffer. Of this mixture, a 200-µL volume was used for analysis. The HPLC system consisted of a constaMetric 4100 solvent delivery system (LDC Analytical, Riviera Beach, FL), a Waters 717Plus autosampler (Bedford, MA), and a Jasco FP 920 fluorescence detector (Jasco). Chromatographic separations were achieved at 35°C on a Shandon Hypersil BDS column (100×3 mm, internal diameter; 3 µm particle size) from Applied Science (Breda, The Netherlands), with a mobile phase composed of 10 mM aqueous potassium dihydrogen phosphate containing 22% (v/v) methanol and 0.2% triethylamine, with the pH adjusted to 6.0 (orthophosphoric acid). The mobile phase was filtered [0.45-µm Millipore HA filters (Milford, MA)] and degassed by ultrasonication. The flow rate was set at 0.7 mL/min, and the column effluent was monitored at excitation and emission wavelengths of 381 and 525 nm, respectively with the emission band width set at 40 nm. Peak detection was performed with the Fisons ChromCard data analysis system (Milan, Italy). Drug concentrations in unknown samples were determined by interpolation on linear calibration curves, constructed in blank human plasma, by least-squares linear regression of peak heights *versus* 1/*x*. The mean percentage deviation from nominal values (accuracy) and precision (within-run and between-run variability) were always <15%. The lower limit of quantitation for both the lactone and carboxylate forms were 100 pg/mL.

Non-protein bound and total cisplatin concentrations in plasma were determined by a validated analytical procedure based on measurement of platinum atoms by flameless atomic-absorption spectrometry (AAS) as described [38,39]. For measurement of unbound cisplatin, 500-µL aliquots of plasma were extracted with neat 1000 µL ice-cold ethanol in a 2-mL polypropylene vial. After a 2-hour incubation at -20°C, the supernatant was collected by centrifugation at 23,000×*g* for 5 min (4°C), and transferred to a clean vial. A volume of 600 µL was evaporated to dryness under nitrogen at 60°C, and the residue reconstituted in 200 or 600 µL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride by vigorous mixing. A volume of 20 µL was eventually injected into the AAS. For determination of total cisplatin, a 100-µL volume of plasma was added to 900 µL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride, followed by vortex-mixing for 10 s. Of this solution, a volume of 20 µL was injected into the AAS. Samples were analyzed on a Perkin Elmer Model 4110 ZL spectrometer with Zeeman-background correction using peak area signal measurements at a wavelength of 265.9 nm and a slid width of 0.7 nm [38,39]. The injection temperature was set at 20°C. Platinum DNA adduct levels in peripheral leukocytes were determined as described [40], with modifications [41]. Following DNA isolation from buffy coat preparations, samples were digested with DNAse I and zinc chloride and injected into the furnace using a 4-times multiple sampling feature of the Perkin Elmer AAS. The cisplatin DNA-adduct levels were expressed as picogram platinum per microgram DNA (pg Pt/ µg DNA).

Pharmacokinetic data analysis

Individual plasma concentrations of topotecan were fit to a two-compartment model, using the software package Siphar v4.0 (SIMED, Creteil, France). The concentration-time profiles were obtained after zero-order input, with weighted least-squares analysis applying a weighting factor of $1/y$. The topotecan area under the plasma concentration-time curve (AUC) was determined for both the lactone ($AUC_{(L)}$) and carboxylate forms ($AUC_{(c)}$) on the basis of the best fitted curves. The apparent plasma clearance ($CL/f_{(L)}$) of topotecan lactone was calculated by dividing the dose administered (expressed in free base equivalents) by the observed AUC. The terminal disposition half-life [$T_{1/2}(z)$] of topotecan was calculated as $\ln 2/k$, where k is the terminal elimination rate constant (expressed in h^{-1}). The peak plasma concentrations (C_{max}) and the time to peak plasma concentration (T_{max}) were determined graphically from the (observed) experimental values. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as $AUC_{(L)}/[AUC_{(L)}+AUC_{(C)}]$.

Kinetic profiles of CDDP were obtained similarly using a two-compartment linear model with extended least-squares regression analysis as reported earlier [42]. The AUC of cisplatin was calculated to the last sampling time point with detectable drug levels (C_{last}) by the linear trapezoid method and extended to infinity by addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression of the final plasma concentration values.

Statistical considerations

Pharmacokinetic parameters for all compounds are reported as mean values ± S.D. The difference in pharmacokinetic parameters between sequences was evaluated statistically using a paired Student's t-test. Probability values (two-sided) of less than 0.05 were regarded as statistically significant. All calculations were performed using the statistical packages NCSS version 5.X (J.L. Hintze, Kaysville, UT) and STATGRAPHICS Plus version 2.0 (Manugistics Inc., Rockville, MA).

RESULTS

Forty-nine patients entered this study between January 1997 and February 1999. Patient characteristics are listed in Table 1. One patient was not eligible due to reduced renal function at the time of study entry, 1 patient was not assessable for toxicity because of the occurrence of a cerebrovascular accident after two days of treatment with topotecan and was taken off study. Forty-seven patients were assessable for toxicity and 45 patients for response.

The majority of the patients was either asymptomatic or had only mild symptoms. Nineteen patients were female and 28 were male. Seventeen patients had received prior chemotherapy, one line only. No patients were pretreated with drugs known to be highly myelosuppressive i.e. carboplatin, mitomycin C, nitrosoureas or high dose cyclophosphamide. The most common tumor type was head and neck cancer. Dose levels of topotecan studied were 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.3 mg/m²/day, respectively. The total number of assessable courses was 175. The median number of courses per patient was 4 (range 1-6).

Both myelosuppression and diarrhea were the principal DLTs of this regimen. Seven patients required dose reductions after experiencing dose-limiting toxicity. Once dose reduction had taken place, the courses in these patients were evaluated for toxicity at the lower dose level.

Table 1 Patient characteristics

Characteristic		No. of Patients
No. Entered		49
No. Assessable		47
Age, years		
Median	57	
Range	28-70	
Sex		
Female		19
Male		28
Performance status		
Median	1	
Range	0-1	
Tumor type		
Head/Neck		11
(N)SCLC		10
ACUP		10
Cervical		3
Miscellaneous		13
Previous therapy		
Chemotherapy		9
Radiation		12
Chemotherapy and radiation		8
None		18

Hematologic toxicity and drug sequencing

The severity of the observed hematologic toxicity was clearly dependent on the sequence of drug administration. At each dose level studied, both neutropenia and thrombocytopenia were more severe when cisplatin administration preceded the administration of topotecan (CT sequence) (Table 2 and Figure 1) reflected in both a significantly lower nadir and percentage decrements in neutrophil and platelet counts in this sequence [P=<0.00001 (neutropenia), P=<0.00001 (thrombocytopenia)].

Table 2 Toxicity (worst per cycle) and drug sequencing

Topotecan (mg/m^2/day)	Sequence	No. of Pts/ No. of cycles	Neutropenia (grade 3)	Neutropenia (grade 4)	Thrombocytopenia (grade 3)	Thrombocytopenia (grade 4)	Nausea (grade 1)	Nausea (grade 2)	Nausea (grade 3)	Vomiting (grade 1)	Vomiting (grade 2)	Vomiting (grade 3)	Vomiting (grade 4)	Diarrhea (grade 1)	Diarrhea (grade 2)	Diarrhea (grade 3)	Diarrhea (grade 4)	Fatigue (grade 1)	Fatigue (grade 2)	Fatigue (grade 3)	DLT Per patient No. of Pts/ Total No. of Patients	DLT 1st Cycle No. of Pts/ No. of cycles	DLT All Cycles No. of cycles/ Total No. of cycles
0.75	CT	6/9	1	1	1	0	7	0	0	3	0	0	0	0	0	0	0	5	2	0	1/6	0/6	1/9
0.75	TC	7/19	0	1	0	0	11	3	0	6	1	0	0	0	0	0	0	6	4	0	0/7	0/6	0/19
1.0	CT	7/7	2	0	1	0	4	2	1	1	5	0	1	1	0	0	0	0	3	0	2/7	2/6	2/7
1.0	TC	7/22	1	3	0	0	12	5	0	5	13	0	0	4	1	0	0	7	2	0	2/7	0/6	2/22
1.25	CT	9/17	7	0	4	1	6	6	1	2	3	0	1	7	3	1	0	5	2	0	4/9	3/9	5/17
1.25	TC	9/17	1	5	0	0	6	5	1	2	5	1	0	4	0	0	0	7	2	0	3/9	1/9	3/17
1.5	TC	3/13	3	1	0	0	4	2	0	0	0	0	0	2	0	0	0	8	1	0	1/3	0/3	1/13
1.75	TC	7/17	2	1	2	0	4	9	3	9	2	2	2	4	0	0	0	3	6	0	3/7	2/6	4/17
2.0	TC	12/36	11	3	1	2	15	12	3	10	9	3	2	8	6	1	2	9	13	1	7/12	4/10	8/36
2.3	TC	7/17	8	2	1	1	7	5	3	8	5	0	2	2	1	0	3	4	2	1	4/7	4/7	5/17

Note: Several patients experiencing DLT required dose reductions. Once dose reduction had taken place, patients were evaluated for toxicity at the lower dose level.

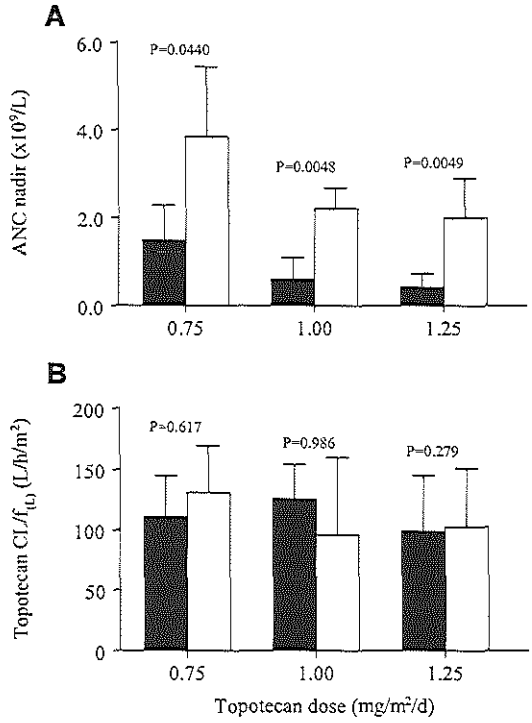

Figure 1 (A) Absolute neutrophil count (ANC) nadirs and (B) topotecan clearance ($CL/f_{(L)}$) during the first and second courses in sequences CT (■) and TC (□). Data are expressed as mean (bars) ± SD (error bars).

At the dose level of 1.0 mg/m² in the sequence CT, two out of six patients experienced neutropenia grade 4 lasting for 5 days or more. By protocol definition, these patients were judged as having DLT. At the next dose level combining topotecan 1.25 mg/m²/day with cisplatin 75 mg/m² in the sequence CT, of the six patients treated, one patient experienced neutropenic fever and another patient had neutropenia grade 4 lasting for more than 5 days in combination with diarrhea grade 3 and vomiting grade 4. Four additional patients were treated at this dose level. One of these patients, patient number 10, had DLT because of a neutropenia grade 4 lasting longer than 5 days. Another patient, who formally was ineligible due to reduced renal function at study entry, experienced grade 4 neutropenia and thrombocytopenia in the second course, and died as a result of the complications of this toxicity.

Since 3 out of 9 (4 out of 10 taking into account the ineligible patient) patients experienced DLT no further dose escalation was pursued. The protocol defined MTD as the

dose level below that level at which 3/6 patients experienced DLT. The dose level combining cisplatin 75 mg/m² day 1 with by topotecan 1.25 mg/m²/day day 1 to 5 is considered the recommended dose for this sequence, but only in non- or marginally pretreated patients in good physical condition and under strict conditions of control as the patients under study.

After the determination of the recommended dose in the sequence CT, dose escalation of topotecan continued in the reversed sequence. At the dose level 1.25 mg/m² 1 out of 6 patients experienced DLT consisting of neutropenic fever in this sequence. At the dose level 1.5 mg/m² no DLT was observed. One patient had a neutropenia grade 4 lasting for 5 days or more and vomiting grade 3 at dose level 1.75 mg/m² in the sequence TC. At the dose level combining topotecan 2.0 mg/m²/day with cisplatin, no DLT occurred in the initial 3 patients. It was decided to escalate the dose of topotecan to the dose recommended for use as a single agent, 2.3 mg/m²/day. Of the first three patients, only one patient developed DLT (diarrhea grade 4). However, three of the four additional patients treated at this dose level, were considered to have DLT, on the basis of vomiting grade 4 (1 patient), diarrhea grade 4 (1 patient), neutropenia grade 4 lasting for 5 days or more (2 patients) and thrombocytopenia grade 4 (1 patient). Thus, combining topotecan 2.3 mg/m²/day and cisplatin 75 mg/m² was not considered feasible. Seven additional patients were treated at dose level 2.0/75 mg/m². Four of these patients developed DLTs: vomiting grade 4 (2 patients), diarrhea grade 3 or 4 (3 patients) although manageable with loperamide therapy, neutropenia grade 4 lasting for 5 days or more (2 patients), and thrombocytopenia grade 4 (1 patient), resulting in DLT in 4 out of 10 patients. Thus, in full accordance to the recommendations for the sequence CT, the recommended dose of topotecan is 2.0 mg/m²/day combined with cisplatin 75 mg/m² for the sequence topotecan followed by cisplatin. Since it was felt that these doses are only feasible in non- or minimally pretreated patients in good physical condition under strict medical surveillance, it was decided to expand the dose level combining topotecan 1.75 mg/m² to six patients. One of these patients experienced grade 4 vomiting in the first course. No other DLTs were observed.

Overall, the hematologic toxicity was relatively mild (Table 2). Grade 3 to 4 neutropenia was observed in 55 of 175 courses (31%). It was complicated by neutropenic fever in only 4 patients. The onsets of neutropenia and thrombocytopenia were relatively late. The nadir of the neutrophils usually occurred around day 19 (range 4-30) after the start of the treatment and lasted for median 5 days (range 1-15). Thrombocytopenia was mild, being grade 3-4 in only 8% of the cycles, all in conjunction with neutropenia. Despite the limited severity of myelosuppression, treatment had to be delayed in 34% of the courses due to prolonged myelosuppression. A marked inhibition of erythropoiesis was observed. The percentage of patients requiring erythrocyte transfusions was 72%, in 74 of 175 courses.

Non-hematologic toxicity

Gastrointestinal toxicity was mild to moderate (Table 2) and generally comparable to the toxicities that result from similar single agent doses of topotecan and cisplatin. No sequence dependent effects were noted. Nausea grade 2 or 3 was observed in 60 of 175 courses (34%) and vomiting grade 3 or 4 in 15 of 175 courses (9%). Both were in time related to the

administration of cisplatin. Diarrhea grade 3 or 4 was encountered in 7 cycles (4%) and had a median day of onset on day 8 (range 7-14) and a median duration of 4 days (range 2-10). The diarrhea was self-limiting or resolved after low dose loperamide therapy in all but two patients, who were hospitalized for i.v. rehydration.

Consistent with the profile of cisplatin 75 mg/m^2, seventeen patients developed nephrotoxicity grade 1, and 4 patients grade 2 after a median of 2 cycles (range 1-6). Peripheral neurotoxicity grade 1 was encountered in 18 patients. Twenty patients had mostly reversible ototoxicity grade 2 (tinnitus) and 2 patients ototoxicity grade 3 after receiving median 2 cycles (range 1-6). One patient at dose level 2.3 mg/m^2 developed grade 4 bilirubinemia, due to obstruction of a biliary stent. One patient with a nasopharyngeal cancer, treated at dose level 2.0 mg/m^2 developed progressive dyspnoe accompanied by fever during the second course. An X-ray of the chest revealed interstitial enhancement with a reticulonodular pattern, especially more prominent at the bases. The pulmonary function demonstrated reduced lung volumes compatible with restrictive lung disease. Bronchoscopy revealed no abnormalities. Despite therapy with antibiotics and low dose corticosteroids, the patient's condition worsened and it was decided to perform an open lung biopsy. Pathologic examination revealed interstitial fibrosis with a marked infiltration with eosinophils, which was considered related to topotecan treatment. The patient was treated with high dose corticosteroids resulting in an amelioration of the symptoms.

Other side effects were mucositis (8 % of cycles), alopecia (19 patients grade 1, 7 patients grade 2), and fatigue.

Anti-tumor activity

Six patients achieved a partial response. The tumor types included, non-small cell and small cell lung cancer, ACUP, renal and pancreatic cancer. The patient with small cell lung cancer was pretreated twice with combination chemotherapy consisting of doxorubicin, etoposide and cyclophosphamide resulting in major responses of 30 and 25 weeks duration, respectively. Her response on cisplatin/topotecan chemotherapy lasted 22 weeks. The patient with pancreatic cancer was pretreated with 5-fluorouracil in combination with a DPD-inhibitor resulting in a partial response for 19 weeks. On therapy with cisplatin/topotecan he achieved a partial response for 26 weeks. The patient with renal cancer did not respond to prior therapy with a farnesyltransferase inhibitor. His response on cisplatin/topotecan lasted 20 weeks. The other 3 patients were not pretreated. The duration of their responses was 21 (NSCLC), 30 (ACUP) and 30+ (ACUP) weeks. Twenty-nine patients showed disease stabilization with a median duration of 20 weeks (range 8-68 weeks).

Topotecan and cisplatin pharmacokinetics

The possible effect of drug sequence on the pharmacokinetics of topotecan and cisplatin was investigated in the first 18 patients, that were randomized in a cross-over design for the administration sequence. These patients were all treated at the fixed cisplatin dose of 75 mg/m2 and topotecan doses of 0.75, 1.0 or 1.25 mg/m2/d×5. Table 3 lists the main pharmacokinetic parameters from a compartmental analysis of the two drugs, with topotecan

given at 0.75 mg/m2/d×5. The sequence of drug administration did not significantly influence the disposition of topotecan lactone, indicating mean (±SD) AUC values of 7.52±2.51 and 6.18±1.56 ng.h/mL (P=0.31) using the CT and TC sequence, respectively (Fig. 2). The apparent clearance (CL/f) of topotecan lactone was clearly dose-independent in the range of 0.75 to 1.25 mg/m2, similar to single agent data, and not significantly different between study courses (107±33.0 (CT) vs 109±53.5 L/h/m2 (TC); P=0.38, paired Student's t-test) (Fig. 1). Similarly, the lactone to total drug AUC ratio (L/T ratio) was independent of the sequence and averaged 0.36±0.04 (CT) vs 0.33±0.08 (TC). Topotecan pharmacokinetic parameters obtained on the fifth administration day were essentially similar to the data from day 1 (Data not shown). In order to rule out a potential effect of the interval time between drug administration, kinetic data were obtained from the first 18 patients (3 receiving the CT sequence and another 3 receiving the reversed order at each dose level) treated with a topotecan dose of 0.75, 1.0 or 1.25 mg/m2/d×5. Data of unpaired analysis in these patients indicated that a change of the interval time to 24 hours had no significant influence on any of the studied parameters (P>0.05, Mann-Whitney's U-test; Table 3).

Table 3 Effect of drug sequence and interval time on the pharmacokinetics of topotecan and cisplatin at a topotecan dose of 0.75 mg/m^2/d×5 and a single fixed cisplatin dose of 75 mg/m^2.

	3-Hour Interval (n = 6)		24-Hour Interval (n = 6)	
Parameter	Sequence CT day 1)	Sequence TC (day 1)	Sequence CT (days 1/2)	Sequence TC (day2 1/2)
Topotecan				
C_{max} ng/ml	2.05 ± 0.96	1.97 ± 0.55	3.01 ± 1.85	2.86 ± 1.10
$AUC_{(L)}$, ng.h/ml	7.52 ± 2.51	6.18 ± 1.56	9.20 ± 3.45	9.89 ± 3.09
$CL/_{f(L)}$, L/h/m^2	111 ± 34.0	131 ± 38.3	96.3 ± 40.8	83.5 ± 25.2
L:T ratio	0.36 ± 0.04	0.33 ± 0.08	0.33 ± 0.03	0.33 ± 0.22
Cisplatin				
AUC_{fu}, µg.h/ml	2.30 ± 1.17	2.29 ± 0.20	3.24 ± 0.27	3.07 ± 0.51
CL_{fu}, ml/min	817 ± 463	747 ± 177	823 ± 52.2	765 ± 139
AUC_{tot}, µg.h/ml	33.3 ± 10.5	29.6 ± 4.12	46.5 ± 1.26	45.3 ± 4.08
A_{max}, pg/µg DNA	2.91 ± 2.33	1.93 ± 1.28	2.41 ± 0.81	3.20 ± 2.78

Data are mean values ± S.D. Abbreviations: C_{max}, peak plasma level; AUC, area under the plasma concentration-time curve; CL/f, apparent clearance; L/T ratio, topotecan lactone to total drug AUC ratio; fu, unbound platinum fraction; tot, total platinum fraction; A_{max}, peak platinum DNA adduct levels in peripheral leukocytes.

The peak plasma levels and the plasma clearance of unbound cisplatin were also independent of the drug sequence with a 3-hour or a 24-hour interval time between administration (Table 3). Over the 3 dose levels studied, the cisplatin clearance was not dependent on the topotecan dose, and averaged 817±463 (CT) vs 747±177 mL/min (TC) (P=0.19) with the 3-hour interval time (Fig. 2). Similarly, sequence and topotecan dose had no influence on the protein binding of cisplatin (overall mean: 93.1±2.8%) and on the peak

platinum DNA-adduct levels in peripheral blood leukocytes [4.58±4.12 (CT) vs 5.72± 4.66 pg Pt/µg DNA (TC) across all 3 dose levels; P=0.55].

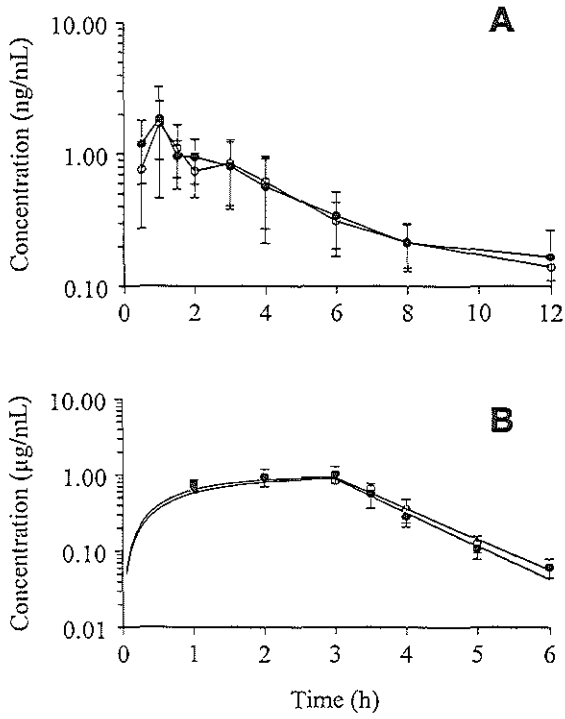

Figure 2 Plasma concentration-time curves of topotecan lactone (A) and unbound cisplatin (B) (closed symbols: sequence CT; open symbols: sequence TC) in 6 patients treated with topotecan 0.75mg/m²/day and cisplatin 75 mg/m². Data represent mean values (symbol) ± S.D. (error bars).

Table 4 Summary of topotecan pharmacokinetics during the first course as a function of treatment cohort.

Topotecan ($mg/m^2/dx5$)	*n*	$C_{max\,(L)}$ (ng/mL)	$AUC_{(L)}$ (ng.h/mL)	$CL/f_{(L)}$ ($L/h/m^2$)	L/T ratio
CT sequence					
0.75	6	2.05±0.96	7.52±2.51	111±34.0	0.36±0.04
1.00	6	2.26±0.73	8.38±1.93	126±28.4	0.39±0.05
1.25	10	4.10±1.72	14.5±4.42	99.0±46.0	0.42±0.07
TC sequence					
0.75	6	1.97±0.55	6.18±1.56	131±38.3	0.33±0.08
1.00	6	3.88±2.43	8.27±4.47	95.8±63.8	0.35±0.16
1.25	6	4.33±2.43	14.7±5.64	102±48.3	0.38±0.05
1.50	3	6.87±5.74	18.2±6.11	106±29.0	0.37±0.05
2.00	6	5.92±3.10	18.4±6.74	131±64.6	0.38±0.04
2.30	7	3.72±1.00	18.4±6.15	145±60.1	0.39±0.07

Data are mean values ± S.D. Abbreviations: C_{max}, peak plasma level; AUC, area under the plasma concentration-time curve; CL/f, apparent clearance; L/T ratio, topotecan lactone to total drug AUC ratio; *n*, number of patients studied.

To further assess the effects of cisplatin administration and drug sequence on topotecan pharmacokinetics, all additional patients enrolled in the study had complete sampling performed, with the exception of 3 patients treated at the 2.0 mg/m2/day topotecan dose and 1 patient (only second course missing) at the 2.3 mg/m2/day dose. A summary of the topotecan pharmacokinetic data from the first course is provided in Table 4. In both sequence groups, substantial interpatient variability in kinetic parameters was apparent, with more than 2-fold variation in AUC values, although mean values were correlated to the administered dose (Spearman's ρ(rho)=0.76; TC sequence). There were no significant differences in any of the parameters between the topotecan dose levels (P>0.05, Kruskal-Wallis' test), consistent with a linear and dose-independent behavior of the compound. Pharmacokinetic parameters between sequences were again not significantly different. Parameters between the day of topotecan dosing were not significantly different as indicated by the ratio of the topotecan lactone AUC measured on days 1 and 5 (Data not shown), although the mean ratios slightly deviated from 1.0 probably as a result of a minor topotecan accumulation during the consecutive treatment days. Pharmacokinetic data obtained during the second treatment course, again with sampling performed on days 1 and 5, were essentially similar to the first course (Not shown).

The effect of the topotecan dose on the disposition of unbound and total cisplatin in plasma during the first treatment course is shown in Table 5. None of the pharmacokinetic parameters between the sequences and the various topotecan dose levels was significantly different.

Table 5 Effect of topotecan dose on the pharmacokinetics of cisplatin during the first treatment course at a single fixed cisplatin dose of 75 mg/m^2.

Topotecan (mg/m2/dx5)	n	AUCfu (μg.h/mL)	CLfu (mL/min)	AUCtot (μg.h/mL)	CLtot (mL/min)
CT sequence					
0.75	6	2.30±1.17	817±463	33.3±10.5	88.1±45.1
1.00	6	2.64±1.27	623±310	41.5±6.51	58.6±13.5
1.25	10	2.97±1.12	962±660	37.9±4.39	56.3±7.72
TC sequence					
0.75	6	2.71±0.20	925±177	37.0±4.12	67.5±9.87
1.00	6	2.87±0.35	833±143	35.5±4.45	67.3±11.5
1.25	6	2.58±0.45	874±183	41.6±9.79	55.6±14.8
1.50	3	2.75±0.46	919±174	32.9±3.54	75.3±8.06
1.75	3	3.22±0.27	722±75.0	47.6±4.28	48.8±4.36
2.00	7	3.13±0.50	815±87.0	37.1±6.44	69.7±11.3
2.30	7	2.58±0.23	975±130	36.1±4.89	69.8±7.30

Data are mean values ± S.D. Abbreviations: AUC, area under the plasma concentration-time curve; CL, clearance; fu, unbound platinum fraction; tot, total platinum fraction; *n*, number of patients studied

Table 6 Topotecan and cisplatin pharmacokinetics at the recommended doses during the first treatment course: (1) 75 mg/m^2 cisplatin followed by 1.25 mg/m^2/dx5 topotecan and (2) 2.00 mg/m^2/dx5 topotecan followed by 75 mg/m^2 cisplatin. Data are mean values ± S.D.

	(1) CT sequence (n=10)			*(2) TC sequence [n=6 (T) or 7 (C)]*		
Parameter	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5
Topotecan						
C_{max} ng/ml	4.10 ± 1.72	3.31 ± 2.31	3.50 ± 2.25	5.11 ± 3.50	7.52 ± 3.55	6.16 ± 3.30
$AUC_{(L)}$, ng.h/ml	14.5 ± 4.42	13.8 ± 4.22	14.4 ± 4.42	15.9 ± 8.79	24.6 ± 8.24	22.6 ± 6.10
L:T ratio	0.40 ± 0.03	0.39 ± 0.07	0.40 ± 0.08	0.36 ± 0.02	0.37 ± 0.04	0.41 ± 0.05
Cisplatin						
AUC_{fu}, μg.h/ml	2.97 ± 1.12	-	-	-	-	3.13 ± 0.50
CL_{fu}, ml/min	962 ± 660	-	-	-	-	815 ± 87.0
AUC_{tot}, μg.h/ml	37.9 ± 4.39	-	-	-	-	37.1 ± 6.44
A_{max}, pg/μg DNA	3.12 ± 6.52	-	-	-	-	2.34 ± 2.67

Topotecan pharmacokinetic parameters on day 2 were only available from 4 patients. Abbreviations: C_{max}, peak plasma level; AUC, area under the plasma concentration-time curve; L/T ratio, topotecan lactone to total drug AUC ratio; fu, unbound platinum fraction; tot, total platinum fraction; CL, clearance; A_{max}, peak platinum DNA adduct levels in peripheral leukocytes

At the recommended doses for further clinical studies, viz. 75 mg/m2 cisplatin followed by 1.25 mg/m2/day topotecan (CT) and 2.0 mg/m2/day topotecan followed by 75 mg/m2 cisplatin (TC), plasma sampling was also performed on day 2 to ensure that the topotecan disposition did not alter before day 5. Paired analysis showed that all relevant parameters were essentially similar between days of drug administration in both sequences (Table 6), although in the (less myelotoxic) TC sequence, the topotecan lactone peak plasma level and AUC values were slightly higher on day 2 as compared to days 1 and 5. This is most likely caused by the small number of patients studied (n=4 on day 2), in combination with large intrapatient and interpatient variability in topotecan kinetics.

DISCUSSION

Both cisplatin and topotecan have broad antitumor activity. Because topoisomerase I inhibitors might interfere in the repair of cisplatin induced DNA interstrand cross-links, there has been considerable interest in the effects of combining these classes of drugs. Interaction of topoisomerase I inhibitors with platin derivatives have been studied in vitro and in vivo. The combination of topotecan and cisplatin was synergistic in teratocarcinoma [10], non-small cell lung cancer [5,11,14,17], ovarian cancer [5,9], esophageal cancer [16], breast cancer [5] and melanoma cell lines and in a human tumor xenograft of small cell lung cancer [13] and ovarian cancer [9]. In contrast, patterns of cross-resistance observed in studies with resistant small cell lung cancer cell lines suggested that topoisomerase I inhibitor-cisplatin combinations might be disadvantageous [12]. The cytotoxicity of the combination of topotecan and cisplatin was also dependent on the schedule used. When V79 chinese hamster lung fibroblast were exposed to cisplatin early in the course of topotecan treatment synergy was most prominent [43]. This phenomenon was also confirmed in IGROV-1 ovarian cancer and MCF7 breast cancer cell lines. Incubation of these cells with cisplatin followed by topotecan resulted in optimal synergism [5]. However, in other cell lines variations in the scheduling of cisplatin and topotecan did not influence the observed interaction [5,17]. The potential importance of sequence dependence for the combination of cisplatin and the intravenous formulation of topotecan in the clinical setting was studied by Rowinsky et al., revealing enhanced myelosuppression when cisplatin administration preceded topotecan [17]. Recently, an oral formulation of topotecan with a bioavailability of 32-44% became available, which is a more convenient method of drug administration [31-33]. The reported phase I study was performed to explore the influence of alternate sequences of oral topotecan in a daily times five schedule and cisplatin on the observed side-effects and pharmacokinetic behavior of both drugs and to determine the maximum tolerated dose of topotecan in combination with cisplatin 75 mg/m^2 once every 3 weeks in both sequences.

Both neutropenia and diarrhea were the DLTs of oral topotecan combined with cisplatin in this schedule. Other toxicity was usually mild to moderate and consisted of nausea and vomiting, mucositis, fatigue, neuro- and nephrotoxicity and alopecia.

Myelosuppression was significantly more severe when cisplatin preceded topotecan administration. This observation is in accordance with the data reported for the combination

of the intravenous formulation of topotecan and cisplatin [17]. The onset of the neutropenia was relatively late with a median day of onset of the nadir on day 19 (range 4-30). These data are in line with the data reported by Miller et al. [44]. The combination of topotecan, administered intravenously on day 1 to 5, with cisplatin on day 1 resulted in a neutrophil nadir around day 12 (range 8-25). Compared to the median time to neutrophil nadir of 12 days (range 9-15) for single agent oral topotecan [34] and day 9 (range 6-10) [19] for single agent intravenously administered topotecan, the nadir in our study was delayed. This resulted in treatment delay due to prolonged myelosuppression in 34% of the courses. Despite grade 3 or 4 neutropenia was observed in 31% of the courses, the incidence of neutropenic fever was only 2%.

The doses in this sequence, cisplatin followed by topotecan, we can recommend for phase II studies are oral topotecan 1.25 mg/m²/day day 1 to 5 and cisplatin 75 mg/m², but only in non- or minimally pretreated patients in good clinical condition and under strict medical surveillance, comparable to the patients treated in this study. In other circumstances dose adjustment of topotecan should be considered. In other phase I studies, combining cisplatin 50 mg/m² on day 1 with i.v. topotecan as a 30-min infusion daily for 5 consecutive days, neutropenia and thrombocytopenia constituted the principal toxicities. The recommended dose of topotecan for further trials was 0.75 to 1.0 mg/m²/day combined with cisplatin 50 mg/m², accounting for 50-66% of the single agent intravenous dose of topotecan [17,44]. This percentage is similar to our results where the recommended dose of oral topotecan in this schedule is 54% of single agent oral topotecan in a daily times five schedule [34]. We realize that oral availability of topotecan is not taken into account. However, the relevance of bioavailability becomes questionable in view of the results of recent studies indicating that the oral administration of topotecan at a dose of 2.3 mg/m²/day is as effective as the intravenous administration of topotecan 1.5 mg/m²/day [35, 36] in both ovarian cancer and small cell lung cancer.

For the reversed sequence, the recommended doses are oral topotecan 2.0 mg/m²/day day 1 to 5 followed by cisplatin 75 mg/m². This constitutes a topotecan dose of 87% of the single agent dose. However, as indicated, it is recommended that the use of topotecan and cisplatin at these doses should be limited to patients similar to those studied in this trial, i.e., untreated or minimally pretreated patients with a good performance status under strict medical surveillance. In all other circumstances, topotecan dose reduction is recommended. This sequence of drug administration was also studied for the combination of the intravenous formulation of topotecan for 5 consecutive days in escalating doses and cisplatin 50 mg/m² in an alternating schedule with carboplatin, cisplatin, teniposide and vincristine in patients with small-cell lung cancer [45]. Preliminary data indicate that it is feasible to combine i.v. topotecan 1.5 mg/m²/day, the recommended dose of single-agent topotecan, with cisplatin 50 mg/m². Thus, the observed hematological toxicity is sequence dependent both for the intravenous and the oral formulation of topotecan in combination with cisplatin, resulting in a higher dose intensity of topotecan when administered before cisplatin.The observed pharmacokinetic parameters of the lactone and the carboxylate form of topotecan demonstrated linear and dose independent behavior over the total dose range studied and

were similar to single agent data [46] and also comparable to the data obtained in the schedule with 24 hour interval between the administration of topotecan and cisplatin in our study, indicating no apparent pharmacokinetic interaction between topotecan and cisplatin. The sequence of drug administration also had no influence on the pharmacokinetics of topotecan at the dose levels administered, neither on day 1, 2 or 5. This is in contrast with the reported reduction of the clearance of intravenously administered topotecan observed after preceding cisplatin administration [17]. Sequence-dependent differences in toxicity and pharmacokinetics can be obscured by a large intra-patient variability in AUC. However, the intrapatient variability in AUC of topotecan lactone for oral topotecan expressed as coefficient of variation (CV) is 18.5% [34] and is comparable with the intrapatient variability in AUC observed after intravenous administration of topotecan (CV is 12.6%) [47]. Since patients were treated in a cross-over design, sequence dependent toxicological and pharmacological differences could be assessed as accurately as in an IV study.

Also the ratio of topotecan AUC of lactone to total drug correspond very well with data of a previous study in which oral topotecan was administered as a single agent [34] and did not vary with the sequence of drug administration.

The plasma clearance and volume of distribution of unbound cisplatin as well as the AUC up to the last measured time point of total cisplatin in plasma indicated no significant influence of topotecan on the protein binding and plasma disposition of cisplatin. Preclinical studies indicated that the reversal of cisplatin-induced DNA interstrand cross-links was delayed by concomitant incubation with a topoisomerase I inhibitor [48-50], without modifying their formation. However, in our study the values of the maximal platinum DNA-adduct formation in peripheral leukocytes and the area under the DNA-adduct versus time curve were consistent with single agent data [42], and were independent of the drug sequence. Although the preclinical observations might not be extrapolated to the clinical setting, it is possible that the extreme variability in platinum DNA-adduct values would not allow any small alteration in adduct formation to be observed even if it was present. It is also possible that other mechanisms may contribute to the enhanced toxicity observed for the sequence CT. In in vitro studies induction of topoisomerase I [51] and enhanced topoisomerase I inhibitory activity [52] were observed after incubation with cisplatin followed by the administration of a topoisomerase I inhibitor. Simultaneous incubation of platinum derivatives and topoisomerase I inhibitors resulted in enhanced S-phase arrest in human colon and ovarian cancer cell lines indicative of increased topoisomerase I inhibitor-induced cytotoxicity [48,49]. This observation might indicate that the synergistic toxicity observed for the combination of topoisomerase I inhibitors and platinum derivatives can partly be explained by a modification in cellular response to DNA damage.

Based on the available data, the importance of the sequence of drug administration and the enhanced toxicity observed when cisplatin is followed by topotecan can not simply be extrapolated to the antitumor activity of the combination. However, a sequence dependent effect on antitumor activity can not be ruled out. Further randomized phase II studies in patients with topotecan sensitive tumor types are needed to elucidate the importance of drug sequencing and possible cytotoxic interaction, and the potential relevance of the higher dose

intensity of both drugs, that can be achieved when the less toxic sequence of drug administration is used.

In conclusion, the recommended dose for phase II studies in selected patients is oral topotecan 1.25 mg/m²/day for 5 consecutive days combined with cisplatin 75 mg/m² on day 1, once every 3 weeks, or topotecan 2.0 mg/m²/day day 1 to 5 followed by the same dose cisplatin on day 5. No pharmacokinetic interaction could explain the enhanced myelosuppression observed in the sequence CT.

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Chapter 5

Phase I and pharmacological study of increased dose oral topotecan in combination with intravenous cisplatin

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SUMMARY

Background: A recent clinical study with cisplatin-topotecan chemotherapy has shown that the maximum tolerated dose was cisplatin 75 mg/m^2 on day 1 followed by oral topotecan 1.25 mg/m^2/day for 5 days (10). Since preclinical studies have shown a relationship between topotecan exposure and antitumour response, we tried to increase the topotecan dose when given with a reduced fixed dose of cisplatin 50 mg/m^2.

Patients and Methods: A total of 12 patients were treated with oral topotecan administered daily for 5 consecutive days in escalating doses starting at 1.50 mg/m^2/day and cisplatin was given as a 3-h infusion before topotecan. Serial plasma samples were obtained from all patients for pharmacokinetic analysis of the lactone and carboxylate forms of topotecan and unbound and total cisplatin.

Results: Dose-limiting toxicity, consisting of thrombocytopenia, neutropenia and/or diarrhea, was encountered at the second dose level (topotecan 1.75 mg/m^2/day, cisplatin 50 mg/m^2). Hematological pharmacodynamics indicated a delayed onset of neutropenia with this combination as compared to single agent data. The apparent clearance of topotecan was independent of dose, with mean values of 109±65.8 and 73.5±14.4 L/h/m^2 (P=0.27) at the 1.50 and 1.75 mg/m^2/day doses, respectively. The topotecan dose did not affect any of the cisplatin pharmacokinetic parameters (P≥0.10), suggesting lack of a kinetic interaction.

Conclusion: The maximum-tolerated dose of this combination is cisplatin 50 mg/m^2 on day 1 immediately followed by oral topotecan 1.50 mg/m^2/day for 5 days. A 33% reduction in cisplatin dose allowed only a 20% dose increase in oral topotecan. These results indicate that the studied regimen has no advantage as compared to the combination with cisplatin 75 mg/m^2.

INTRODUCTION

Topotecan [(S)-9-dimethylaminomethyl-10-hydroxycamptothecin, Hycamtin®] is a water-soluble semi-synthetic derivative of camptothecin, an inhibitor of the nuclear enzyme topoisomerase I known to interfere with the process of DNA breakage and resealing, resulting in irreversible DNA-single strand breaks and ultimately in cell death (1). Since cisplatin is a DNA damaging agent, synergy was hypothesised for the combination with topotecan. This synergism was demonstrated in *in vitro* studies in various cancer cell lines (2). *In vitro* studies indicated that topoisomerase I inhibitors delayed the reversal of cisplatin induced DNA interstrand cross-links without modifying their formation, as a possible mechanism for the observed synergism. Also, simultaneous treatment prolonged the DNA and RNA synthesis inhibition induced by either drug alone (3,4). When observed, synergy seemed to be sequence dependent and cytotoxicity increased when topotecan administration was preceded by cisplatin (5). Driven by these promising *in vitro* data and by the clinical knowledge that topoisomerase I inhibitors and cisplatin have different toxicity profiles and share a broad spectrum of antitumour activity, several phase I studies combining i.v. topotecan and cisplatin were initiated (6-9). All studies showed that a considerable dose reduction of i.v. topotecan was required as compared to the single agent dose, and that

cisplatin given before topotecan produced a greater incidence of severe neutropenia in the studies in which the sequence dependent effects were studied (6,10).

Based on *in vitro* observations showing more pronounced antitumour efficacy with protracted exposure of topoisomerase I inhibitors at low concentration, several clinical studies on low dose continuous infusion of topotecan were performed (11-14). Since oral delivery is a more convenient method for prolonged drug administration, and preferred by patients (15,16), an oral formulation of topotecan was developed. Topotecan, supplied in gelatin capsules, has a bioavailability of 42 ± 13% (17). The maximally tolerated dose for oral topotecan, administered for 5 days every 21 days as a single agent was defined as 2.3 mg/m^2/day, with myelosuppression (in particular neutropenia) as the dose limiting toxicity (18). Two randomised studies on single agent topotecan suggested that the oral formulation is equipotent to the intravenous formulation in patients with ovarian- and small cell lung cancer, whilst associated with less grade 3 and 4 neutropenia (19,20). Recently, a phase I study combining oral topotecan (T) given for 5 days every 3 weeks combined with cisplatin (C) at 75 mg/m^2 on day 1 (sequence CT) or day 5 (sequence TC) was completed (10). In congruency with the results of the study by Rowinsky with i.v. topotecan (6), the maximum tolerated dose (MTD) for topotecan in the CT sequence of 1.25 mg/m^2/day x 5 was considerably lower than that for the alternate sequence (2.00 mg/m^2/day x 5). No indication of a pharmacokinetic interaction between cisplatin and topotecan was observed (10).

Finally, topotecan systemic exposure was directly associated with antitumour activity in *in vivo* studies (21). Given this exposure-response relationship, we tried to increase the topotecan dose in the combination cisplatin-oral topotecan by using a lower cisplatin dose. We performed a phase I study in patients with solid tumours with oral topotecan preceded by a fixed dose of i.v. cisplatin at 50 mg/m^2.

PATIENTS AND METHODS

Patient selection

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumour refractory to standard forms of therapy, or for whom no better option than cisplatin-topotecan was available, were eligible. Other eligibility criteria included the following: age between 18-75 years; Eastern Cooperative Oncology Group performance status ≤ 2; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycin C); no previous therapy with topoisomerase I inhibitors; and adequate hematopoietic (absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L and platelet count ≥ 100 x 10^9/L), renal (creatinine clearance ≥ 60 mL/min) and hepatic (total serum bilirubin ≤ 1.25 x upper normal limit and serum ASAT and ALAT ≤ 2.0 x upper normal limits, in case of liver metastasis: serum ASAT and ALAT ≤ 3.0 x upper normal limits) function. Specific exclusion criteria included the existence of gross ascites and/or any gastrointestinal condition that would alter drug absorption. The study protocol was approved by the institutional ethics board. All patients gave written informed consent before study entry.

Dosage and dose escalation

Escalating doses of oral topotecan were administered on day 1-5, every 21 days preceded by a fixed dose of 50 mg/m² cisplatin administered intravenously over 3-h given on day 1. The starting dose of topotecan was 1.50 mg/m²/day, which is 120% of the recommended dose for the combination with cisplatin at 75 mg/m² given day 1 in the same drug sequence (10). Dose escalation was based on the prior dose level toxicities. At least three patients were treated at each dose level. If one of three patients experienced dose limiting toxicity (DLT), three additional patients were entered at that dose level. The maximum-tolerated dose (MTD) was defined as one dose level below the dose that induced DLTs in 3 out of 6 patients during the first course. DLTs were defined as NCI-CTC version 1994 grade 4 neutropenia lasting for five days or more, or complicated with fever requiring hospitalisation, grade 4 thrombocytopenia and/or non-hematological toxicity ≥ grade 3 (grade 2 for renal toxicity), excluding nausea. Intrapatient dose escalation was not permitted. If a patient encountered DLT, the dose of topotecan was decreased one dose level at retreatment. The treatment was resumed when the neutrophil count had recovered to ≥ 1.0 x 10^9/L and the platelet count to ≥ 100 x 10^9/L. A maximum of 6 cycles was administered to each patient.

Drug administration

All patients received cisplatin as a 3-h infusion diluted in 250 mL of hypertonic saline [3% (w/v) sodium chloride] on day 1, immediately followed by the oral administration of topotecan, which was given for five consecutive days on an empty stomach, at least 10 min before meals.

Pre-medication consisted of ondansetron (8 mg i.v.) combined with dexamethasone (10 mg i.v.) administered 30 min before the start of the cisplatin infusion. The administration of cisplatin was preceded by infusion of 1 L of a mixture of 5% (w/v) dextrose and 0.9% (w/v) sodium chloride over 4 h, and followed by another 3 L with the addition of 20 mM potassium chloride and 2 g/L magnesium sulphate applied over 16 h. Topotecan capsules containing either 0.25 or 1.00 mg of the active compound were supplied by SmithKline Beecham Pharmaceuticals (Harlow, UK). Cisplatin (Platosin) was purchased as a powder from Pharmachemie (Haarlem, The Netherlands).

Treatment assessment and pharmacokinetic data analysis

Treatment assessment and blood sampling was performed as described in the previous study (10). Blood samples for pharmacokinetic analysis were taken at the following time points: for cisplatin before infusion; at 1, 2, and 3 h after start of the infusion; and at 0.5, 1, 2, 3, 4, and 18 h after the end of the infusion, and for topotecan prior to dosing; and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after administration of topotecan.

Samples for topotecan kinetics were analysed by a validated reversed-phase high-performance liquid chromatographic method, that allowed simultaneous determination of the lactone and the hydrolysed ring-opened carboxylate forms, as described (22) with minor modifications (10). Non-protein bound and total cisplatin concentrations in plasma were

determined by a validated analytical procedure based on measurement of platinum atoms by flameless atomic-absorption spectrometry (23).

Individual plasma concentrations of topotecan were fit to a one- or two-compartment model, using the software package Siphar v4.0 (SIMED, Creteil, France). The concentration-time profiles were obtained after zero-order input, with weighted least-squares analysis applying a weighting factor of 1/y. The topotecan area under the plasma concentration-time curve (AUC) was determined for both the lactone (AUC(L)) and carboxylate forms (AUC(c)). The apparent plasma clearance (CL/f(L)) of topotecan lactone was calculated by dividing the dose administered by the observed AUC. The terminal disposition half-life [T1/2(z)] of topotecan was calculated as ln2/k, where k is the rate constant of the terminal disposition phase (expressed in h-1). The peak plasma concentrations (Cmax) and the time to peak plasma concentration (Tmax) were determined graphically from the (observed) experimental values. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as AUC(L)/[AUC(L)+AUC(c)].

Kinetic profiles of CDDP were obtained similarly using a two-compartment linear model with extended least-squares regression analysis as reported earlier (10). The AUCs of unbound and total cisplatin were calculated to the last sampling time point with detectable drug levels (C_{last}) by the linear trapezoid method and extended to infinity by addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression of the final plasma concentration values.

Statistical considerations

Pharmacokinetic parameters for all compounds are reported as mean values ± S.D. The difference in pharmacokinetic parameters was evaluated statistically using a Student's t-test and a Friedman's two-way analysis of variance test. Probability values (two-sided) of less than 0.05 were regarded as statistically significant. All calculations were performed using the statistical package NCSS version 5.X (JL Hintze, Kaysville, UT, 1992).

RESULTS

Twelve patients (8 males, 4 females) entered this study between March and September 1999. Patient characteristics are listed in Table 1. All patients were eligible, 11 patients were assessable for toxicity (1 patient died due to a suspected pulmonary embolism on day 11) and 10 patients were assessable for response (1 patient stopped treatment after one course at her own request and refused CT-evaluation). The majority of patients had mild symptoms. Fifty percent of patients, equally distributed over both dose levels, had received prior chemo- and/or radiotherapy. Overall, all patients were non- or minimally pretreated and in good clinical condition. The patient population was thus comparable to the 'highly selected' group of patients at the recommended dose level of topotecan 1.25 mg/m^2/day on day 1-5 in combination with cisplatin 75 mg/m^2 on day 1 in our previous study (10). Dose levels of topotecan studied were 1.50 and 1.75 mg/m^2/day. The total number of assessable courses was 43, with a median number of courses per patient of 4 (range 1-6).

Gastrointestinal toxicity and myelosuppression were the principle DLTs of this regimen. Three patients, treated at dose level 1.75, required dose reductions after experiencing DLT, and one patient even had a second dose reduction to 1.25 mg/m^2/day.

Table 1 Patients characteristics

Characteristic		No. of patients
No. entered		12
No. assessable		12
Age, years		
Median	56	
Range	38-74	
Sex		
Female		4
Male		8
Performance status		
Median	1	
Range	0-2	
Tumour type		
Carcinoma of unknown primary		4
Mesothelioma		2
Non-small cell lung cancer		2
Head/Neck		1
Head/Neck+NSCLC		1
Cervical		1
Colon		1
Previous therapy		
Chemotherapy		2
Radiation		1
Chemotherapy and radiation		3
None		6

Abbreviations: NCSLC = non-small cell lung cancer.

Hematological toxicity

Hematological toxicity observed during all courses is shown in Table 2A. Neutropenia was the main hematological toxicity. The percentage of courses associated with grade 3 or 4 neutropenia (no DLTs) and grade 3 or 4 thrombocytopenia at the topotecan dose level of 1.50 mg/m^2 was respectively, 36% and 9%, and 32% for grade 1 or 2 thrombocytopenia. At the next dose level of topotecan 1.75 mg/m^2, grade 3 or 4 neutropenia occurred in 50% of courses, associated with grade 3 or 4 thrombocytopenia in 11% of courses. Grade 1 or 2 thrombocytopenia was observed in 22% of courses. At this dose level, 2 patients were judged as having experienced DLT: 1 had grade 4 neutropenia lasting for more than 5 days

and 1 had neutropenic fever and grade 4 thrombocytopenia. The patients who had their topotecan dose reduced due to hematological toxicity had a much higher incidence of grade 3-4 hematological toxicity in subsequent courses compared to patients who were initially treated at a lower dose level. All of these patients had had prior chemo- and/or radiotherapy. The percentage decrease in leukocyte, neutrophil and platelet count during the first course in 10 patients (1 patient was inassessable for toxicity and 1 patient did not have all blood samples taken exactly according to the protocol) is shown in Table 2B. The median day of onset of neutropenia during the first course was relatively late (day 18, range 14-21) as compared to topotecan single agent data, in contrary to thrombocytopenia nadir which occurred after an interval similar to the one of single agent nadir (day 14, range 12-18). An example of hematological pharmacodynamic-time profiles from 2 patients receiving topotecan at either 1.50 (◦) or 1.75 (•) mg/m^2/day x 5 is shown in fig. 1.

Table 2a Hematological toxicity (worst per cycle)

Topotecan	No. of	Neutropenia		Thrombocytopenia	
mg/m^2 day	pts/cycles	3	4	3	4
1.50	6/16	2	1	0	0
1.75	6/18	3	6	1	1
1.50 (after dose reduction)	3/6	0	5	0	2
1.25 (after dose reduction)	1/3	0	3	2	1

Table 2b Absolute hematological toxicity during course 1

Topotecan	No	Nadir WBC		Nadir ANC		Nadir platelets	
mg/m^2/day	pts	day	% decrease	day	% decrease	day	%decrease
1.50	5	18±3	74±16	19±2	84±11	15±3	62±20
1.75	5	15±1	83±13	16±2	93±10	13±1	83±6.9

Abbreviations: WBC, white blood cell count; ANC, absolute neutrophil count

Overall, at the recommended dose of topotecan 1.50 mg/m^2 on day 1-5, hematological toxicity was acceptable with 4 chemo- and/or radiotherapy pretreated patients experiencing neutropenia grade 4 for less than 5 days and grade 3-4 thrombocytopenia in only 2 of the 22 courses. Three patients with grade 2 anemia received blood transfusions. Only 1 course had to be delayed at this dose level for hematological reasons. Patients starting at the second dose level of topotecan 1.75 mg/m^2 and requiring dose reduction due to hematological toxicity were prone to have hematological toxicity at the lower dose levels. These patients accounted for 63% of all grade 3 or 4 neutropenia's at this dose level, and for all grade 3 or 4 thrombocytopenia's.

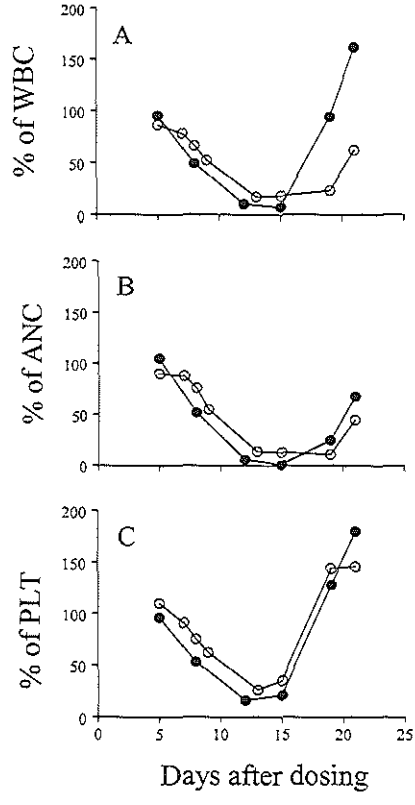

Figure 1 An example of hematological pharmacodynamic-time profiles from 2 patients receiving topotecan at either 1.50 (°) or 1.75 (•) mg/m^2/day x 5.
Abbreviations: WBC, white blood cells; ANC, absolute neutrophil count; PLT, platelets.

Non-hematological toxicity

Fatigue and gastrointestinal toxicity were the most frequently reported non-hematological side-effects (data shown in Table 3). One patient treated with topotecan 1.50 mg/m^2 experienced grade 3 diarrhea in the first course, which was considered DLT as defined according to the protocol. The diarrhea occurred on day 7 and resolved within one day with loperamide treatment, therefore topotecan dose was not reduced. The patient had no diarrhea in the second course. On the next dose level of topotecan at 1.75 mg/m^2, 3 out of 6 patients experienced DLT, 1 had grade 3 mucositis and vomiting, 1 had grade 3 diarrhea and was treated with loperamide from day 14-16 (this patient also had grade 4 neutropenia lasting for more than 5 days), and 1 had diarrhea grade 3 on day 12 (and neutropenic fever).

One extensively pretreated patient with progressive metastatic colon cancer died 11 days after the first administration of cisplatin and topotecan, presumably due to a pulmonary embolism, which was not considered related to the treatment. Since the patient died at home, the clinical diagnosis was not confirmed by additional investigations.

Other side effects were alopecia (grade 1 or 2 in 50% of patients) and peripheral neurotoxicity (grade 1 or 2 in 16% patients). No nephrotoxicity other than grade 1 was encountered.

Overall, non-hematological toxicity at the recommended dose level was infrequent. Other than the one patient described with grade 3 diarrhea on day 7, who had grade 3 nausea in the second course, no toxicity graded > 2 was observed.

Table 3 Non-hematological toxicity (worst per cycle)

Topotecan mg/m^2 day	No. of pts/cycles	Nausea			Vomiting			Diarrhea			Fatigue		
		1	2	3	1	2	3	1	2	3	1	2	3
1.50	6/16	5	2	1	2	1	0	1	0	1	9	0	0
1.75	6/18	6	4	1	1	2	1	5	3	2	6	2	0
1.50 (after dose reduction)	3/6	2	0	0	1	0	0	1	0	0	1	0	0
1.25 (after dose reduction)	1/3	0	0	0	0	0	0	0	0	0	1	0	0

Antitumour activity

One patient with a mesothelioma achieved a partial response, which is currently still ongoing after course 6. Another patient with pulmonary metastases of a head and neck tumour and a simultaneous secondary primary non-small cell lung cancer also achieved a partial response, which was not confirmed since she had radiotherapy for consolidation of the response. Six patients showed disease stabilisation. Two patients were not evaluable for response, including the one patient that presumably died due to a pulmonary embolism.

Topotecan and cisplatin pharmacokinetics

Pharmacokinetic analysis was performed in 11 out of 12 patients in course 1 and in 1 patient also in course 6. Table 4 and 5 list the main pharmacokinetic parameters of the two drugs with topotecan given at 1.50 or 1.75 mg/m^2/day x 5 in combination with a fixed dose of cisplatin 50 mg/m^2 day every 3 weeks. The average plasma concentration-time curves of topotecan lactone and unbound cisplatin on day 1 at the recommended dose level of topotecan at 1.50 mg/m^2 are presented in fig. 2.

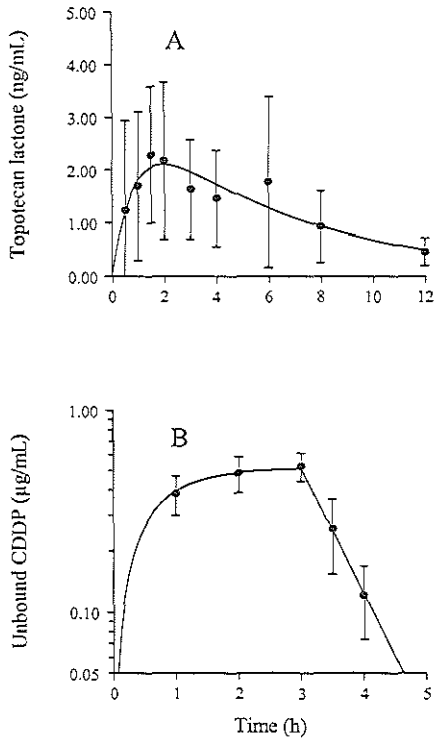

Figure 2 Average plasma concentration-time curves of (A) topotecan lactone and (B) unbound cisplatin (CDDP) in 6 patients on day 1 at the recommended dose level of topotecan at 1.50 mg/m^2.

Table 4 Summary of topotecan lactone pharmacokinetics of course 1 day 1 (data present mean values ± SD)

	Topotecan (mg/m^2/day)		
	1.50 (n=6)	1.75 (n=5)	p
C_{max} (ng/mL)	3.42 ± 1.36	5.08 ± 3.52	0.31
T_{max} (h)	2.61 ± 2.12	2.59 ± 2.14	0.99
AUC (ng.h/mL)	17.0 ± 6.83	24.6 ± 4.68	0.065
CL/f (L/h/m^2)	109 ± 65.8	73.5 ± 14.4	0.27
$t½_{(z)}$ (h)	3.53 ± 1.18	3.23 ± 1.24	0.69
L/T ratio	0.38 ± 0.034	0.42 ± 0.058	0.19

Abbreviations: C_{max}, peak plasma level; T_{max}, time to maximum concentration; AUC, area under the plasma concentratio-time curve; CL/f, apparent clearance; $t½_{(z)}$, half-life of the terminal disposition phase; L/T ratio, topotecan lactone to total drug AUC ratio.

The apparent clearance (CL/f) of the pharmacologically active topotecan lactone and the lactone to total drug AUC ratio (L/T ratio) were clearly dose independent and similar to single agent data (18). The interpatient variability in AUC was 40% at 1.50 mg/m^2 and 19% at (1.75 mg/m^2). The apparent clearance of topotecan (Cl/f) on days 1, 2 and 5 averaged 93.1±51.0 (n=11), 81.0±30.8 (n=9) and 76.7±14.4 L/h/m^2 (n=9), and were not significantly different (P = 0.20, Friedman's two way analysis of variance test), suggesting lack of drug accumulation (fig. 3). All data were consistent with a dose-independent and linear behavior of topotecan.

Table 5 Summary of ciplatin pharmacokinetics of course 1 (data present mean values ± SD)

	Topotecan (mg/m2/day)		
	1.50 (n=6)	1.75 (n=5)	p
Cisplatin total			
Cmax (µg/mL)	1.87 ± 0.197	1.93 ± 0.318	0.71
AUC (µg.h/mL)	25.2 ± 3.55	23.2 ± 4.86	0.45
CL (mL/min)	65.3 ± 11.5	72.1 ± 24.5	0.56
t½(z) (h)	58.4 ± 32.2	34.6 ± 16.5	0.17
Cisplatin unbound			
Cmax (µg/mL)	0.55 ± 0.087	0.62 ± 0.13	0.31
AUC (µg.h/mL)	1.35 ± 0.271	1.51 ± 0.305	0.38
CL (mL/min)	1221 ± 192.9	1068 ± 184.9	0.21
t½(z) (h)	0.77 ± 0.29	0.52 ± 0.11	0.10

C_{max}, peak plasma level; AUC, area under the plasma concentration-time curve; CL, clearance; $t_{½(z)}$, half-life of the terminal disposition phase.

The effect of the topotecan dose on the pharmacokinetics of total and unbound cisplatin in plasma during the first treatment course is shown in Table 5. Cisplatin clearance was not dependent on the topotecan dose, with mean values of 65.3 and 72.1 mL/min when given in combination with 1.50 and 1.75 mg/m^2/day of topotecan, respectively.

Pharmacokinetic data for topotecan and cisplatin obtained during the sixth treatment course in one patient were similar to the first course, indicating no alteration in topotecan and cisplatin pharmacokinetics, topotecan CL/f on 3 separate days was 159±65.4 ng/h/m^2 and 168±14.0 ng/h/m^2, during the first and sixth course, respectively. Overall, pharmacokinetics of both compounds was dose independent and similar to single agent data (18).

DISCUSSION

The combination of i.v. cisplatin and oral topotecan is attractive given their broad anti-tumour activity, preclinical synergism, their different toxicity profiles and patient's preference of oral chemotherapy (when at least equally effective). In all phase I studies combining cisplatin and topotecan, the topotecan dose at MTD was considerately reduced as compared

to single agent topotecan regimens. Since preclinical studies suggest existence of a topotecan systemic exposure-response relationship, and while clinical studies in topotecan-sensitive tumour types still have to confirm this issue, we tried to increase the topotecan dose by decreasing the cisplatin dose to 50 mg/m^2. Although cisplatin at this dose is effective in combination regimens in non-small-cell lung cancer (24) and ovarian cancer (25), a reduced cisplatin dose is considered to be related with a loss of response and survival in, for example, ovarian cancer (26).

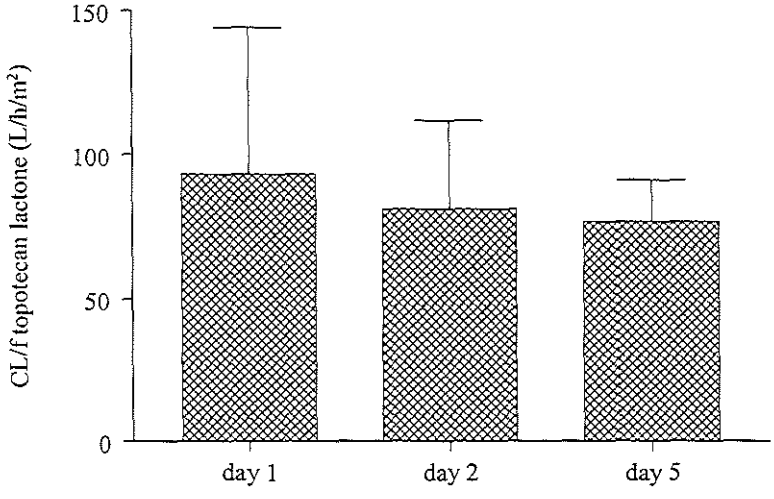

Figure 3 The apparent clearance (CL/f) of topotecan lactone obtained on the first, second and fifth administration day in the first course of respectively 11, 9 and 9 patients treated with cisplatin 50 mg/m^2 on day 1 and topotecan 1.50 or 1.75 mg/m^2/d on days 1-5. Data represent mean values (bars) ± standard deviation (error bars).

In the present phase I study both myelosuppression and gastrointestinal toxicity were dose limiting in 3 out of 6 patients treated with topotecan at 1.75 mg/m^2 administered on day 1-5 every 3 weeks, preceded by i.v. cisplatin 50 mg/m^2 on day 1. Other toxicities such as nephrotoxicity, neurotoxicity, fatigue and alopecia were mild and infrequent. All patients who were treated at the 1.75 dose level and had their topotecan dose reduced due to dose limiting toxicity, had prior chemo- and/or radiotherapy, and experienced significant

hematological toxicity at the lower dose levels, while the 6 patients who were initially treated at the recommended dose level of topotecan 1.50 mg/m^2 experienced less toxicity. Despite the late onset of the neutrophil nadir, compared to single agent data, only one course was delayed at the 1.50 mg/m^2 dose level. No other important toxicity was encountered at this dose level, except for one patient experiencing grade 3 diarrhea for one day only and grade 3 nausea.

The observed pharmacokinetic parameters of the lactone and the carboxylate form of topotecan demonstrated linear and dose independent behavior over the total dose range studied and were similar to single agent data. Furthermore, topotecan dose had no influence on the unbound fraction of cisplatin, as suggested previously (10).

In conclusion, the MTD of topotecan is 1.50 mg/m^2 on day 1-5, preceded by cisplatin 50 mg/m^2 on day 1. This regimen seems to have no advantage as compared to our previous recommended schedule of topotecan 1.25 mg/m^2, preceded by cisplatin 75 mg/m^2 in a similar patient population (10), since the dose reduction of 33% of cisplatin only allows a minor dose escalation of topotecan.

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Chapter 6

Inter- and intra-patient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens

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ABSTRACT

Anticancer drugs still are dosed based on the body-surface area (BSA) of the individual patient, while the BSA is not the main predictor of the clearance for the majority of drugs. The relevance of BSA-based dosing has not been evaluated for topotecan yet. A retrospective pharmacologic analysis was performed of kinetic data from 4 clinical phase I studies in which topotecan was administered orally as single agent combined with data from a combination study of topotecan and cisplatin. A strong correlation (r=0.91) was found between the area under the plasma concentration time curve of the lactone and carboxylate forms of topotecan by plotting 326 data sets obtained from 112 patients receiving oral topotecan at dose levels ranging from 0.15 - 2.70 mg/m^2. The intra-patient variability, studied in 47 patients sampled for 3 or more days, for the apparent lactone clearance (CL/F), ranged 7.4 - 69% (mean 24 ± 13%, median 20%). The inter-patient variabilities in the CL/F, calculated with the data of all studied patients, expressed in L/h/m^2 and in L/h were 38% and 42%, respectively. In view of the relatively high inter- and intra-patient variabilities in topotecan clearance, in contrast to a variability of only 12% in the BSA of the studied patients, no advantage of BSA-based dosing was found over fixed dose regimens.

INTRODUCTION

Most anticancer drugs are dosed based on the body-surface area (BSA) of the individual patient, with the aim of reducing inter-patient variability of drug exposure. However, this strategy failed for most drugs [1], except for docetaxel (Taxotere®), where BSA has been shown to be a main predictor of the clearance in a pharmacokinetic model [2]. The relevance of this concept of BSA-based dosing has not been evaluated for topotecan (Hycamtin®), one of the most promising agents that entered clinical practice in the recent years. Topotecan has antitumor activity against various human malignancies, and relationships have clinically been found between systemic exposure of topotecan and hematological toxicity [reviewed in 3-5], and with antitumor activity in preclinical models [6]. The bioavailability of oral topotecan in adult cancer patients ranged from 30 - 44% with inter-patient variabilities of 26 - 31% [7-9]. However, since pharmacokinetic analysis in most patients [10-12] has only been carried out for 2 days, a reliable estimate of the intra-patient variability of orally administered topotecan has not yet been established. The recommended dose for single agent oral topotecan is 2.3 mg/m2/day for 5 days every 3 weeks. As alternative a fixed dose of 4 mg/day for 5 days every three weeks was proposed [10]. However, thusfar studies on oral topotecan were performed by dosing patients based on their body-surface area instead of using fixed dose regimens. Since most patients will take their oral medication in an outpatient setting, it is important to keep dosing regimens as simple as possible and because of this it would be most practical to use fixed dosing regimens [13]. The aim of the present analysis of kinetic data from several phase I studies was to investigate, whether dosing of oral topotecan in the treatment of adult cancer patients based on body-surface area of individual patients has any advantage over fixed dose regimens.

PATIENTS AND METHODS

Patient selection

The patient selection criteria were fully described elsewhere for the oral phase I studies in which topotecan was administered as single agent [3] and for the study in which oral topotecan was combined with i.v. cisplatin [14]. In short, patients with a confirmed diagnosis of a malignant solid tumor resistant to standard chemotherapy regimens were eligible for these studies. Age should be between 18 and 75 years and performance status, defined by the Eastern Cooperative Oncology Group, had to be ≤ 2. No previous anticancer therapy for at least 4 weeks was allowed. Adequate hematopoietic and renal functions were required, patients with mildly impaired liver functions (i.e. total serum bilirubin ≤ 1.25 x upper normal limit, ASAT and ALAT ≤ 2 x upper normal limits and in case of liver metastases ≤ 3 x upper normal limits) were allowed to participate in the described studies. A specific exclusion criterion was the existence of any gastrointestinal circumstance, which could alter the absorption of topotecan. All patients signed informed consent.

Treatment schedules in the single agent phase I studies

Oral administration, using the i.v. formulation, of single agent topotecan was studied in 4 phase I studies [3]. The first study involved a twice-daily dosing of topotecan at dose levels of 0.15 - 0.60 mg/m2 for 21 days, repeated every 28 days [3, 11]. In the second study, topotecan was administered twice-daily for 10 days, every 21 days, at dose levels of 0.50 - 0.80 mg/m2 [3, 12]. In the third study the administration of topotecan was reduced to once a day for 10 days at dose levels ranging from 1.00 - 1.60 mg/m2 [3, 12]. The final phase I study of single agent oral topotecan involved a once daily administration for 5 days, also repeated every 21 days, and included dose levels ranging from 1.20 - 2.70 mg/m2 [3, 10]. In the daily times 5 schedule also patients were included who were treated with a fixed dose of 4 mg/day.

Treatment schedule in the combination phase I study

The latter mentioned regimen of oral topotecan, this time at dose levels ranging form 0.75 - 2.30 mg/m2/day for 5 days, using the drug formulated in gelatin capsules, was also studied combined with a fixed dose of 75 mg/m2 of i.v. cisplatin [14]. In the present report we also included pharmacokinetic-data of patients, treated with oral topotecan daily times 5, at dose levels of 1.50 and 1.75 mg/m2 preceded by a 3 h cisplatin infusion at 50 mg/m2 on day 1 of each course in an ongoing study using the same eligibility criteria as previously reported [14].

Pharmacokinetic sample collection and analysis

Blood samples were collected, upto 12 h after dosing [10-12, 14], in 4.5 ml glass tubes containing lithium heparin as anticoagulant and were centrifuged within 10 minutes to separate the plasma. Subsequently, the plasma was deproteinized by 4 fold dilution in ice-cold (-20°C) methanol, resulting in a stabilized lactone to carboxylate ratio [15], and stored at –80°C upon analysis. Simultaneous determination of the lactone and carboxylate form of topotecan was performed by a reversed-phase high-performance liquid chromatographic

(HPLC) method as described [15], with minor modifications for the analysis of drug levels in the combination phase I study [14].

On the basis of the best fitted curves, two and three compartmental analysis models after zero-order input were used for the calculation of the area under the plasma concentration-time curves (AUC)0-infinity of the lactone as well the carboxylate form of topotecan as described [14]. The apparent oral clearance (CL/F) of topotecan lactone was calculated by dividing the dose per m2 by the observed lactone AUC, expressed in L/h/m2. The absolute CL/F, expressed in L/h, was calculated by dividing the absolute dose by the AUC of topotecan lactone.

Statistical analysis

Linear regression analysis was performed, using the NCSS package (Version 5.X; J.L. Hintze, East Kaysville, UT, 1992), to test potential relationships between evaluated parameters. One way analysis of variance was performed to evaluate statistically significant differences ($p<0.05$) between groups, using the same program.

Table 1 Evaluable pharmacokinetic days in the 4 single agent phase I studies of orally administered topotecan.

Dose (mg/m^2)	twice daily	once daily	No. of pts	total number of evaluable kinetic courses				
				day 1	day 4	day 8	day 21	total
0.15	21 days		2	2		1		3
0.30	21 days		3	3		3		6
0.40	21 days		2	3		2	2	7
0.50	21 days		4	4		4		8
0.60	21 days		3	3		3		6
0.50	10 days		1	1		1		2
0.60	10 days		4	3		4		7
0.70	10 days		4	4		4		8
0.80	10 days		1	1				1
1.00		10 days	3	3		3		6
1.40		10 days	3	3		3		6
1.60		10 days	4	4		4		8
1.20		5 days	3	3	3			6
1.80		5 days	3	3	3			6
2.30		5 days	6	6	6			12
2.70		5 days	4	4	4			8
1.25 [a]		10 days	1	1		1		2
4.00 [a]		5 days	6	6	6			12
total [b]			56	57	22	33	2	114

a: Fixed dose

b: One patient in the twice daily 0.40 mg/m2 was also sampled during course 2 and one patient in the once daily 1.40 mg/m2 was also sampled during a course with a fixed dose of 1.25 mg

RESULTS

In the 4 phase I studies on single agent oral topotecan 56 patients were evaluable for pharmacokinetic analysis for a total of 114 kinetic days (table 1). The majority of evaluable days were obtained in the phase I trial studying the combination of oral topotecan and i.v. cisplatin, in which 56 patients were sampled for a total of 228 days (table 2).

By plotting 326 data sets (95% of total) of kinetic days for which both the lactone and the carboxylate AUC could be assessed, a strong linear relationship was found between the AUCs of the pharmacologic active lactone form of topotecan and its inactive ring-opened carboxylate form (AUCcarboxylate = 1.62 + 1.57*AUClactone, r=0.91, fig 1). For further estimation of inter- and intra-patient variabilities and their implication for BSA dosage regimens, pharmacokinetic parameters of the lactone form were used in this analysis.

Table 2 Evaluable pharmacokinetic days in the phase I study in which oral topotecan was combined with i.v. cisplatin.

Dose (mg/m2)	No of pts	Total number of evaluable kinetic days										
		C1d1	C1d2	C1d5	C2d1	C2d5	C3d1	C3d5	C6d1	C6d2	C6d5	Total
0.75	6	6		6	6	6	6	6				36
1.00	6	6		6	6	6	6	6				36
1.25	11	10	4	11	5	5	4	4	2	2	1	48
1.50	9	9	5	8	3	3			1	1	1	31
1.75	10	10	6	9	3	3						31
2.00	8	6	3	7	4	4						24
2.30	7	7		7	4	4						22
Total	56a	54	18	54	31	31	16	16	3	3	2	228

a: 1 patient received 2.30 (Course 1) and 2.00 mg/m2 (Course 2)

Inter and intra-patient variability

Since the majority of the patients in the single agent oral phase I studies were sampled for pharmacokinetic analysis only for 2 days during 1 cycle, the intra-patient pharmacokinetic parameter variabilities for topotecan lactone were studied using the data obtained in the topotecan/cisplatin combination phase I study, in which pharmacokinetic sampling was performed during several cycles. As already reported, there was no pharmacokinetic interaction between oral topotecan and i.v. cisplatin [14]. In view of this, all kinetic days (with and without cisplatin) were used for the present analysis. The intra-patient variability in AUC and CL/F of topotecan lactone was calculated as the standard deviation divided by the average, only using data of the 47 patients which at least had 3 evaluable kinetic courses of the AUC of topotecan lactone. The averaged intra-patient variability in the lactone AUC

across all dose-levels was 24 ± 13% (median 20%, range 7.6 - 61%). The averaged intra-patient variability in the CL/F, expressed in L/h/m2 and in L/h, was 24 ± 13% (median 20%, range 7.4 - 69%).

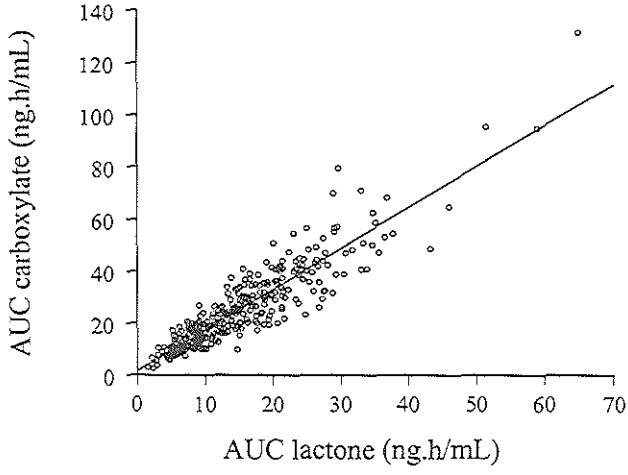

Figure 1 Relationship between the AUCs of the lactone and carboxylate form of topotecan in 326 evaluable data sets

The inter-patient variability in CL/F, expressed in L/h/m^2 as well as in L/h, was calculated using the data of all the patients, by using the averaged apparent CL/F of all kinetic days of each patient as single value. The averaged apparent CL/F was 103 ± 39.0 L/h/m^2 (CV=38%, n=107, fig 2a), with no significant difference (p=0.074) in the CL/F over the 19 studied dose-levels. The averaged apparent CL/F, studied over 27 different individual dosages, was 194 ± 80.4 L/h (CV=42%, n=107, fig 2b) or 195 ± 81.1 L/h (CV=42%, n=114, fig 2b), by inclusion of the patients treated with a fixed dose.

In addition, no alteration in topotecan lactone kinetics was found (p=0.30) after multiple (up to 6) courses (fig 3), using the data of patients treated in the combination phase I study, in which patients were samples for multiple courses.

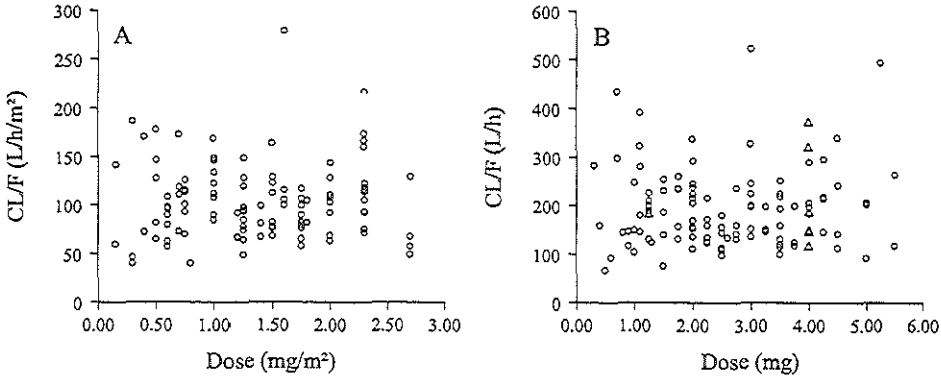

Figure 2 Plot of dose/m2 (A) and absolute dose (B) and the averaged apparent CL/F (patients with a fixed dose are indicated by triangles).

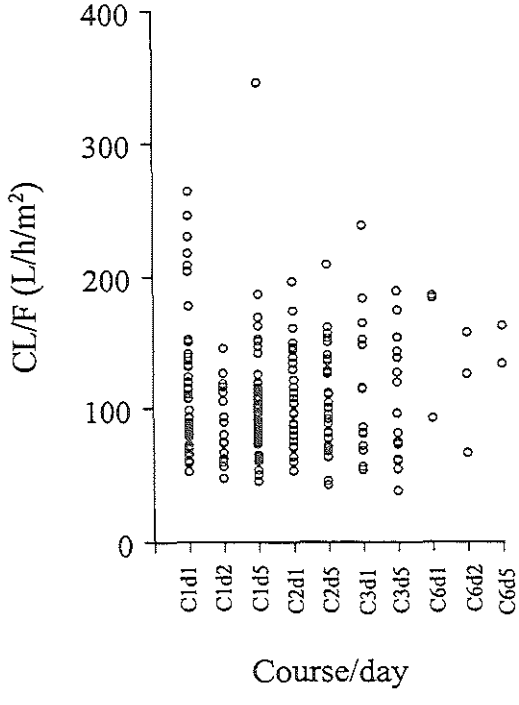

Figure 3 Apparent CL/F during multiple courses and days.

BSA as determinant for dose calculations

As shown in fig 2 and 3, the apparent CL/F was constant over the studied dose-ranges and courses. In fig 4 the BSA is plotted versus absolute apparent CL/F in L/h (mean ± S.D.), calculated with the actual dose given to each individual patient. A poor positive relationship was found between BSA and the average apparent CL/F (CL/F = 52.4 + 75.1*BSA, r=0.29), with large variabilities in the apparent CL/F across all studied BSA values in the 47 patients with 3 or more pharmacologically evaluable courses.

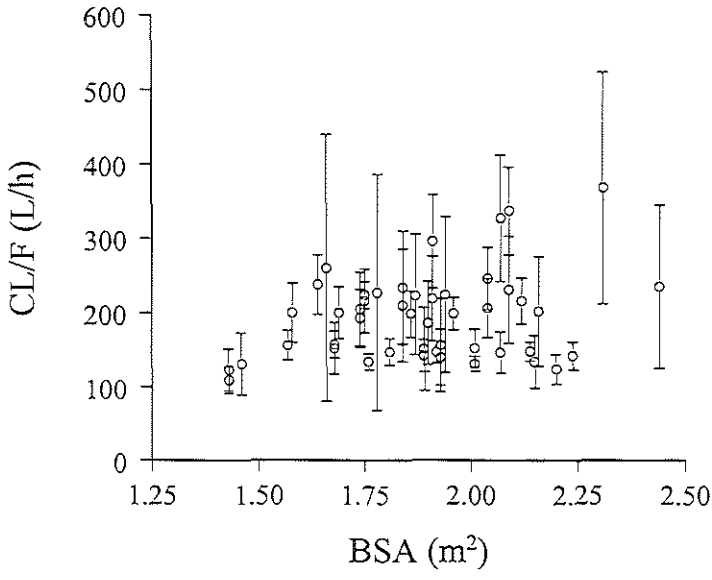

Figure 4 Body-surface area (BSA) versus absolute apparent CL/F expressed in L/h (mean ± S.D.).

DISCUSSION

Dosing of most cytotoxic agents is commonly based on the BSA of patients, intending to reduce inter-patient pharmacokinetic variabilities of a compound. This, in turn, is based on an assumed relationship between the clearance of a compound and the BSA of the individual patient [1, 13]. However, calculation of the exact clearance of topotecan is not feasible since the compound has a reversible conversion from the lactone to the carboxylate form. Moreover, accurate dosing of oral topotecan based on BSA is also not feasible, since for oral use the drug is now only available as gelatin capsules containing 1.0 and 0.25 mg

respectively, resulting in the necessity of rounding of the absolute dose to the nearest quartile mg. Since excretion by the kidneys is a major route of elimination of topotecan [reviewed in 4] and alterations in the pharmacokinetic parameters for topotecan have only been described in patients with a renal dysfunction, i.e. creatinine clearance < 60 mL/min [16], in the set of studies we performed an altered topotecan clearance was not expected. The apparent CL/F of oral topotecan, in patients with a normal renal function, is highly dependent on the absorption of the lactone form from the gastrointestinal tract.

The oral bioavailability of topotecan in adult cancer patients for drinking of the i.v. solution ranged from 30 ± 7.7% [7] to 44% [9] and was found to be 42 ± 13% for the drug formulated in gelatin capsules [8]. In these clinical studies inter-patient variabilities in the oral availability in adults ranged from 26 - 31% [7, 8], which is not dissimilar to the inter-patient variability of 38 and 42% for the apparent CL/F expressed in L/h/m2 and in L/h, respectively, in our studies. Since the inter-patient variability was calculated with the averaged apparent CL/F of topotecan lactone, using the data of patients which were studied up to 6 times, this variability might even be underestimated.

Only limited information was available on the intra-patient variability of orally administered topotecan in adult cancer patients. Gerrits et al. [3] reported intra-patient variabilities in AUC of topotecan lactone of 25 ± 31% (n=22) and 35 ± 25% (n=10) in clinical phase I studies in which topotecan was administered either once daily times 5 or 10 respectively, and of 97 ± 70% (n=10) and 60 ± 51% (n=13) in twice daily times 10 and 21 schedules, respectively. Since samples were only collected for pharmacokinetic analysis on 2 treatment days during one cycle, an accurate estimation of the intra-patient variability was not possible. In this present analysis we assessed the intra-patient variability using data of 47 patients, who were sampled on 3 - 6 days each, resulting in an average intra-patient variability of the lactone AUC of 24 ± 13% (median 20%) with a range of 7.6 - 61%.

The broad range in the intra-patient variability in lactone AUC after oral administration of topotecan is probably related to the fact the carboxylate form is poorly absorbed from the small intestine, while the lipophilic pharmacologic active lactone form of topotecan is able to pass the membranes of the small intestine [4]. Since the pH in the small intestine ranges from pH 5 - 7 and the rate of interconversion between the lactone and carboxylate form of topotecan is pH dependent, the amount of topotecan which is available for absorption is related to a fluctuation in the pH.

We did not find saturation of the absorption, tissue distribution or elimination of orally administered topotecan over the studied dose-range of 0.15 - 2.70 mg/m2 apparent from a lack in significant difference in the observed CL/F over the dose range studied. Also administration of multiple (up to 6) courses of orally administered topotecan did not alter the apparent topotecan lactone CL/F.

The inter-patient variability in the topotecan CL/F of 38 and 42%, expressed in L/h/m2 and L/h respectively, is much larger than the 12% inter-patient variability in BSA of our patients (average BSA 1.9 ± 0.22 m2, n=107). In view of the intra-patient variability of 24 ± 13% in the apparent lactone CL/F, with individual variabilities upto 69%, the inter-patient variability in the bioavailability of 26 - 31% and the poor relationship between the BSA and

the average apparent CL/F, we feel that there is no scientific rationale for BSA-based dosing of orally administered topotecan in adult patients. This confirms our previous observation of similar pharmacokinetics after oral administration of either 2.3 mg/m2 of topotecan or a fixed dose of 4 mg [10], which was already suggesting that fixed-dose regimens could be applied.

In conclusion, in view of the relatively high intra- and inter-patient variabilities in the AUC and CL/F of topotecan lactone and the relatively small range in observed BSA, oral topotecan can be added to the list of agents where BSA-adjusted dosing does not appear definitely better [1]. We recommend a fixed dose regimen for future use in clinical trials, which is more convenient for the oncologist and the pharmacist, is more cost-effective and last but not least, a fixed dose regimen is less cumbersome for the patients. Further randomized clinical studies in a large population are needed to fully explore the advantages of fixed dose regimens of orally administered topotecan, in which simultaneously the need for potential dosage adjustments at extreme BSA values have to be investigated.

A careful study of inter-patient variability of topotecan AUC in patients of the same BSA, renal and hepatic function, to look at the effects of factors as age, gender, protein binding, and inherited or acquired metabolic function in addition to expression of the MDR-1 P-glycoprotein and BCRP drug-transporting proteins [17] in intestinal tissues as an explanation for this variability, is currently being conducted.

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Chapter 7

Gender-dependent pharmacokinetics of topotecan in adult patients

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ABSTRACT

Background Gender-dependent differences in the clinical pharmacokinetic behavior of various drugs have been documented previously. Most commonly, these differences are associated with differences in body composition, renal elimination, drug absorption or hepatic metabolism. Gender-dependent differences in the pharmacokinetics of topotecan (Hycamtin®) have not yet been described.

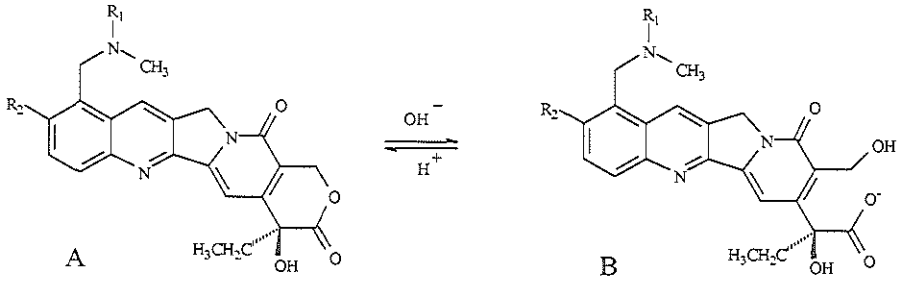
Patients and methods Pharmacokinetic data of the lactone and carboxylate forms of topotecan were derived from clinical studies in which topotecan was administered either orally or intravenously to a total of 55 males and 37 females.

Results A significant difference (P=0.0082) of 38% was found between the apparent clearance of topotecan lactone after oral administration in males (237±105 L/h) and females (163±62.5 L/h). When adjusted for body-surface area, this difference remained significant (P=0.031). Similarly, differences were noted in the percentage of topotecan in the lactone form (37.1±5.32% versus 41.7±6.51%, P=0.0076). Statistical analysis revealed that individual hematocrit values, which were consistently lower in females (P<0.023), were a significant predictor of the apparent topotecan lactone clearance. This was confirmed experimentally in *in vitro* incubation studies in whole blood using artificially altered hematocrit values and in blood samples from both male and female volunteers.

Conclusion Topotecan is thus subject to significant gender-dependent differences in pharmacokinetics that arise as a result of a physiological difference in hematocrit values between males and females. This finding may have significant implications for the interpretation of the relationships between pharmacokinetics and pharmacodynamic outcome of topotecan treatment, and may provide a basis for the development and refinement of future clinical protocols.

INTRODUCTION

Topotecan (Hycamtin®, fig 1), a water-soluble semisynthetic analogue of the topoisomerase I inhibitor camptothecin, is one of the most promising new anticancer agents. Single agent topotecan, administered intravenously (i.v.), has demonstrated antitumor activity against various solid tumors in adult cancer patients, including metastatic ovarian and small cell lung cancer. Most responses were achieved using a daily times 5 schedule in which topotecan was administered as a 30-min infusion [reviewed in 1]. Since daily i.v. administration of topotecan for 5 days, repeated every 21 days, is inconvenient for patients, an oral formulation of topotecan has been developed with a bioavailability of 42 ± 13% [2]. Different administration schedules of oral topotecan have been evaluated in clinical studies, including once daily times 5 and 10 and twice daily times 10 and 21 administrations, from which the once daily times 5 schedule was recommended for future clinical studies [reviewed in 3]. The need for further clinical development of the oral topotecan formulation became even more important in view of recent findings that the oral formulation has similar efficacy as compared to the i.v. formulation, while less hematological toxicity was observed [4,5].



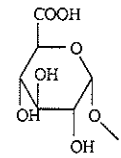
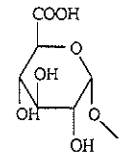
Compound	R1	R2
Topotecan	CH_3	OH
N-desmethyl topotecan	H	OH
Topotecan-*O*-glucuronide	CH_3	
N-desmethyl topotecan-*O*-glucuronide	H	

Figure 1 Chemical structures of the lactone (A) and carboxylate (B) forms of topotecan and its known human metabolites.

The pharmacokinetic profile of topotecan is quite complex since it can undergo a spontaneous pH-dependent interconversion between a pharmacologic active lactone form and an inactive carboxylate form (fig 1). Gender-dependent differences in pharmacokinetic behavior have been described for a wide variety of compounds over the last few decades. Most commonly, these have been shown to be associated with differences in body composition, renal elimination, drug absorption or hepatic function [reviewed in 6]. In this report, we describe gender-dependent differences in topotecan pharmacokinetics after both oral and i.v. administration, and present in vitro studies to provide a formal explanation of this phenomenon.

PATIENTS AND METHODS

Patient selection criteria

All patients included in the studies had a histologically or cytologically confirmed diagnosis of a malignant solid tumor, refractory to standard therapy or for which no recognized therapy was available. The patients participated in either a phase I study, in which oral topotecan was combined with i.v. cisplatin [7], or a phase II study of single agent topotecan administered as a 21-day continuous i.v. infusion [8]. The eligibility criteria, treatment plans and detailed clinical profiles have been fully described elsewhere [7,8].

Drug administration

SmithKline Beecham Pharmaceuticals (Harlow, UK) supplied capsules containing either 0.25 or 1.0 mg of topotecan lactone and a lyophilized vial preparation containing 5 mg of topotecan lactone. Orally administered topotecan was studied at dose levels of 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 or 2.30 mg/m^2/day for 5 days, repeated every 3 weeks, in combination with a fixed dose of 75 mg/m^2 cisplatin by a 3 h infusion, in 49 patients [7]. A total of 10 patients, treated with oral topotecan daily times 5, at dose levels of 1.50 or 1.75 mg/m^2, preceded by i.v. cisplatin at a dose of 50 mg/m^2 on day 1 of each course, was also included in this study. In the i.v. phase II study, topotecan was administered as a 21-days continuous infusion at dose levels of 0.50 and 0.60 mg/m^2/day at an infusion rate of 6 mL/24 h, using ambulatory pumps, repeated every 28 days [8].

Blood sample collection and analysis

Blood samples were collected in 4.5-mL glass tubes containing lithium heparin as anticoagulant. Following oral administration, samples were obtained prior to dosing, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after dosing on day 1 [7]. Similarly, in the i.v. trial blood was withdrawn prior to infusion, and at steady state on day 8 after start of dosing [8]. The blood samples were centrifuged immediately at the site of the patient to separate the plasma. The plasma samples were directly deproteinized by 4-fold dilution in ice-cold (-20°C) methanol, resulting in a stabilized lactone to carboxylate ratio [9], and stored at –80°C upon analysis. Simultaneous determination of the lactone and carboxylate form of topotecan was performed by a reversed-phase high-performance liquid chromatographic method as described [9].

Pharmacokinetic analysis

In the present pharmacokinetic analysis we used the pharmacokinetic data of day 1 of course 1 in the oral phase I study [7] and of day 8 in the continuous infusion phase II study [8]. The area under the plasma concentration-time curve (AUC) of total topotecan, i.e. lactone plus carboxylate, and the topotecan lactone and carboxylate forms in the oral phase I study were calculated by noncompartmental and two or three compartmental analysis models after zero-order input. The apparent clearance of topotecan lactone and the clearance of total topotecan in the oral phase I study were calculated by dividing the dose in mg/m2 or the absolute dose in mg by the observed AUC and were expressed in L/h/m2 and L/h, respectively. While in the i.v. study, the clearances were calculated by dividing the rate of infusion divided by the steady-state plasma concentration. The apparent terminal disposition half-life of topotecan lactone and carboxylate were calculated as ln2/k, in which k represents the rate constant of the terminal disposition phase. The lactone to total ratio in the oral phase I study was defined as the AUCL/AUCT, while the times to reach the maximum concentration for topotecan lactone and carboxylate were determined graphically. The lactone to total concentration ratio in the 21-days continuous infusion study was calculated as the concentration of the lactone form divided by the concentration of total topotecan.

In vitro experiments

From 5 male volunteers a volume of 12 ml heparinized blood was collected, from which 6 mL was centrifuged for 5 min at 2000g to separate plasma and blood cells. The plasma supernatants, combined with the buffy-coat, and the remaining red blood cell fractions were collected. Fractions of the whole blood, the red blood cells and the plasma were combined to create different hematocrit values in blood of the same individual, ranging between 0.20 and 0.60 L/L. A volume of 1 mL of these samples was incubated simultaneously with 5 ng/mL of topotecan lactone and 5 ng/mL of topotecan carboxylate for 15 minutes at 37°C to study the impact of the hematocrit on the lactone to total concentration ratio in the plasma compartment. The blood samples were further processed as described above for the blood samples of the patients. The remaining fractions were used for the determination of hematocrit values. To confirm the gender-related difference in the topotecan pharmacokinetics, 1 mL of normal heparinized whole blood of 5 female and 5 male volunteers was incubated with 5 ng/mL of topotecan lactone and carboxylate and further processed as described above.

Statistical analysis

All parameters are reported as mean values ± SD. Two-tailed unpaired Student's t-tests were performed to evaluate statistically significant differences ($P<0.05$) in pharmacokinetic and biochemical parameters between males and females, using the NCSS package (Version 5.X; J.L. Hintze, East Kaysville, UT, 1992). Linear regression analysis was performed to test potential relationships between parameters, using the same program.

RESULTS

Clinical pharmacokinetics

A total of 54 patients (36 male and 18 females) enrolled in the oral phase I study was evaluated for pharmacokinetic analysis during day 1 of course 1. Since cisplatin has no effect on the pharmacokinetics of topotecan [7], courses without and in combination with i.v. administered cisplatin were used for the determination of gender-dependent differences in topotecan pharmacokinetics. The pharmacokinetic and biochemical characteristics of the evaluable patients are listed in table 1.

Table 1 Pharmacokinetic and biochemical characteristics

	Males	Females	p
Oral phase I study			
L/T (%)	37.1 ± 5.32 (n=36)	41.7 ± 6.51 (n=18)	0.0076
CL/F_L (L/h)	237 ± 105 (n=36)	163 ± 62.5 (n=18)	0.0082
CL/F_L (L/h/m^2)	123 ± 53.3 (n=36)	92.4 ± 33.3 (n=18)	0.031
CL/F_T (L/h)	85.0 ± 33.3 (n=36)	69.1 ± 29.9 (n=18)	NS
CL/F_T (L/h/m^2)	44.7 ± 17.9 (n=36)	38.5 ± 14.6 (n=18)	NS
T_{maxL} (h)	1.63 ± 1.25 (n=36)	1.92 ± 1.60 (n=18)	NS
T_{maxC} (h)	2.79 ± 1.43 (n=36)	2.96 ± 1.67 (n=18)	NS
$T_{½L}$ (h)	2.97 ± 1.12 (n=36)	3.11 ± 0.912 (n=18)	NS
$T_{½C}$ (h)	3.65 ± 1.05 (n=36)	3.47 ± 0.722 (n=18)	NS
BSA (m^2)	1.96 ± 0.204 (n=36)	1.77 ± 0.204 (n=18)	0.0003
Ht (L/L)	0.39 ±0.037 (n=30)	0.36 ± 0.041 (n=16)	0.015
CL_{creat} (mL/min)	89 ± 20 (n=21)	80 ± 19 (n=10)	NS
Albumin (g/L)	41 ± 4.0 (n=34)	41 ± 2.9 (n=15)	NS
Total protein (g/L)	76 ± 5.3 (n=34)	75 ± 4.8 (n=15)	NS
21-Days continuous intravenous infusion			
L/T (%)	29.6 ± 6.67 (n=18)	34.1 ± 5.70 (n=19)	0.034
CL_L (L/h)	73.9 ± 27.8 (n=19)	59.9 ± 13.6 (n=19)	NS
CL_L (L/h/m^2)	37.5 ± 14.3 (n=19)	34.3 ± 8.14 (n=19)	NS
CL_T (L/h)	21.3 ± 5.78 (n=18)	20.0 ± 3.85 (n=19)	NS
CL_T (L/h/m^2)	10.4 ± 3.88 (n=18)	11.5 ± 2.56 (n=19)	NS
BSA (m^2)	1.98 ± 0.139 (n=19)	1.76 ± 0.164 (n=19)	0.0001
Ht (L/L)	0.37 ±0.038 (n=12)	0.34 ± 0.028 (n=16)	0.023
CL_{creat} (mL/min)	98 ± 16 (n=9)	87 ± 22 (n=14)	NS
Albumin (g/L)	43 ± 4.0 (n=12)	41 ± 6.1 (n=16)	NS
Total protein (g/L)	70 ± 8.9 (n=12)	71 ± 16 (n=16)	NS

L/T (%) = lactone to total ratio, CL/F and CL = (apparent) clearance of topotecan lactone and total, Tmax = time to reach the maximal plasma concentration of topotecan lactone and carboxylate, T½ = terminal half life of topotecan lactone and carboxylate, BSA = body-surface area, Ht = hematocrit, CL_{creat} = creatinine clearance, NS = no significant difference.

The apparent clearance of topotecan lactone was significantly 1.4-fold faster in males as compared to females (P=0.0082), while after correction for the body-surface area the apparent clearance of the lactone form was remained significantly 1.3-fold faster in males (P=0.031). Interestingly, no significant differences were observed in the clearance of total topotecan. The lactone to total ratio of the AUC was significantly 1.3-fold higher in females (P=0.0076) and a significant correlation (r=0.35, P=0.0086) was found between the lactone to total AUC ratio and the apparent clearance of topotecan lactone (fig 2a). Linear regression analysis was performed between, respectively, the significantly different biochemical characteristics body-surface area and hematocrit and the apparent clearance of topotecan, expressed in L/h as well as in L/h/m^2.

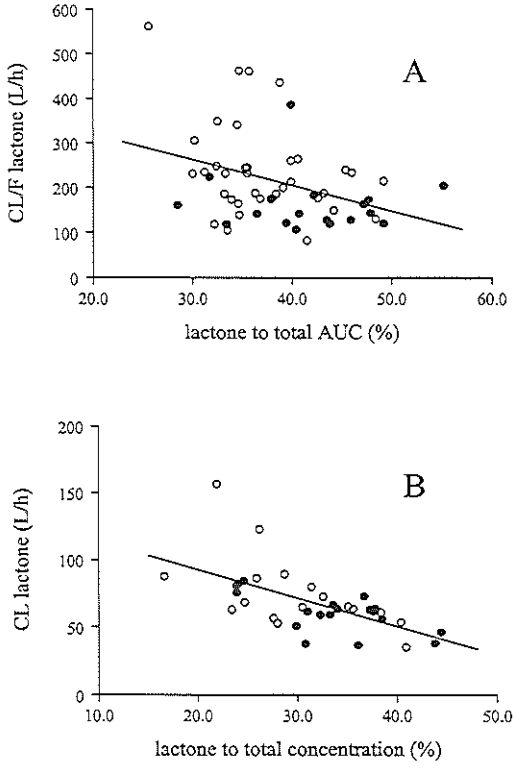


Figure 2 Relationship between the lactone to total ratios and the apparent topotecan lactone clearance in patients treated with oral topotecan (A) or i.v. topotecan (B). Male patients are indicated by open circles and females by closed circles.

A significant relationship was found between the body-surface area and the absolute apparent clearance expressed in L/h (P=0.013), while no significant relationship was found after correction of the apparent clearance for the body-surface area. In contrast, the relationship between hematocrit and the absolute as well the corrected apparent clearance was significantly correlated (P=0.040 and P=0.030 respectively). In the continuous i.v. phase II study, 38 patients (19 males and 19 females) had evaluable topotecan pharmacokinetics on day 8 of course 1, with a 1.2-fold higher lactone to total concentration in females (table 1). As in the oral study, a significant correlation (r=0.61, P<0.0001) between the lactone to total concentration and the apparent clearance of topotecan lactone was found (fig 2b).

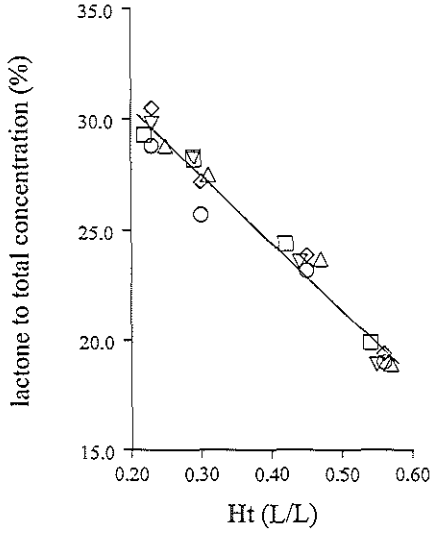


Figure 3 Relationship between the hematocrit (Ht) and the lactone to total concentration ratio in plasma. Each symbol represents the created hematocrit value versus the lactone to total ratio in the plasma of 1 healthy volunteer.

In vitro studies

We sought to define an in vitro model that would explain the differences between males and females in topotecan pharmacokinetics. In the experiments with the whole blood samples of the healthy male volunteers, with artificially altered hematocrit values, a strong significant correlation (r=0.98, P<0.0001) was found between the hematocrit value and the lactone to total concentrations after a 15-min incubation at 37°C with 5 ng/mL of both topotecan lactone and carboxylate (fig 3). By comparing normal heparinized whole blood

samples of 5 male and 5 female volunteers, a significant difference (P=0.0015) in the hematocrit value was found, with mean values of 0.44 ± 0.014 L/L for males and 0.39 ± 0.019 L/L for females. After a 15-min incubation at 37°C with 5 ng/mL of topotecan lactone and carboxylate, the lactone to total concentration ratio in the plasma compartment was significantly higher (P<0.0001) in females as compared to males, with respective values of 27.8 ± 0.41 % and 25.2 ± 0.36 %. In addition, the change in the lactone to total concentration ratio was accompanied by a significantly (P=0.010) higher exposure of the lactone form in the plasma compartment of the blood samples of the female volunteers, with mean concentrations of 3.41 ± 0.103 and 3.22 ± 0.074 ng/mL for the blood samples obtained from the female and male volunteers.

DISCUSSION

In the present study, we have demonstrated for the first time that topotecan clearance is significantly slower in females as compared to males. These data complement previous knowledge of the clinical pharmacology of topotecan, and may have important clinical implications for its optimal use. Previous studies have revealed that major factors responsible for gender-dependent pharmacokinetics are related to differences in body composition, renal elimination, drug absorption and hepatic function [reviewed in 6]. Indeed, a significant difference in the body-surface area between male and female patients was found in the present study. However, the apparent clearance of topotecan lactone after oral administration was still 1.3-fold higher in male patients as compared to female patients after correction for body-surface area, indicating that body-surface area was not the major predictor for the gender-dependent clearance of topotecan lactone. Likewise, no significant linear relationship was found between body-surface area and the apparent clearance of topotecan lactone after correction of the apparent clearance for body-surface area.

Pharmacokinetic studies performed during previous clinical trials of topotecan have consistently failed to recognize the discrepant drug disposition in males and females. This is most likely caused by the fact that in most studies only a limited number of patients was sampled or combined measurement of topotecan lactone plus carboxylate was performed. Recently, gender-dependent differences in topotecan pharmacokinetics were not found in a population of children and adults in the range of 3 weeks to 22 years of age [10]. This is probably related to the fact that no gender differences in hematocrit values are reported in children under the age of 12 years and only marginal differences were observed between the 12 and 18 years [11].

Topotecan is mainly eliminated by the kidneys, with 40% (range 26-80%) of the dose excreted in the urine as parent compound within 24 h after a 30-min i.v. infusion [reviewed in 1]. One of the known metabolic pathways of topotecan is the loss of the methyl moiety linked to the nitrogen in the core structure of topotecan by the cytochrome P450 enzyme system, resulting in N-desmethyl topotecan (fig 1). Concentrations of this metabolite in plasma and urine were very low; after a 30-min i.v. infusion, peak plasma concentrations of N-desmethyl topotecan accounted for less than 1% of the maximal total drug concentration, and in urine

only 1 to 4% of the delivered dose was excreted as N-desmethyl topotecan [12]. Recently, a new metabolic conjugation pathway has been described [13], resulting in the formation of topotecan-O-glucuronide and N-desmethyl topotecan-O-glucuronide (fig 1). Since relatively low amounts of these metabolites were excreted in the urine, with maximal concentrations of 10 and 3.5%, respectively, in comparison with urinary concentrations of the parent compound, coupled with the fact that altered topotecan clearance only has been described in patients with severely-impaired renal function [14], gender-dependent differences in the known metabolic pathways of topotecan are unlikely to occur. However, minor gender-related differences in renal clearance could be expected, since the glomerular filtration rate of the kidneys is related to the body-weight, and thus higher topotecan clearance in males could be due to their higher body-weight [reviewed in 6]. Nevertheless, as described above, the apparent clearance of topotecan lactone was significantly higher in males, even after correction for the body-surface area (i.e. body-weight), while no significant difference was found for the clearance of total topotecan.

Differences in intestinal drug absorption between males and females after oral administration have been reported and shown to be possibly related to a slower gastric emptying rate in females, different levels of gut enzymes and differences in the hepatic first-pass effect. In the present analysis, however, differences in the gastric emptying rate are less likely, since the time to reach the maximum concentration of topotecan lactone after oral administration did not differ significantly. Moreover, previously we did not find differences in the oral bioavailability of the i.v. dosing solution between males and females of topotecan lactone, with bioavailabilities of 31 ± 8.4 % (n=7) and 30 ± 7.5% (n=5) for males and females, respectively [data compiled from 15].

Gender-related differences caused by different levels of liver and gut enzymes are not expected since metabolism is a minor route of elimination of topotecan. As described above, low amounts of the known metabolites of topotecan were detected in urine and plasma of patients. Likewise, in a phase I and pharmacologic study in patients with impaired hepatic function, similar topotecan pharmacokinetics were observed in patients with and without liver injury [16], also suggesting a minor role of liver enzymes in the overall elimination of topotecan.

A significant relationship was found following linear regression analysis of body-surface area versus the absolute apparent clearance of topotecan lactone, while after correction for body-surface area this relationship did not remain statistically significant. However, significant relationships were noted between the hematocrit and the absolute apparent clearance of topotecan lactone, as well as the apparent clearance corrected for body-surface area, indicating that hematocrit was a significant predictor for the apparent clearance of topotecan lactone.

To further evaluate the role of hematocrit in topotecan pharmacokinetics as a potentially important contributing factor to the observed gender-dependency, various additional in vitro studies were performed. Hematocrit values in healthy humans are known to be different in males and females, with respectively values of 0.44 ± 0.02 L/L and 0.39 ± 0.02 L/L [17]. Furthermore, erythrocytes are known to be carriers for a variety of endogenous compounds

and drugs, including topotecan [18-20]. Drugs and endogenous compounds in the plasma compartment are in equilibrium between plasma proteins and plasma water, i.e. in a bound and unbound form. The plasma water is the central compartment, from which the unbound drug is able to move across cell membranes, including those of red blood cells. Topotecan has a plasma protein binding of approximately 35% [21], and hence 65% of the drug in principle is directly available for cellular uptake. To demonstrate the relationship between hematocrit value and the lactone to total concentration ratio in plasma, whole blood of 5 male volunteers, with artificially altered hematocrit values, was incubated with topotecan lactone and carboxylate. The hematocrit appeared to be a principle predictor of the resulting topotecan lactone to total concentration ratio in the plasma compartment, with higher ratios at lower hematocrit values. This phenomenon was confirmed by in vitro incubation of topotecan in whole blood of males and females, showing significantly higher lactone to total topotecan concentrations in females as compared to males. The higher lactone to total topotecan ratios in blood with lower hematocrit values is most likely caused by the fact that the carboxylate form, which is charged, is not able to pass cell membranes and thus remains in the plasma compartment [22]. Hence, the absolute amount of the carboxylate form in the plasma compartment in the in vitro experiments is independent of the hematocrit value, resulting in lower carboxylate concentrations in blood samples with lower hematocrit values. In addition, we found significantly higher topotecan lactone concentrations in the plasma compartment of the blood samples of the female volunteers. This phenomenon is consistent with the in vivo finding of lower topotecan lactone clearance in females, as a result of higher exposure of the lactone form in females compared to males.

In conclusion, we have shown that topotecan is subject to significant gender-dependent differences in pharmacokinetic behavior that result from a physiologic difference in hematocrit values between males and females. This finding may have implications for interpretation of the relationship between pharmacokinetic parameters and pharmacodynamic outcome of topotecan treatment. A potential gender-dependent relationship between the pharmacokinetics and pharmacodynamics has to be investigated in a study using single agent topotecan at a fixed dose. Eventually, pharmacologic data generated in this investigation and the recognition of the gender-dependency in topotecan pharmacokinetics may provide a basis for the development and refinement of clinical protocols allowing more rational and selective treatment with topotecan.

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Chapter 8

Topotecan lacks third-space sequestration

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ABSTRACT

The objective of this study was to determine the influence of pleural and ascitic fluid on the pharmacokinetics of the antitumor camptothecin derivative topotecan. Four patients with histologic proof of malignant solid tumor received topotecan 0.45 or 1.5 mg/m^2 orally on several occasions both in the presence and absence of third space volumes. Serial plasma and pleural or ascitic fluid samples were collected during each dosing and analyzed by high-performance liquid chromatography for both the intact lactone form of topotecan and its ring-opened carboxylate form. The apparent topotecan clearance (CL/f) demonstrated substantial interpatient variability, but remained unchanged within the same patient in the presence [110±55.6 L/h/m^2 (mean±SD of 8 courses)] or absence of pleural and ascitic fluid [118±31.1 L/h/m^2 (7 courses)]. Similarly, terminal half-lives and AUC ratios of lactone to total drug in plasma were similar between courses within each patient. Topotecan penetration into pleural and ascitic fluid demonstrated a mean lag time of 1.61 h (range, 1.37 to 1.86 h) and ratios with plasma concentration increased with time after dosing in all patients. The mean ratio of third space topotecan total drug AUC to that in plasma was 0.55 (range, 0.26 to 0.87). These data indicate that topotecan can be safely administered to patients with pleural effusions or ascites, and that there is substantial penetration of topotecan into these third spaces that may proof beneficial for local antitumor effects.

INTRODUCTION

The increased risk of toxicity following chemotherapy in patients with pleural effusions and massive ascites is widely known, and has been well documented for several compounds including methotrexate [1,2] and fludarabine [3]. This phenomenon is most likely related to greater drug accumulation in the peripheral compartment and a slower transport back to the central compartment, ultimately resulting in prolonged drug exposure. For this reason, it is advised to evacuate large pleural and ascitic effusions prior to administration of these agents. On the other hand, penetration of the delivered chemotherapeutic agent should be sufficient to produce adequate drug distribution into the pleural or ascitic fluid to induce relevant local antitumor effects [4].

Diffusion of orally or systemically administered drugs into the peritoneum may be diminished by fibrous tissue due to prior surgery or prior regional i.p. chemotherapy, as reported for mitomycin C [4]. In addition, several other factors including molecular weight, hydrophobicity, blood and lymph flow and capacity of the capillary wall and intervening interstitium have been shown to affect the peritoneal-blood barrier [5]. The same factors may also be applicable for pleural effusions and the pleural fluid-blood barrier, although only few paired plasma/pleural fluid pharmacokinetic data are available for antineoplastic agents [5-7].

In the absence of any pharmacokinetic data on third space sequestration for topotecan, a topoisomerase I inhibitor with substantial antitumor activity against various malignancies [reviewed in Ref. 8], we have prospectively evaluated the extent of penetration of this drug in pleural and ascitic fluid in cancer patients, and assessed the influence of these third spaces on topotecan plasma pharmacokinetics.

MATERIALS AND METHODS

Patients and treatment

A total of 4 patients with a histologically confirmed diagnosis of a malignant solid tumor that was refractory to standard forms of therapy was studied (Table 1). All patients had adequate hematopoietic, hepatic and renal functions [9]. The study drug topotecan was supplied as capsules containing either 0.25 or 1.0 mg of the active compound (SmithKline Beecham Pharmaceuticals Inc., Harlow, UK), and was administered orally once daily, after an overnight fast, either for 5 consecutive days and repeated every 3 weeks (3 patients) or for 2 consecutive days and repeated every week (1 patient). In all 4 patients, comedication was uniform and consisted of cisplatin (50 or 70 mg/m^2 administered as a 3-h i.v. infusion immediately before topotecan on day 1 of every course) and ondansetron (8 mg, i.v.) combined with dexamethasone (10 mg, i.v.) given 30 min before cisplatin. During therapy, the patients did not use any other medication that might have interfered with topotecan absorption and disposition. The clinical protocol was approved by the institutional review board, and patients signed informed consent before entering the study.

Table 1 Patient characteristics

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age (yrs)	41	65	40	35
Gender (M/F)	M	M	M	F
Carcinoma	ACUP[a]	rectum	ACUP	ovarian
Third space	pleural	pleural	ascites	ascites
Treatment schedule	d1-5 q3w[b]	d1-5 q3w	d1-5 q3w	d1-2 q1w[c]
Drug dose (mg/m^2/d)	1.50	1.50	1.50	0.45
Drug dose (mg/d)	3.00	2.75	3.25	0.75 (d1) 1.00 (d2)[d]

a: ACUP, adenocarcinoma of unknown primary origin; b: d1-5 q3w, once daily for 5 consecutive days, repeated every 3 weeks; c: d1-2 q1w, once daily for 2 consecutive days, repeated every week; d: As a result of body-surface area-based dosing, and given the availability of 0.25-mg and 1.0-mg topotecan capsules only, the calculated weekly dose was split into unequal daily doses.

Sample collection

Material for pharmacokinetic analysis was collected during the first treatment course on days 1, 2 and 5 from patients on the 5-day schedule, and during courses 1, 2 and 3 on days 1 and 2 from the patient on the 2-day schedule. Blood samples were collected in 4.5-mL glass tubes containing lithium heparin as anticoagulant (Becton Dickinson, Meylan, France) and were obtained at the following time points: prior to dosing, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after topotecan administration. The blood samples were immediately placed in an ice-bath and centrifuged within 10 min at 3000x*g* for 5 min at 4°C, to separate the plasma. Subsequently, a volume of 250-µL of the plasma sample was added to 750-µl of ice-cold (-

20°C) methanol in 2.0 mL polypropylene vials (Eppendorf, Hamburg, Germany). After vortex-mixing for 10 s, the samples were stored at -80°C until the day of analysis. Pleural and ascitic samples were obtained at the same time points as described for blood samples using a Medicut 16GA cannula (45×1.7mm internal diameter; Sherwood Medical, Tullamore, Ireland) and were collected in 4.5-mL polypropylene tubes, after discarding the first 10-mL of fluid. These samples were processed as described above for plasma.

Topotecan assay

The samples, plasma as well as pleural liquid and ascites, were analyzed using a reversed-phase HPLC assay with fluorescence detection, as described earlier [10], with minor modifications. In brief, samples were centrifuged for 5 min at 23,000 x *g* at 4°C, followed by a 5-fold dilution in phosphate-buffered saline prior to injection of 200-µL aliquots into the HPLC system. Chromatographic separations of topotecan carboxylate and lactone forms and endogenous compounds were achieved on a Hypersil BDS column (100x3 mm ID, 3 µm particle size; Shandon, Cheshire, UK), which was maintained at 35°C. The mobile phase, composed of 10 mM potassium dihydrogenphosphate-methanol-triethylamine (1750:500:4, v/v/v) with the pH adjusted to 6.0 (orthophosphoric acid), was delivered at a flow rate of 0.70 mL/min. The excitation and emission wavelengths of the Jasco FP920 fluorescence detector (Tokyo, Japan) were set at 381 and 525 nm, respectively, with an emission band width of 40 nm. Chromatographic data analysis was performed based on peak height measurements relative to injected standards using the ChromCard system of Fisons (Milan, Italy).

Pharmacokinetic analysis

Individual plasma concentrations of topotecan lactone and carboxylate forms were fit to a linear two-exponential equation, using the software package Siphar version 4 (SIMED, Creteil, France), based on a variety of considerations including Akaike's and Schwarz' information criterion. The concentration-time profiles were obtained after zero-order input, with a weighted least-squares algorithm applying a weighting factor of $1/y$. The area under the concentration-time curve (AUC) were determined for both the lactone (AUC_L) and carboxylate forms (AUC_C) on the basis of the best fitted curves. The apparent plasma clearance of topotecan lactone (CL/f) was calculated by dividing the dose administered by the observed AUC. The apparent terminal disposition half-life ($T_{1/2}$) was calculated as $\ln 2/k_{el}$, where k_{el} is the observed elimination rate constant of the terminal phase. The peak plasma concentrations (C_{max}) were determined graphically from the observed experimental values. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as AUC_L / (AUC_L + AUC_C). The fraction of drug penetrating into pleural or ascitic fluid was derived from the ratio of the topotecan total drug AUCs in the third space and plasma.

RESULTS

Plasma pharmacokinetics

Peak plasma concentrations and AUCs of topotecan lactone following an oral dose of 1.50 mg/m^2 to patients 1 and 2 were similar before and after pleural fluid was drained (fluid volumes, 3.1 and 1.1 L, respectively) (Table 2). Data from patient 3, who had recurrent ascites during all topotecan administrations with volumes of 8.4 and 9.4 L drained on days 2 and 6, respectively, indicated no difference in pharmacokinetic parameters between treatment days. Similarly, ascites (estimated to be 4.0, 1.0 and 1.0 L on 3 occasions by ultrasonography and percutaneous drainage) had no measurable effect on topotecan plasma pharmacokinetics in patient 4 (Table 2). Overall, the apparent topotecan clearance (CL/f) demonstrated substantial interpatient variability, but remained unchanged within the same patient in the presence [110±55.6 L/h/m^2 (mean±SD; 8 courses)] or absence of pleural or ascitic fluid [118±31.1 L/h/m^2 (7 courses)]. Topotecan L/T ratios in plasma were very similar between courses within each patient and averaged 40.0±3.89% (drained) and 40.0±6.11% (not drained), respectively.

Table 2 Summary of topotecan plasma pharmacokinetics in the presence or absence of pleura or ascitic fluid

Pat no.	Third space	No. of curves	AUC_L (ng.h/mL)	CL/f (L/h/m^2)	C_{max} (ng/mL)	$T_{1/2}$ (h)	L/T ratio (%)
1	pleural	1	12.9	136	2.06	1.83	44.7
	none	2	14.7, 18.3	119, 95.5	3.33, 6.50	2.17, 1.77	44.8,43.5
2	pleural	1	23.3	64.3	2.71	4.53	36.3
	none	2	18.7, 17.0	80.0, 88.1	2.04, 2.30	4.43, 5.90	34.6,35.8
3	ascites	3	22.1±1.92	68.2±5.82	2.43±0.32	4.38±0.36	40.3±2.4
4	ascites	3	5.80±1.82	155±61.2	1.51±0.65	3.00±0.58	39.2±5.3
	none	3	6.28±1.42	148±12.1	1.16±0.15	3.40±0.66	40.5±8.4

Data were obtained both in the presence and absence of third space fluids in each individual patient treated with topotecan doses and treatment schedules as given in Table 1. The AUC values were calculated by compartmental analysis, and data of patients 3 and 4 represent mean values ± SD. Abbreviations: AUCL, area under the topotecan lactone plasma concentration-time curve; CL/f, apparent plasma clearance of topotecan lactone; Cmax, peak plasma concentration of topotecan lactone; T1/2, apparent terminal disposition half-life; L/T ratio, percent of total drug (lactone plus carboxylate forms) circulating as topotecan lactone.

Pleural and ascitic fluid penetration

Given the low plasma protein binding of topotecan [~35%; (11)] and the relatively high total protein content in pleural fluid and ascites of the patients (range, 38 to 45 mg/mL), no correction for protein binding was performed. Topotecan concentrations in pleural fluid and

ascites peaked at ≥6 h after oral dosing, demonstrated a mean lag time of 1.61 h (range, 1.37 to 1.86 h; overall mean±SD in plasma, 0.63±0.28 h), and rose slowly to equal that in plasma by ~8 h (Figs. 1A to 1C). Topotecan disappearance from pleural fluid [$T_{1/2}$, 11.9 h (n=1)] and ascites [$T_{1/2}$, 7.94 h (n=1)] was slower than that from plasma. As a result, third space penetration, expressed as the ratio of concomitant pleural fluid or ascites and plasma concentration of total topotecan depended greatly on the sampling time point, and increased significantly with time in all patients (Figs. 1D to 1F). Overall, the mean ratio of third space topotecan total drug AUC to that in plasma was 0.55 (range, 0.26 to 0.87). The hydrolysis of topotecan to the ring-opened form was rapid and L/T AUC ratios were 18.1% and 23.5% in pleural fluid and 29.2% in ascites. Measurement of topotecan in ascites from patient 3 indicated that less than 1% of the administered dose was present in ascites at 6 to 8 h after dosing, indicating lack of a sink effect.

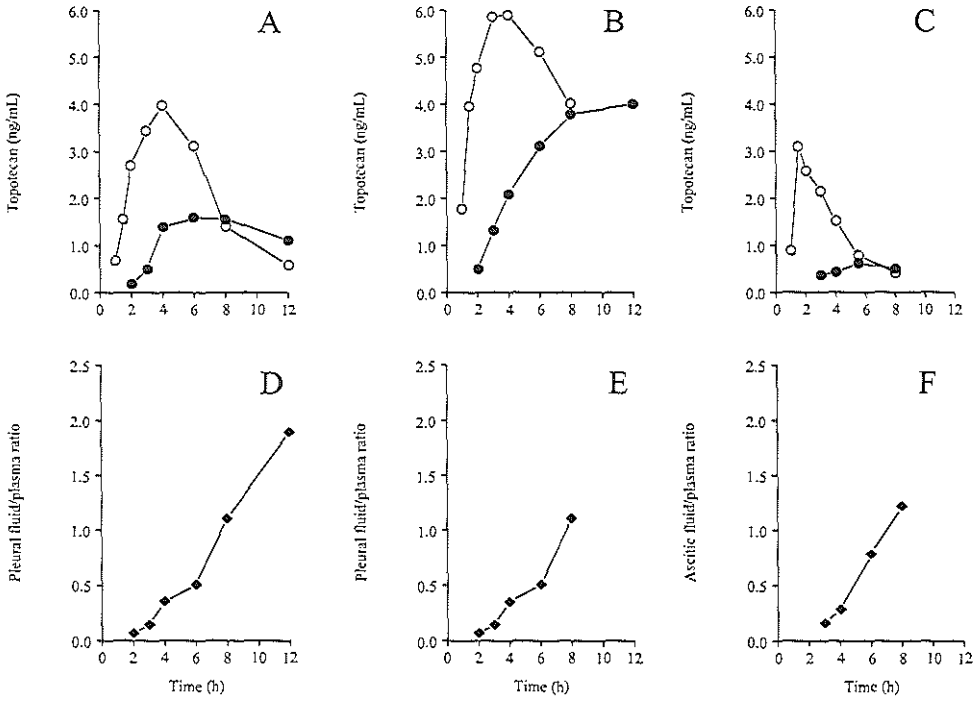

Figure 1 Concentration *versus* time plots of topotecan expressed as total drug (lactone plus carboxylate) in plasma (open circles) and ascites (closed circles, patients 1 and 2) or pleural fluid (closed circles, patient 4) and corresponding topotecan third-space fluid:plasma concentration ratios in patients 1 (A and D), 2 (B and E), and 4 (C and F).

Toxicity

Overall, treatment was very well tolerated in the 4 patients. No severe hematological toxicity or other organ toxicity was observed following oral topotecan administration at these doses. The third patient experienced fatigue graded 2 on a 4-point scale (NCI-Common Toxicity Criteria), whereas the fourth patient had mild nausea and vomiting.

DISCUSSION

This study was performed to explore the influence of pleural and ascitic fluid on the pharmacokinetic behavior of topotecan in cancer patients. Although the topotecan administration was preceded by cisplatin infusion in this study in all patients, important pharmacokinetic interactions that may have influenced the generated data are not very likely; (*i*) comparison of the kinetics of topotecan in clinical combination therapy regimens with cisplatin to single agent therapy did not reveal an apparent interaction [12], and (*ii*) using a randomized cross-over design for the administration order, no statistically significant differences in clinical pharmacokinetics were observed between sequences of oral topotecan and i.v. cisplatin [9].

Topotecan concentrations in pleural fluid and ascites were initially less than in plasma, and several hours were required for equilibrium to be attained between these fluids and plasma. The limited surface area for topotecan diffusion relative to the volumes of fluid, and the fact that pleural fluid and ascites are not well stirred, in addition to the hydrophilic nature of the drug likely contributed to the slow equilibrium kinetics. Overall, both pleural fluid and ascites represented only a small additional compartment for topotecan distribution, particularly in view of the already large topotecan steady-state volume of distribution of 73-133 L [13]. Nonetheless, concentrations equivalent to that in plasma were achieved after 8 hours, and topotecan elimination was found to be more slowly from the pleural and peritoneal cavity than from plasma. This is in keeping with earlier findings indicating slow peritoneal clearance of topotecan and high peritoneal:plasma concentration ratios of >10 following i.p. drug administration [14, 15].

Topotecan has been detected previously in ascites of 2 patients treated with a combination of i.v. topotecan and oral etoposide [16]. However, the reported ascitic fluid:plasma concentration ratios were established by single point measurements at different times after administration. Since these concentration ratios were shown in our patients to be by no means constant parameters during the dosing interval (Figs. 1D to 1F), single-point data are clearly inappropriate to directly compare the extent of penetration by topotecan. Hence, the approach of using paired AUC values in third space fluids and plasma, as done in the current study, should be considered the gold standard to report these ratios. Although the described data on topotecan accumulation is limited to only 4 patients, our results suggest that oral administration of topotecan can produce adequate drug distribution in pleural fluid and ascites at concentrations associated with significant antitumor activity in experimental models [7, 18]. In this context, it is of particular interest that topoisomerase I inhibitors were previously shown to be highly S-phase specific and that cytotoxicity is a function of the time

to drug exposure above a certain threshold concentration [19]. The topotecan penetration and subsequent accumulation in the third spaces might thus offer a potential therapeutic advantage in that tumor cells in the thoracic and peritoneal cavity are exposed to high local drug levels for prolonged time periods. This concept has also been described recently for systemic therapy with the structurally-related camptothecin derivative, irinotecan (CPT-11), although in contrast to topotecan, concentrations appeared to decline in parallel with those in plasma [20]. The reason for this discrepant behavior is unknown, but it likely reflects intrinsic differences in physicochemical and/or pharmacokinetic properties of both compounds, including differential binding to (plasma) proteins.

The plasma pharmacokinetics of topotecan revealed a substantial degree of interindividual variability, in line with previous observations [9, 21]. By comparing topotecan plasma levels in the same patient before and after drainage of pleural or ascitic fluid, no differences in rate of absorption and elimination became apparent. The lack of increased systemic exposure to topotecan in patients with massive third space volumes was further substantiated by the lack of excess toxicity. Hence, in contrast to clinical information on irinotecan treatment that suggested an increased risk of severe toxicity in patients with large pleural effusions or ascites [20], there was no evidence that the severity of toxicity was different between study courses with and without third space volumes in our patients treated with topotecan.

In conclusion, we have shown that (*i*) topotecan plasma pharmacokinetics are unaltered in patients with third space volumes, (*ii*) it can be safely administered to patients with large pleural effusions or massive ascites, and (*iii*) there appears to be substantial penetration of topotecan into these third spaces that may proof beneficial for local antitumor effects.

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Chapter 9

Modulation of cisplatin pharmacodynamics by Cremophor EL: experimental and clinical studies

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ABSTRACT

The paclitaxel vehicle Cremophor EL (CrEL) has been shown to selectively inhibit accumulation of cisplatin in peripheral blood leukocytes but not in tumor cells *in vitro*, and we hypothesized that this phenomenon is responsible for the improvement of the therapeutic index of cisplatin observed in combination studies with paclitaxel. Here, we report on studies assessing the interaction between CrEL and cisplatin in a murine model, and involving the potential clinical applicability of CrEL as a protector for cisplatin-associated hematologic side effects. In mice, CrEL (0.17 mL/kg, i.v.) given in combination with cisplatin (10 mg/kg, i.p.) did not change cisplatin pharmacokinetics. Cisplatin-induced hematologic toxicity, expressed as WBC at nadir, was significantly reduced by CrEL from 5.05±0.95 to 6.50±1.31 $\times 10^9$/liter (P=0.0009). Data obtained from cancer patients treated with cisplatin (70 mg/m^2, 3-h i.v.) and topotecan (0.45 or 0.60 mg/m^2/d×2) preceded by CrEL (12 mL, 3-h i.v.) (n=6) or without CrEL (n=10) similarly indicated significant differences in the percent decrease in WBC between the groups (46.5±18.7 vs 67.2±15.0%; P=0.023). Likewise, the percent decrease in platelet count was significantly greater in the absence of CrEL (23.9±5.38 vs 73.3±15.5%; P=0.0003). Pharmacokinetic parameters of unbound and total cisplatin and of topotecan lactone and total drug were not significantly different from historic control values ($P \geq 0.245$). Overall, this study provides further evidence on the important role of CrEL in the pharmacological and toxicological profile of cisplatin, and implies that reformulation of cisplatin with CrEL for systemic treatment might achieve improvement of its therapeutic index, particularly in the setting of a weekly dose-dense concept.

INTRODUCTION

Cisplatin is one of the most frequently used drugs in the treatment of a wide variety of solid tumors, including testicular cancer, ovarian cancer, (non-) small cell lung cancer, head and neck cancer and bladder cancer [1]. Major side effects of the conventional 3-weekly application are nausea/vomiting, renal toxicity, neurotoxicity and ototoxicity. There is a substantial body of evidence that for cisplatin, similar to other cytotoxic agents, the dose-response relationship is steep. Clinical studies have indicated in tumor types such as head and neck cancer and ovarian cancer that higher dose intensity, if achievable, leads to a higher response rate [2]. Most frequently, reaching a higher dose-intensity was attempted to be reached by increasing the dose per administration. However, in practice this led to unacceptable side effects, preventing further use of this strategy [3]. Another way of increasing dose intensity is by shortening treatment intervals without changing the dose per administration. Since 1988 we have been performing a large clinical and preclinical program focussing on interval shortening for cisplatin, with the drug given weekly. In a phase I study the maximum tolerable dose was found to be 80 mg/m^2/week. Dose limiting toxicity was myelosuppression, while the commonly known side effects of cisplatin were not dose limiting using this schedule [4]. Phase II studies have shown major activity in head and neck cancer [5], and even in a tumor type such as mesothelioma that is commonly considered to be chemotherapy-resistant [6]. By adding oral etoposide to the

weekly administration of cisplatin [7], high response rates were also obtained in non-small cell lung cancer [8], and even in ovarian cancer resistant to conventional cisplatin schedules [9]. Recently, we have added weekly administered Taxol (i.e. paclitaxel formulated in CrEL-ethanol, 1:1, v/v) to our regimen of weekly cisplatin, and observed a marked reduction in myelosuppression as compared to what would have been expected based upon the single agent toxicities [10].[3] Similar observations have been made in clinical studies with conventional 3-weekly schedules [11]. In general, the pharmacokinetics of both drugs during the two tested sequences (Taxol preceding and following cisplatin) was unaltered, indicating that a pharmacodynamic interaction must have occurred. Up to now, convincing data regarding the fundamental reasons for these clinically important pharmacodynamic interactions are lacking. Based upon previous *in vitro* work indicating selective inhibition of cisplatin accumulation by CrEL in peripheral WBCs but not in tumor cells [12,13], we speculated that CrEL is responsible for improvement of the therapeutic index of cisplatin observed in combination studies with Taxol. Here, we report on studies assessing the interaction between CrEL and cisplatin in a murine model, and involving the potential clinical applicability of CrEL as a protector for cisplatin-associated hematologic side effects.

MATERIALS AND METHODS

Chemicals

Cisplatin (Platosin) was purchased as a powder from Pharmachemie (Haarlem, The Netherlands). Solutions of cisplatin for experimental use were prepared in isotonic saline at a concentration of 10 mg/mL. CrEL (lot 16H0043) was obtained from Sigma Chemical Co. (St. Louis, MO), and topotecan reference material was kindly provided by SmithKline Beecham (Harlow, UK). All other chemicals and reagents were of analytical grade or HPLC grade and were obtained from Rathburn (Walkerburn, UK). Water was filtered and deionized by the Milli-Q-UF Plus system (Millipore, Bedford, MA) and was used in all aqueous solutions.

In Vitro Blood Distribution

Aliquots (1 mL) of freshly prepared heparinized human whole blood were incubated for 15 min at 37°C with 5 ng/mL of topotecan lactone (L) and 5 ng/mL of topotecan carboxylate (C) in the combination with CrEL at final whole blood concentrations of 0, 0.5, 1.0, 2.0 and 4.0 µL/mL. Subsequently, the samples were centrifuged for 5 min at 2500*g* (4°C) to separate the plasma. The plasma samples were directly deproteinized with a 3-fold volume of ice-cold (-20°C) methanol, and were stored at -80°C prior to analysis. The lactone to total drug concentration ratio in plasma was calculated as: L/(L+C). The remaining plasma was used for the determination of the CrEL concentrations.

In a separate set of experiments, a volume of 5 mL whole blood was incubated with 5 µg/mL of cisplatin in the absence and presence of CrEL. Aliquots of 1 mL were centrifuged for 5 min at 2500*g* (4°C) for the measurement of total and unbound platinum concentrations in the plasma compartment at the following time points: immediately after start of the incubation, and at 0.5, 1 and 2 h after the start of incubation. For the determination of the

unbound cisplatin concentrations, 500 µL aliquots of the plasma supernatant were added to 1000 µL of ice-cold (-20°C) ethanol directly after collection of the plasma, and were stored at -80°C prior to analysis.

Animal Studies

Female FVB mice (10-12 weeks of age; weight, 23.2±1.87 g) were obtained from Harlan Nederland (Horst, The Netherlands), and were used in all experiments. The animals were divided in groups of 8 mice, and placed in methacrylate cages covered with filter bonnets in a controlled environment maintained on an automatic 12-h lighting cycle at a temperature of 22±2°C according to institutional guidelines. The animals were given a standard chow (Hope Farms B.V., Woerden, The Netherlands) and acidified water *ad libitum*. All solutions were prepared within 1 h prior to injection and stored on ice until use. Mice in groups of 5-20 were injected intravenously under light diethyl ether anesthesia with isotonic saline or a CrEL solution containing 50 µL/mL in isotonic saline (injection volume, 100 µL/30 g body weight). Administration was performed by injection into the tail vein after the mice had been gently warmed under a red 100-W lamp and had their tails soaked briefly in a warm water bath. The mice were then randomly divided into two groups; at 1 h after the first administration, one group was injected intraperitoneally with a cisplatin solution containing 1 mg/mL in isotonic saline (injection volume, 300 µL/30 g body weight), and the other group was injected isotonic saline as a control. The final CrEL and cisplatin doses were 0.17 mL/kg and 10 mg/kg, respectively. The cisplatin dose was previously determined as the i.p. dose that results in death of 50% of mice within 9 days [14], and was chosen based on preliminary experiments (using 4 mice per dose level each at 0, 2.5, 5.0, and 10 mg/kg) indicating that it would result in a substantial degree of myelosuppression (%decrease in WBC at nadir: 25.9, 31.8, and 55.5, respectively at 2.5, 5.0, and 10.0 mg/kg). Mice were bled under light diethyl ether anesthesia at approximately 10 a.m. on the days specified. Blood samples of 40 µL were obtained from the tail vein (days 1-4) or the retro-orbital venous plexus (day 5) into a heparinized glass micropipet for daily determination of peripheral blood-cell counts using an autoanalyzer. For pharmacokinetic purposes, additional blood samples were obtained in mice at 5, 15, 30 and 45 min, and 1, 2, 4, 6, 8, 16, and 24 h after i.v. CrEL administration (dose, 0.17 mL/kg body weight), using 4 animals per time point into polypropylene microtubes containing 7 USP units of lithium heparin as an anticoagulant. Similarly, blood samples were taken at the third and fifth day following i.p. cisplatin administration both in the absence and presence of CrEL. These samples were placed on ice, and plasma was separated within 10 min by centrifugation for 5 min at 2500*g* (4°C), and then stored at -20°C until analysis. It was confirmed that the observed concentrations of cisplatin and CrEL in all dosing solutions were within ±5% of their nominal target values. The experimental protocol was approved by the ethical committee of the Animal Welfare Office (Erasmus University, Rotterdam, The Netherlands).

Clinical Studies

Patients with recurrent or progressive ovarian cancer were treated with 6 cycles of weekly cisplatin (70 mg/m^2 infused over 3 h) with or without CrEL (12 mL), in combination with oral topotecan (0.45 or 0.60 mg/m^2/day for 2 days). CrEL was administered as a 3-h i.v. infusion immediately prior to cisplatin, and the dose was similar to that administered with paclitaxel at a dose level of 90 mg/m^2. Blood samples for pharmacokinetic analysis were obtained from an indwelling i.v. canula and collected in vials containing lithium heparin as anticoagulant at the following time points: prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after topotecan administration; and at 1, 2, 3, 3.5, 4, 5, 6, and 21 h after start of the cisplatin infusion. Blood samples for assessment of hematologic parameters were obtained (at least) on a weekly basis.

Pharmacologic Analysis

The analytical assay for unbound cisplatin and total cisplatin was based on flameless atomic absorption spectrometry [15]. Simultaneous determination of the lactone and carboxylate forms of topotecan was performed by reversed-phase high-performance liquid chromatography with fluorescence detection [16], with minor modifications as described [15]. CrEL concentrations in plasma samples were measured using a colorimetric dye-binding microassay [17]. The plasma concentration-time profiles of unbound and total cisplatin were fitted to two-compartmental linear models with extended least-squares regression analysis using the Siphar v4.0 software package (Innaphase, Champs-sur-Marne, France). The AUC of cisplatin was calculated to the last sampling time point with detectable drug levels (C_{last}) by the linear trapezoidal method, and extrapolated to infinity by addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression analysis of the final plasma concentration values. Unbound cisplatin clearance was calculated by dividing the administered dose by the observed AUC, and the terminal disposition half-life was calculated as ln2/k, where k is the rate constant of the terminal disposition phase. The peak plasma concentration was determined graphically from the observed experimental values. The potential for drug accumulation or altered disposition following repeated cisplatin administration was assessed by the ratio of AUC values between treatment courses (i.e., AUC_{c1}/AUC_{c2} and AUC_{c1}/AUC_{c3}). For topotecan, the concentration-time profiles were obtained after zero-order input with weighted least-squares regression analysis applying a weight factor of $1/y$. The AUCs of the lactone (AUC_L) and carboxylate (AUC_C) forms were determined on the basis of the best fitted curves, and the apparent clearance of topotecan lactone (CL/f) was determined using the dose expressed in free base equivalents and the fitted AUC. Other parameters were assessed as outlined for cisplatin. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as $AUC_L/(AUC_L+AUC_C)$.

RESULTS

Preclinical Evaluation

Prior to *in vivo* testing, it was confirmed that CrEL at clinically relevant concentrations had no effect on the blood distribution and plasma protein binding of cisplatin in human samples *in vitro* (P>0.3 at 5 tested concentrations; Table 1). In addition, CrEL did not substantially influence erythrocyte partitioning and the lactone to carboxylate interconversion of topotecan at similar concentrations (Table 2), although the topotecan lactone concentration was slightly reduced at a CrEL concentration of 1.0 µL/mL by one-way ANOVA. This effect was not consistently observed with increased CrEL levels, and was considered of minor importance, particularly in view of substantial kinetic variability with the planned topotecan regimen [18].

Table 1 Effect of CrEL on in vitro cisplatin concentrations in plasma[a]

CrEL (µL/mL)	AUC_u (µg.h/mL)	$T_{1/2}$ (h)	AUC_u/AUC_{tot}	$fu_{2\text{-}h}$
0	6.39±0.263	1.37±0.06	0.581±0.013	0.349±0.008
0.5	6.35±0.161	1.36±0.04	0.573±0.026	0.338±0.010
1.0	6.33±0.220	1.35±0.01	0.573±0.006	0.338±0.009
2.0	6.49±0.146	1.35±0.042	0.582±0.016	0.347±0.020
4.0	6.32±0.230	1.30±0.025	0.577±0.005	0.345±0.010
	P=0.83[b]	P=0.31[b]	P=0.91[b]	P=0.99[b]

[a] Data were obtained at several time points after incubating cisplatin (5 µg/mL) in whole blood in the absence and presence of CrEL, followed by centrifugation and analysis of the plasma supernatant. Data are presented as mean values of at least 3 independent observations per group ± S.D.
[b] One-way analysis of variance test.
Abbreviations: AUC, area under the plasma concentration time curve; $T_{1/2}$, disappearance half-life; u, unbound cisplatin; tot, total cisplatin; $fu_{2\text{-}h}$, fraction unbound cisplatin in plasma at 2 h after start of incubation.

Table 2 Effect of CrEL on in vitro topotecan concentrations in plasma[a]

CrEL (µL/mL)	$C_{15\text{-}min}$ L (ng/mL)	$C_{15\text{-}min}$ C (ng/mL)	L/T ratio
0	3.33±0.108	9.65±0.138	0.257±0.0037
0.5	3.19±0.057	9.52±0.099	0.251±0.0018
1.0	3.10±0.018	9.43±0.172	0.247±0.0045
2.0	3.13±0.065	9.37±0.114	0.251±0.0056
4.0	3.20±0.085	9.45±0.370	0.253±0.0026
	P=0.023[b,c]	P=0.51[b]	P=0.14[b]

[a] Data were obtained after incubating topotecan lactone and carboxylate forms (5 ng/mL each) in whole blood for 15 min in the absence and presence of CrEL, followed by centrifugation and analysis of the plasma supernatant. Data are presented as mean values of at least 3 independent observations per group ± S.D.
[b] One-way analysis of variance test.
[c] Control (CrEL concentration, 0 µL/mL) significantly different from the other groups.
Abbreviations: $C_{15\text{-}min}$, topotecan concentration in plasma at 15 min after start of incubation; L, topotecan lactone form; C, topotecan carboxylate form; T, topotecan total drug (lactone plus carboxylate forms).

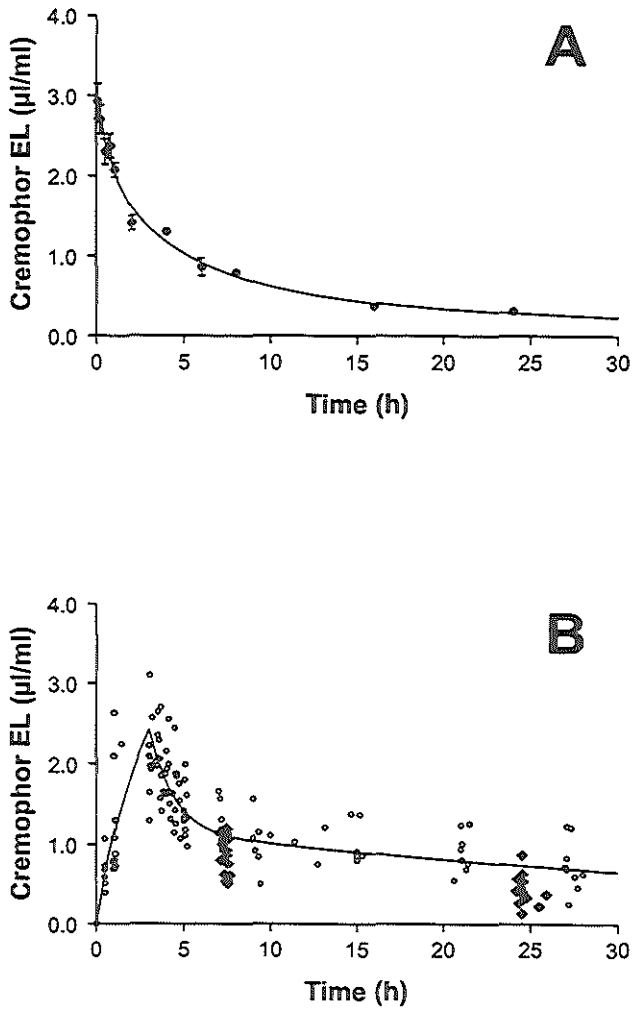

Figure 1 (A) Plasma concentration-time profile of CrEL in female FVB mice after i.v. administration of CrEL at 0.17 mL/kg; data are displayed as mean values (symbols) ± S.D. (error bars); (B) Plasma concentration-time profiles of CrEL in 6 patients (closed symbols) treated with 3-h i.v. infusion of CrEL (dose, 12 mL) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/d×2), and in 11 patients (open symbols and curve fit) receiving single agent paclitaxel formulated in CrEL-ethanol-isotonic saline at a dose of 100 mg/m^2 (CrEL dose, 8.33 mL/m^2) (unpublished data, AS and JV).

The CrEL dose tested in our murine model was shown to be associated with plasma concentrations in the range of 0.5 to 3.0 μL/mL (Fig. 1A), which is within the range required to modulate cellular cisplatin accumulation in peripheral blood leukocytes *in vitro* [12,13]. Cisplatin given alone (10 mg/kg, i.p.) induced significant reduction in the peripheral WBC count at nadir (39.4%), compared to saline-treated control mice ($P<0.0001$), similar to previous findings [20]. The addition of CrEL resulted in significant protection against cisplatin-induced hematologic toxicity as observed from increased WBC nadir values (Table 3). CrEL given alone had no effect on any of the studied parameters (Table 3).

Table 3 Hematologic toxicity of cisplatin in a mouse model[a]

Treatment group	WBC nadir ($\times10^9$/L)	ANC nadir ($\times10^9$/L)	PLC nadir ($\times10^9$/L)	HT (L/L)
Saline	8.33±1.82 (7.12-11.5)	0.90±0.76 (0.20-1.51)	398±419 (29-1057)	0.435±0.007 (0.427-0.443)
CrEL	9.52±2.40 (6.99-13.4)	0.74±0.36 (0.14-1.39)	296±205 (17-550)	0.427±0.031 (0.382-0.485)
Cisplatin	5.05±0.95[b,c] (3.22-6.93)	0.64±0.42 (0.14-2.04)	484±293 (28-1200)	0.411±0.063 (0.223-0.504)
Cisplatin + CrEL	6.50±1.31[d] (5.10-9.35)	1.10±0.89 (0.31-3.33)	383±240 (72-776)	0.436±0.031 (0.392-0.492)

[a] *Data were obtained on the third, fourth or fifth day after administration of cisplatin (10 mg/kg, i.p.) with or without CrEL (0.17 mL/kg, i.v.) to female FVB mice. Data are presented as mean values of 5 to 20 observations per group ± S.D., with the observed range shown in parenthesis.*
[b] *Significantly different from the saline group at P<0.0001.*
[c] *Significantly different from the CrEL group at P<0.0001.*
[d] *Significantly different from the cisplatin group at P=0.0009.*
Abbreviations: *WBC, white blood cell count; ANC, absolute neutrophil count; PLC, platelet count; HT, hematocrit.*

Clinical Studies

Complete pharmacokinetic data were obtained from 3 patients treated with cisplatin and oral topotecan at 0.45 mg/m^2/d×2, from 3 patients treated with the same regimen preceded by a 3-h i.v. infusion of CrEL (12 mL), and from 3 patients treated with the same combination after topotecan dose escalation (0.60 mg/m^2/d×2). Cisplatin pharmacokinetic parameters were compared to unpublished data from 77 patients treated at the Rotterdam Cancer Institute with single agent cisplatin (70 mg/m^2). No statistically significant differences were found in the parameters for both unbound and total cisplatin between the control and CrEL-treated groups (Table 4 & Fig. 2A).

In addition, topotecan lactone and total drug pharmacokinetic profiles were also unaffected by CrEL when compared to single agent topotecan (Table 4 & Fig. 2B). The observed plasma concentrations of CrEL following its administration were in the same range described previously (Fig. 1B).

Table 4 Summary of cisplatin and topotecan pharmacokinetics in the presence and absence of CrEL[a]

Parameter	With CrEL	Without CrEL[b]	P[c]
Cisplatin			
C_{max} (µg/mL)	0.90±0.22	0.81±0.18	0.249
AUC_{fu} (µg.h/mL)	3.00±1.52	2.34±0.43	0.338
CL_{fu} (L/h/m^2)	32.2±24.8	31.1±6.63	0.771
fu/tot ratio	0.082±0.045	0.076±0.013	0.403
AUC_{c1}/AUC_{c2}	0.886±0.528	0.774±0.049	0.626
AUC_{c1}/AUC_{c3}	0.930±0.590	0.718±0.128	0.420
Topotecan			
CL/f_L (L/h/m^2)	142±95.7	107±22.5	0.448
L/T ratio	0.36±0.05	0.36±0.02	0.999
AUC_{c1}/AUC_{c2}	0.883±0.503	0.716±0.192	0.504
AUC_{c1}/AUC_{c3}	0.692±0.198	NA	NA

[a] Data were obtained from 6 patients treated with cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/d×2) preceded by a 3-h i.v. infusion of CrEL (dose, 12 mL) (n=6) Data are from day 1 of the first course and presented as mean values ± S.D.
[b] Unpublished data from 77 patients treated at the Rotterdam Cancer Institute with single agent cisplatin at a dose level of 70 mg/m^2 and data from Creemers et al. (40) and Gerrits et al. (41) from 5 patients treated with single agent oral topotecan at a dose level of 0.50 mg/m^2.
[c] Unpaired two-tailed Student's t test.
Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; fu, fraction unbound cisplatin; CL, total plasma clearance; tot, total cisplatin (unbound plus bound fractions); c, treatment course number; CL/f, apparent oral clearance; L, topotecan lactone form; T, topotecan total drug (lactone plus carboxylate forms); NA, not available.

Comparative hematologic pharmacodynamic data obtained from treatment courses with (n=6) or without CrEL (n=10) indicated that the percent decrease in WBC was significantly reduced in the CrEL-treated group at the same topotecan dose (46.5±18.7 vs 67.2±15.0%; P=0.023) (Table 5). Likewise, the percent decrease in platelet count was significantly worse in the absence of CrEL (23.9±5.38 vs 73.3±15.5%; P=0.0003).

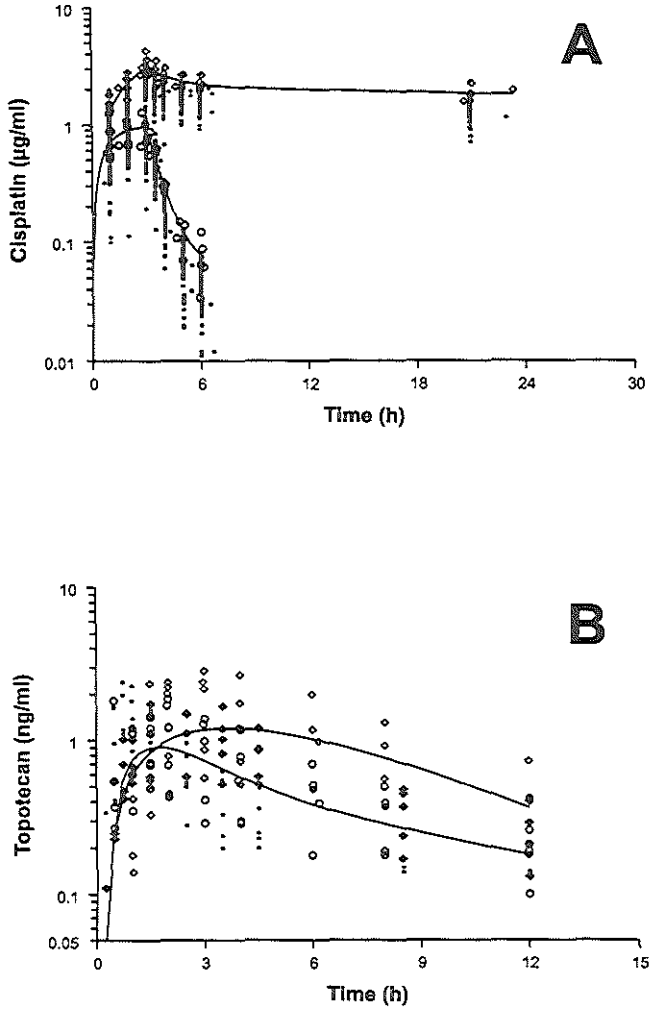

Figure 2 (**A**) Plasma concentration-time profiles of unbound cisplatin (circles) and total cisplatin (triangles) in 6 patients (open symbols and curve fits) treated with 3-h i.v. infusion of CrEL (dose, 12 mL) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/d×2), and in 77 patients (closed symbols) receiving single agent cisplatin at a dose of 70 mg/m^2 (unpublished data, AS and JV); (**B**) Plasma concentration-time profiles of topotecan lactone (circles) and carboxylate forms (triangles) in 6 patients (open symbols and curve fits) treated with 3-h i.v. infusion of CrEL (dose, 12 mL) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/d×2), and in 5 patients (closed symbols) receiving single agent topotecan at a dose of 0.50 mg/m^2.

Table 5 Summary of hematologic pharmacodynamics[a]

Parameter	With CrEL		Without CrEL
Topotecan dose	0.45 mg/m^2	0.60 mg/m^2	0.45 mg/m^2
Leukocytes			
Nadir (×10^9/l)	3.17±1.42	2.57±0.78	2.13±0.98
	(2.3-4.8)	(1.7-3.2)	(0.41-3.4)
%decrease WBC	46.5±18.7	70.5±6.77	67.2±15.0[b]
	(27.3-72.7)	(65.4-78.2)	(46.8-92.5)
Neutrophils			
Nadir (×10^9/l)	1.10±0.20	0.70±0.46	0.83±0.63
	(0.9-1.3)	(0.3-1.2)	(0.1-1.9)
%decrease ANC	69.0±11.0	88.0±7.59	81.8±13.1
	(59.3-80.9)	(80.0-95.1)	(63.6-97.4)
Thrombocytes			
Nadir (×10^9/l)	73.3±7.51	57.0±24.2	79.7±59.5
	(69-82)	(43-85)	(13-188)
%decrease PLC	23.9±5.38	75.4±10.3	73.3±15.5[c]
	(70.1-80.5)	(63.5-81.5)	(45.5-93.8)

[a] Data were obtained from 6 patients treated with cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/d×2) preceded by a 3-h i.v. infusion of CrEL (dose, 12 mL) (n=6) or without CrEL (n=10). The relative hematologic toxicity (i.e., the percentage decrease in blood cell count) was defined as: %decrease = [(pretherapy value – nadir value) / (pretherapy value)] × 100%. Data are presented as mean values ± S.D., with the observed range shown in parenthesis.
[b] Significantly different from the 0.45 mg/m^2/d×2 plus CrEL group at P=0.029.
[c] Significantly different from the 0.45 mg/m^2/d×2 plus CrEL group at P=0.0003.
Abbreviations: WBC, white blood cell count; ANC, absolute neutrophil count; PLC, platelet count.

DISCUSSION

The present study provides further evidence of the important role of the paclitaxel vehicle CrEL in the pharmacology and toxicology of cisplatin, and suggests that CrEL is responsible for pharmacological interactions observed previously between cisplatin and paclitaxel in cancer patients [10]. Apart from the reduction of cisplatin-induced nephrotoxicity by saline infusions and forced hydration, the selective modulation of side-effects associated with cisplatin chemotherapy, notably myelotoxicity in dose-dense regimens, has not yet resulted in a substantially improved therapeutic index of the drug [1]. Our hypothesis that CrEL may act as a modulator of cisplatin-associated myelotoxicity emerged directly from its use as hematopoietic-protecting agent in irradiated mice and our own preclinical and clinical experience.

Indeed, it has repeatedly been confirmed that the pharmaceutical vehicles present in paclitaxel and docetaxel formulations (Cremophor EL and Tween 80, respectively) may have a major impact on the pharmacology of concomitantly administered drugs. The influence of both vehicles on the reduced cisplatin uptake in cells in a previous study was readily shown from experiments with WBC incubated with CrEL or Tween 80 prior to cisplatin [12]. A significant reduction in the intracellular cisplatin concentration in WBC of up to 42% compared to the control was observed in the presence of CrEL or Tween 80. Furthermore, cisplatin accumulation was unaltered with paclitaxel or docetaxel formulations which did not contain CrEL or Tween 80 (viz. dimethylsulfoxide), suggesting that the inhibition of cisplatin accumulation in WBC is

exclusively caused by the formulation vehicles. We now show that the CrEL concentrations used in these experiments, viz. 0.1 and 0.01 µL/mL, are readily achievable in murine and patient plasma for extended time periods after its administration, in accordance with previous data [20,21] . Thus, although not investigated in the current study, altered adduct formation with critical structures of bone marrow cells, might have contributed to the observed decrease in cisplatin-induced hematologic toxicity in the presence of CrEL.

As an alternative mechanism underlying the decrease in hematologic toxicity, a number of reports published on the effects of CrEL administration on hematopoiesis *in vivo* may be particularly relevant. The first significant observation was the increase in circulating platelet levels recorded following prolonged administration of high doses of CrEL in dogs [22]. In contrast, in mice it was shown that peripheral blood cell parameters, including WBCs, reticulocyte and platelet counts are unaffected by CrEL administration, which is consistent with our current findings. However, i.v. administration of CrEL in mice was associated with a decrease in femoral bone marrow cellularity, an upregulation of B220 (B cells) and 7/4 surface antigen expression (neutrophil and activated macrophage) in the marrow and an increase in the incidence of both primitive and committed progenitors [23]. Furthermore, CrEL protected mice from irradiation-induced death if administered prior to the dose [23]. This intriguing effect is consistent with the interpretation that CrEL activates accessory cells, and modulates accessory factors regulating hematopoietic progenitor cells through the operation of cytokine cascades. The induction of histamine release may also play a role in the hematopoietic response to CrEL administration [24-26] . Histamine is known to trigger colony forming units into cycle, stimulate the proliferation of committed hematopoietic progenitor cells [27,28], and modulate the response of primitive hematopoietic cells to interleukin-3 [29,30]. In mice, no evidence was obtained of localized toxicity or marrow destruction as a result of CrEL injection [23], which is consistent with flow cytometric studies demonstrating that even very high concentrations of CrEL (>10%) did not lyse mammalian cell membranes [31]. In addition, no other obvious effects attributable to CrEL have been noted. In this context, it is also particularly noteworthy that CrEL has been shown to reduce hematologic toxicity profiles in mice following administration of several other chemotherapeutic agents, including carboplatin and 5-fluorouracil [32]. Clearly, additional studies on the combined use of CrEL with cisplatin at the molecular, cellular and *in vivo* level are desired for a better understanding of this clinically potentially important phenomenon.

One important observation from this work is that CrEL does not significantly alter the pharmacokinetic behavior of cisplatin and topotecan, thus ruling out kinetic modifications that might explain the altered toxicity of the combination. Pharmacokinetic studies in patients have revealed that CrEL selectively distributes within the central blood/bone marrow compartment and exists in plasma as large polar micelles [21,33]. In addition, we have demonstrated previously that CrEL can have a profound effect on the pharmacokinetic behavior of paclitaxel, resulting in a nonlinear increase in the AUC [34]. Apparently, in the presence of CrEL, paclitaxel prefers to stay within the plasma compartment, which may be due to inclusion and partitioning in micelles [33]. Recent experiments with doxorubicin [35], etoposide [36], and the photosensitizer C8KC [37], indicate that this effect may not be unique for paclitaxel. If micellar encapsulation of these agents by CrEL is the sole explanation for the kinetic interactions, then it can be

anticipated that water-soluble agents such as cisplatin and topotecan are not readily incorporated in the highly hydrophobic interior of CrEL micelles [38], and subsequently change of pharmacokinetic profiles.

One limitation of our study is the additional administration of topotecan, which itself is a highly myelotoxic agent, to the tested regimen in the patients [15,39] . Therefore, we cannot rule out the possibility that CrEL only protected from cisplatin-induced hematologic toxicity and that topotecan-mediated side effects might have influenced the overall clinical benefit. However, in spite of this drawback, it can be speculated in the light of the current observations that modulation of cisplatin pharmacodynamics alone is sufficient to improve pharmacodynamic outcome of the combination. Future studies are planned to confirm the current findings using a randomized clinical study in combination with dose-escalation of CrEL to define the optimal dose. Based on previous clinical experience, it is expected that doses up to 30 mL/m^2 as a 3-h i.v. infusion can be safely administered without the occurrence of hypersensitivity reactions [35]. This type of study may lead to the clinical use of CrEL as an inexpensive protective agent of cisplatin-associated hematologic toxicity.

In conclusion, this study provides further evidence of the important role of CrEL in the modulation of the pharmacological and toxicicological profile of cisplatin, and implies that reformulation of cisplatin with CrEL for systemic treatment might achieve improvement of its therapeutic index, particularly in the setting of a weekly dose-dense concept.

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Chapter 10

Dose and schedule-finding study of oral topotecan and weekly cisplatin in patients with recurrent ovarian cancer

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ABSTRACT

Both weekly cisplatin chemotherapy and single agent topotecan have proven to be effective in recurrent ovarian cancer. Preclinical data show synergism between cisplatin and topotecan. Side effects for this combination are drug sequence dependent and predominantly haematologic. Since preclinical data suggest that Cremophor EL (CrEL), the formulation vehicle of paclitaxel, has a protective effect on haematological toxicity of cisplatin, CrEL was added to the combination cisplatin and topotecan. In this phase I study, escalating doses of oral topotecan administered on day 1,2,8,9,15,16,29,30,36,37,43,44 were combined with weekly cisplatin 70 mg/m^2/d day 1,8,15,29,36,43 (scheme A) or with the presumably less myelotoxic sequence weekly cisplatin day 2,9,16,30,37,44 (scheme B). In scheme C, CrEL 12 mL was administered prior to cisplatin in the sequence of Scheme A. Eighteen patients have received a total of 85 courses. In scheme A 4/10 patients, all treated with topotecan 0.45 mg/m^2/d, experienced DLT: 1 patient had vomiting grade 4, 1 patient had grade 4 neutropenia > 5 days, 1 patient had > 2 weeks delay due to thrombocytopenia and 1 patient due to neutropenia. Both patients in scheme B (topotecan 0.45 mg/m^2/d) had DLT due to a delay > 2 weeks because of prolonged haematological toxicity. No DLT was observed in the first 3 patients in scheme C (topotecan 0.45 mg/m^2/d). However, 2 out of 3 patients treated at dose level topotecan 0.60 mg/m^2/d in scheme C experienced DLT due to > 2 weeks delay because of persistent thrombocytopenia or neutropenia. We conclude that there is a modest clinical effect of CrEL on haematological toxicity for this cisplatin based combination regimen, which seems to reduce these side effects but does not really enable an increase of the oral topotecan dose.

INTRODUCTION

Ovarian cancer is the most common cause of death among patients with gynaecological malignancies [1]. At the time of presentation the majority of patients have advanced disease, which is not amenable to cure by surgery alone. The standard treatment is a multimodality approach, consisting of cytoreductive surgery and platinum/paclitaxel combination chemotherapy [2]. Salvage chemotherapy may result in prolonged secondary remissions with alleviation of symptoms and improvement of the quality of life. The response to salvage chemotherapy is related to the therapy free interval. The longer this interval, the greater the probability of response to retreatment with platinum-based regimens. Dose-intense combination therapy might increase the reponse rates and the disease and overall survival. There is no standard therapy for patients with early recurrent ovarian cancer. Studies with new agents, including topotecan, have shown activity [3-5]. Cisplatin administered weekly at a dose of 70 mg/m^2 in combination with either continuous oral etoposide or with weekly i.v. paclitaxel to patients with progressive or recurrent ovarian cancer yielded a high dose intensity with response rates up to 84% in second line treatment [6,7].

Since there is pre-clinical evidence of synergistic action between cisplatin and topotecan [8,9] and both agents are active in ovarian cancer, while their toxicity profile is mostly non-

overlapping, we investigated a dose-intense weekly combination of these agents in recurrent ovarian cancer. We administered topotecan orally since preclinical studies suggested that prolonged exposure, as can be achieved more conveniently by oral administration, might result in higher antitumour activity [10].

Combining topotecan with i.v. cisplatin in more conventional 3-weekly schedules required considerable dose reduction of topotecan as compared to the single agent dose, even with G-CSF support [11]. Dose limiting toxicities (DLT) consisted mainly of haematological toxicity, which was sequence dependent, with topotecan following cisplatin as the most haematotoxic sequence [12,13]. Whether drug sequencing clinically is also relevant for antitumour activity is yet unknown. To evaluate the sequence dependent effects we administered the oral topotecan 2 days a week preceding weekly cisplatin or following cisplatin.

The third schedule was based on the preclinical observation that CrEL protected from cisplatin-induced haematological toxicity [14-16]. In these studies CrEL inhibited DNA adduct formation and the intracellular accumulation of cisplatin in human leukocytes, and in mice CrEL protected from cisplatin induced myelotoxicity. We might have observed this effect clinically in a study with weekly cisplatin preceded by paclitaxel [7]. In this study an unexpected high dose of weekly paclitaxel 90 mg/m^2 could be safely combined with weekly cisplatin 70 mg/m^2. Therefore, to evaluate the possibility of an increase of the oral topotecan dose i.v. CrEL was administered prior to the weekly cisplatin infusions in the third cohort of the current study.

PATIENTS AND METHODS

Patient selection

Patients with progressive or recurrent ovarian cancer were eligible for this phase I study. In case of prior treatment with a weekly cisplatin based regimen, a minimal progression free interval of 3 months after the completion of the weekly cisplatin schedule, was mandatory. Other eligibility criteria included: WHO performance status 0-2, evaluable disease, no more than 2 prior chemotherapy regimens, no chemotherapy, hormonal or radiotherapy for at least 4 weeks prior to entry in the study, no signs of bowel obstruction, neutrophils $\geq 1.5.10^9$/l, platelet count $\geq 100.10^9$/l, total bilirubin $<$ 1.25 times the upper limit of normal, creatinine clearance $>$ 60 ml/min, peripheral neurotoxicity $\leq$ grade 1, no condition precluding adequate intake of oral topotecan, and no prior therapy with a topoisomerase I inhibitor. The study was approved by the institution's medical ethics committee and all patients signed written informed consent prior to entry in the study.

Treatment Assessment

Before therapy a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including white blood cell (WBC) differential, and serum biochemistry, which involved sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine transferase (ALAT), γ-glutamyl transferase, glucose and uric acid, were performed, as was 24-hours creatinine clearance. During the induction regimen, weekly evaluations included history, physical examination, toxicity assessment according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0, and serum chemistry. CBC was determined twice weekly. Tumour evaluation according to the World Health Organisation (WHO) criteria for response was performed before and after the induction regimen and every 2 courses during the maintenance regimen.

Drug Administration

Cisplatin (Platosin) was supplied as a powder by Pharmachemie (Haarlem, The Netherlands). All patients received cisplatin dissolved in 250 mL of hypertonic saline [3% (w/v) sodium chloride] as a 3-h infusion on a weekly base during the induction regimen and 3-weekly during the maintenance regimen. Topotecan capsules containing either 0.25 or 1.00 mg of the active compound were supplied by SmithKline Beecham Pharmaceuticals (Harlow, UK). Topotecan was administered orally immediately at the start of the cisplatin infusion and the following day (scheme A and C) or the previous day (scheme B) during the induction regimen and for 5 consecutive days during the maintenance regimen (days 1-5) on an empty stomach, at least 10 min before meals. CrEL was obtained from Duchefa (Haarlem, the Netherlands), and administered through a polyvinylchloride-free infusion system in 3 h before the cisplatin infusions in scheme C.

In all patients standard pre-medication consisted of ondansetron (8 mg i.v.) combined with dexamethasone (10 mg i.v.), and an additional 2 mg of clemastine in scheme C. To avoid cisplatin-induced renal damage, the administration of cisplatin was preceded by the infusion of 1 L of a mixture of 2.5% (w/v) dextrose and 0.45% (w/v) sodium chloride over 4 h, and followed by another 3 L with the addition of 20 mM potassium chloride and 2 g/L magnesium sulphate applied over 16 h. Further anti-emetic therapy consisted of oral dexamethason 3 mg twice daily and ondansetron 8 mg twice daily on the first 2 days following cisplatin infusion, followed by oral metoclopramide or domperidon as needed.

Treatment and Dose Escalation

Patients were treated with an induction regimen consisting of oral topotecan day 1,2,8,9,15,16,29,30,36,37,43,44 in combination with weekly cisplatin 70 mg/m^2/d day 1,8,15,29,36,43 (scheme A) or the same administration scheme of topotecan in the presumably less myelotoxic sequence with weekly cisplatin on day 2,9,16,30,37,44 (scheme B). In scheme C CrEL (12 mL) was administered prior to cisplatin using the sequence of scheme A. The dose of CrEL was similar to that administered with paclitaxel at a dose of 90 mg/m^2, as used in our weekly cisplatin/paclitaxel schedule (8).

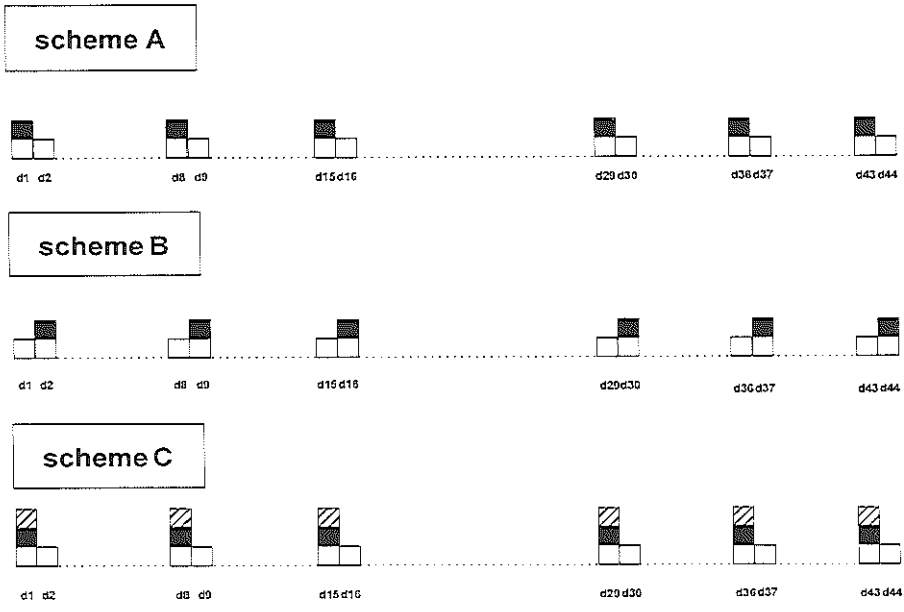

Figure 1 Outline of the different induction regimens: scheme A, B and C.
Cisplatin administration (open symbols), oral topotecan administration (closed symbols) and CrEL administration (hatched symbols).

All schemes are outlined in Fig. 1. The topotecan starting dose was 0.45 mg/m^2/d, a dose deduced from previous phase I studies with single agent topotecan, and our recently completed study with oral topotecan given daily times 5 every 3 weeks in combination with 3-weekly cisplatin [13]. Topotecan dose was escalated depending on the observed toxicity. Weekly treatment was postponed if the neutrophil count had not recovered to $\geq$ 1.0 x 10^9/L and the platelet count to $\geq$ 100 x 10^9/L on the day of retreatment. If the treatment was delayed, haematology was assessed twice a week to enable treatment re-start as soon as possible. Dose limiting toxicity (DLT) was defined as NCI-CTC version 2.0 grade 4 neutropenia lasting for 5 days or more, or complicated with fever requiring hospitalisation, grade 4 thrombocytopenia and/or non-haematological toxicity $\geq$ grade 3, excluding nausea. Treatment delay of more than 2 weeks due to toxicity was also considered DLT. Dose (de-) escalation, if any, was based on the toxicities observed at previous dose levels. In all patients response evaluation took place after completion of the induction regimen. In case of response or stable disease it was left to the judgement of the treating physician to proceed

with a maintenance therapy consisting of cisplatin and/or oral topotecan in a 3-weekly schedule.

Dose Modifications

If neutrophils were < 1.0 x 10^9/L and/or platelet count ≤ 100 x 10^9/L on day 8,15,29,36,43 or preceding each maintenance course, treatment was delayed until recovery.
Prior to the first course creatinine clearance should exceed 60 mL/min, as stated in the inclusion criteria. If the creatinine clearance was impaired due to dehydration or insufficient intake of fluids, prehydration was intensified followed by new creatinine clearance measurements. If the creatinine clearance after additional prehydration remained < 50 mL/min during the induction regimen, cisplatin was withdrawn from the combination regimen.

Sample collection and drug analysis

Blood samples for pharmacokinetics of cisplatin and topotecan were obtained on the day of cisplatin infusion during the first 3 weekly administrations of the induction regimen in scheme C. All blood samples were obtained and analysed as described previously [13].

RESULTS

Eighteen patients entered this study between March 1999 and May 2000. Patient characteristics are listed in Table 1. All patients had progressive or recurrent ovarian cancer after prior cisplatin-based chemotherapy and were eligible. The median WHO performance status was 1 (range 0-2), and age 51 years (range 34-68). The median cisplatin-free interval of 7 months (range 0-17) was short, rendering a poor prognosis. A total of 85 weekly induction administrations (cycles) were given. Overall, only 7 patients received all 6 weekly induction administrations: 2 patients were withdrawn because of an allergic reaction to cisplatin infusion during the 4th administration, 7 patients could not complete all 6 induction administrations due to haematological toxicity, 1 patient was not able to receive her 6th induction administration due to electrolyte disturbances related to the weekly platinum infusions, and 1 patient went off study after the 4th cycle due to early progressive disease.
In scheme A, 10 patients received cisplatin 70 mg/m^2/day 1 followed by oral topotecan at 0.45 mg/m^2/d on day 1 and 2. In scheme B, a total of 2 patients were treated at the same dose level in the reverse sequence. In scheme C, 3 patients were treated at the initial dose level of topotecan at 0.45 mg/m^2/d on day 1 and 2 and another 3 patients at 0.6 mg/m^2/d, all preceded by CrEL and cisplatin on day 1.

Haematological toxicity and in one case vomiting were the dose limiting toxicities (DLTs) of the induction regimen. In scheme A 4 out of 10 patients experienced DLT, in scheme B 2 out of 2 and in scheme C no DLTs were observed at the initial dose level and in 2 out of 3 at the higher dose level. The toxicity of the induction regimen A, B and C will be described separately in more detail in the following paragraphs. The main toxicity of the maintenance regimen was uncomplicated myelotoxicity.

Table 1. Patient characteristics

Characteristic		No. of patients
No. of patients entered		18
No. of patients assessable		18
Cisplatin free interval, months		
< 6		7
6-12		8
>12		3
Age, years		
Median	51	
Range	34-68	
WHO performance status		
0		9
1		7
2		2

Haematological toxicity

The haematological toxicity observed during the induction regimen is shown in table 2. In scheme A, three patients were judged as having DLT for haematological reasons: 1 patient had her 3rd weekly cisplatin administration (cycle) postponed more than 2 weeks due to persistent thrombocytopenia, 1 patient developed grade 4 neutropenia lasting for 5 days or more after cycle 3 and 1 patient had grade 4 thrombocytopenia, following cycle 3. In scheme B both patients had DLT based on delay of their 4th cycle by more than 2 weeks because of prolonged neutropenia (1 patient) or neutro- and thrombocytopenia (1 patient). Both patients continued induction treatment with weekly cisplatin monotherapy. At the first dose level in scheme C, with the addition of CrEL, no DLTs were observed, rendering this dose level feasible. Only 1 grade 4 neutropenia was observed and all patients completed the weekly cisplatin on time. However, in scheme C, at the dose of topotecan at 0.6 mg/m^2/d, again DLT was encountered in 2 out of 3 patients. The 3rd cycle was delayed by more than 2 weeks because of thrombocytopenia in 1 patient and thrombocytopenia followed by neutropenia in another patient. The MTD in scheme C was determined as topotecan at 0.45 mg/m^2/d (days 1 and 2) combined with CrEL 12 mL and cisplatin at 70 mg/m^2/week.

In scheme A, B, C first dose level and C highest dose level, the percentage of cycles associated with grade 3 or 4 neutropenia were respectively: 21, 33, 11 and 11%, but none of the neutropenic periods was complicated by fever. Thrombocytopenia grade 3 or 4 was observed in only a limited number of cycles (9, 17, 0 and 6%), all without bleeding, only 2 patients received a prophylactic platelet transfusion. Mild (grade 1 or 2) anaemia occurred in almost all patients, only 3 patients (1 in scheme A and 2 in scheme C) had grade 3 anaemia. A total of 20 blood transfusions were administered during induction chemotherapy (8 in scheme A, 4 in scheme B and 8 in scheme C).

Table 2 Haematological toxicity (worst per cycle)

Scheme	Topotecan mg/m^2/day	No. of patients/ cycles	Leuko-cytopenia		Neutro-penia		Thrombo-cytopenia	
			3	4	3	4	3	4
A	0.45	10/43	2	1	5	4	3	1
B	0.45	2/6	1	0	1	1	0	1
C	0.45	3/18	0	0	1	1	0	0
C*	0.60	3/18	1	0	1	1	2	0

* Patients who had their topotecan dose reduced to 0.45 mg/m^2/d due to earlier toxicity were included in this Table.

Non-haematological toxicity

Nausea and vomiting were the most frequently reported non-haematological side effects (data shown in table 3). One patient in scheme A was judged as having DLT because of grade 4 vomiting 1 week after cycle 6. The event was only possibly related to the chemotherapy, because she had experienced these symptoms once prior to treatment.

Other side effects included fatigue (grade 1 in 28%, grade 2 in 11%), alopecia in 22%, tinnitus in 17 %, and nephrotoxicity grade 1 in 28%. Mild headache was reported in 50% of patients, but this might also partially be related to the administration of ondansetron.

Two patients were withdrawn from the study because of an allergic reaction to cisplatin. These patients continued treatment with oral topotecan monotherapy.

Anti-tumour activity

Three patients were not assessable for response due to early discontinuation of the induction regimen for other reasons than progression of the disease. Seven patients had early response evaluation after having received more than 3 weekly cycles. Of the 15 patients evaluable for response after the weekly induction regimen, 9 patients (60%) reached a partial response, another 5 (33%) had stable disease, only 1 patient (7%) had early progressive disease. Due to the various maintenance treatments and the small sample size, assessment of the overall response and response duration is not really possible.

Pharmacokinetics

Full kinetic data following the administration of cisplatin and topotecan during the first 3 cycles were obtained from 6 patients in scheme C. Cisplatin and topotecan pharmacokinetic data [apparent clearance of unbound platinum fraction of 32.2±24.8 L/h/m^2 (mean±SD), cisplatin unbound/bound ratio of 0.082±0.045, apparent oral topotecan clearance of

142±95.7 L/h/m^2 and topotecan lactone to total drug AUC ratio of 0.36±0.05] were comparable to single agent data [17,18]. These findings indicate that there was no cisplatin/topotecan/CrEL pharmacokinetic drug interaction.

Table 3. Non-haematological toxicity (worst per cycle)

Scheme	Topotecan mg/m^2/d	No. of patients/ cycles	Nausea Grade			Vomiting Grade			
			1	2	3	1	2	3	4
A	0.45	10/43	15	11	0	7	11	0	1
B	0.45	2/6	2	4	0	1	4	0	0
C	0.45	3/18	4	2	0	2	2	0	0
C*	0.60	3/18	7	2	1	6	1	0	0

* Patients who had their topotecan dose reduced to 0.45 mg/m^2/d due to earlier toxicity were included in this Table.

DISCUSSION

This study, using weekly cisplatin -with and without CrEL- in combination with oral topotecan in different sequences, was based on a number of (pre-)clinical observations: Combining cisplatin and topotecan, two potentially active agents in ovarian cancer in a dose-dense scheme, is attractive given their pre-clinical synergism. Dose-dense weekly cisplatin combination chemotherapy regimens seem to yield improved response rates in recurrent ovarian cancer. Administration of oral topotecan enables prolonged systemic exposure, which is more effective in pre-clinical models and single agent oral topotecan is as effective as i.v., with less grade 4 neutropenia in advanced ovarian cancer [19], while patients have a preference for oral chemotherapy when equally effective [20]. In order to study drug-sequence dependent haematological effects for the combination cisplatin and i.v. topotecan, with cisplatin following topotecan as the least toxic sequence, both administration sequences were evaluated. Since, in the 3-weekly schedules combining cisplatin and topotecan, considerable dose reduction of topotecan was necessary, compared with single agent dose, we also studied the possible myeloprotective effect of co-administration of CrEL in scheme C. CrEL was used since *in vitro* studies indicate that CrEL selectively inhibits cisplatin

accumulation in white blood cells, but not in tumour cells [14,15]. This effect might have been responsible for improvement of therapeutic index observed in a combination study with paclitaxel [7].

In the current study, in this quite heavily pretreated population, haematological toxicity was the main dose-limiting side effect. However with no patients experiencing febrile neutropenia or requiring platelet transfusions because of bleeding, the observed haematological toxicity was, albeit dose limiting, relatively easy manageable. Nevertheless dose delays were needed, thereby limiting the projected dose-intensity in scheme A. Since the projected dose-intensity could already not be achieved at the first dose, lower dose was not studied. Also, using the same dose of topotecan studied in scheme A, in the theoretically less toxic sequence of cisplatin following topotecan (scheme B) resulted in 2 out of 2 patients experiening DLT at the first dose level. Therefore, this schedule was also considered non-feasible. The fact that topotecan was given on the day of cisplatin administration in both schedules, with the only difference consisting of an additional administration of topotecan on the day preceding or following cisplatin, may have accounted for the lack of the initially expected sequence dependent reduction of haematological toxicity in scheme B. As expected, the drug sequence had no apparent influence on the severity and frequency of non-haematological side effects. These side-effects were mostly mild and mainly consisted of nausea, vomiting, alopecia, headache and neurotoxicity.

With the addition of CrEL in scheme C, no DLTs were observed at the first dose level of topotecan at 0.45 mg/m^2 and all patients completed the induction without delays, in contrast to the observation in scheme A and B. A dose escalation of 33% for topotecan however again resulted in 2 DLTs in the first 3 patients because of treatment delay for more than 2 weeks due to persistent haematological toxicity. The non-haematological side-effects in scheme C were comparable to those observed for the other 2 schemes. The number of patients in this study was too small to draw unambiguous conclusions on CrELs myeloprotective effect for this combination. However the fact that 1) no haematological DLTs were seen in scheme C compared to 4 out of 10 patients at the same dose level in scheme A, 2) all patients in scheme C completed the cycles without delay, and 3) 11 and 0% of cycles in scheme C resulted in grade 3 or 4 neutropenia and thrombocytopenia, as compared to respectively 21 and 9% for scheme A at the same dose level, may at least suggest a myeloprotective effect of CrEL. The fact that nevertheless a dose escalation in scheme C was impossible is likely related to the fact that preclinical data indicate a myeloprotective effect of CrEL on cisplatin toxicity but not on topotecan toxicity, while the administered topotecan might have accounted for most of the haematological toxicity. A randomised study in a patient population treated with a cisplatin based regimen would be required to further elucidate CrELs potential myeloprotective effect in patients.

Pharmacokinetic analysis revealed no interaction between the compounds in this combination regimen and the apparent clearance of topotecan was independent of dose and schedule. CrEL did not affect the pharmacokinetics of the cytotoxic agents.

The scheduled cisplatin dose intensity of the patients completing all 6 weekly cycles of cisplatin, calculated over a treatment period of 7 weeks was 52.5 mg/m^2/week. The achieved

median dose intensity of patients completing all 6 weekly induction cycles was 58 mg/m^2/week for scheme A:, 47 mg/m^2/week for scheme B:, 60 mg/m^2/week for scheme C first dose level: and 45 mg/m^2/week for scheme C second dose level:. In comparison to the cisplatin dose intensity 17-25 mg/m^2/week achieved with the standard 3-weekly cisplatin/ oral topotecan combination regimens [13-21], the currently achieved dose intensity of cisplatin in this study is still much higher with all schemes. It is important to note however, that similar dose-intensity of cisplatin can be also achieved in combination with agents such as paclitaxel where the paclitaxel dose-intensity is relatively higher than the currently feasible topotecan dose-intensity. The overall 11% of patients withdrawn from the induction regimen due to an allergic reaction to cisplatin is not higher than reported in the literature for highly cisplatin pretreated populations [22]. Notably, no allergic reactions were observed in the patients treated with weekly cisplatin preceded by CrEL.

The response rate of 60% in this heavily pretreated population with a relatively short cisplatin-free interval is interesting, but not better compared to weekly cisplatin regimens with agents that can more easily added than topotecan [6,7].

In conclusion, this study suggests but does not prove some myeloprotective effect of CrEL on cisplatin induced toxicity. However a weekly schedule of cisplatin with oral topotecan seems less attractive than a weekly combination of cisplatin with either paclitaxel or etoposide in recurrent ovarian cancer.

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PART II

INHIBITORS OF TUBULIN

Chapter 11

Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation

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ABSTRACT

Cremophor EL (CrEL) is a formulation vehicle used for various poorly-water soluble drugs, including the anticancer agent paclitaxel (Taxol). In contrast to earlier reports, CrEL is not an inert vehicle but exerts a range of biological effects, some of which have important clinical implications. Its use has been associated with severe anaphylactoid hypersensitivity reactions, hyperlipidaemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy. The pharmacokinetic behaviour of CrEL is dose independent, although its clearance is highly influenced by infusion duration. This is particularly important since CrEL can affect the disposition of various drugs by changing the unbound drug concentration through micellar encapsulation. In addition, it has been shown that CrEL, as an integral component of paclitaxel chemotherapy, modifies the toxicity profile of certain anticancer agents given concomitantly by mechanisms other than kinetic interference. A clear understanding of the biological and pharmacological role of CrEL is essential to help oncologists to avoid the pitfalls that come with the use of paclitaxel or other agents using this vehicle substance. With the present development of various new anticancer agents, it is recommended that alternative formulation approaches should be pursued to allow better control of toxicity and pharmacological interactions related to the use of CrEL.

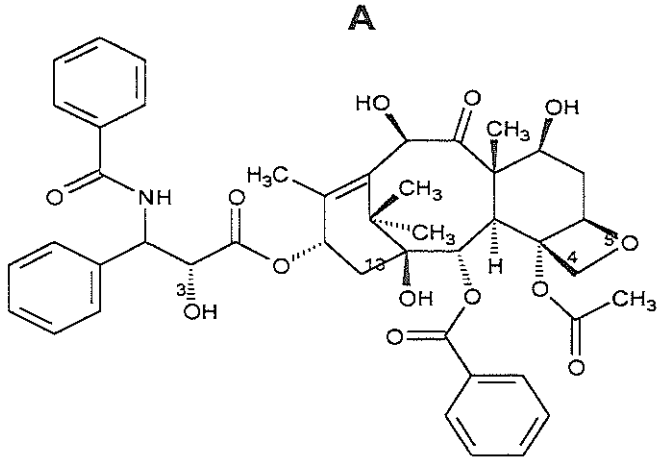
INTRODUCTION

The choice of a suitable pharmaceutical formulation is an essential step in anticancer drug development. This development starts with acquisition of the chemical entity from either natural sources or entirely synthetic routes. Subsequently, the compound is screened for cytotoxic activity *in vitro* and *in vivo*. Once the screening process has been completed the compound should be properly pharmaceutically formulated and produced before entering animal toxicology and pharmacokinetic studies and subsequently human phase I, II and III studies [1,2]. In our opinion, pharmaceutical formulation is a seriously underrated aspect of anticancer drug development.

With only a few exceptions, most new anticancer compounds are initially developed for intravenous (i.v.) use despite some drawbacks such as morbidity of gaining i.v. access, risk of i.v. catheter related infection, thrombosis and extravasation, and patients' preference for oral therapy when equally effective [3]. Important reasons for choosing i.v. use for initial drug development are the fact that usually less gastrointestinal toxicity occurs, there is immediate 100% bioavailability and instantaneous pharmacodynamic effects and there is a possibility to modify the dosing rate or even halt the infusion if needed. Solubility of the compound is a specific demand for i.v. administration, even so for the newer chemotherapeutic agents which are known to be poorly water-soluble. Classical solubility approaches, which will be discussed subsequently, include the use colloidal systems, prodrug development, or solubilization techniques.

Colloidal systems such as liposomes, microcapsules, microspheres, nanoparticles or macromolecule complexes may protect the anticancer drug from premature degradation or

(chemical) inactivation within the systemic circulation. Prodrugs are inactive derivatives that release the active drug following spontaneous degradation or enzymatic reactions. Solubilization is the process of uptake of drugs through complex formation into e.g. oligomers of dextrose and fatty acids, through co-solvent systems (such as ethanol, polyethyleneglycol and glycerol), or through surfactant systems. The surfactant systems consist of either amphoteric compounds (e.g. lecithin or gelatin), ionic surfactants (e.g. sodium palmitate) or nonionic surfactants [e.g. Tween 80 and Cremophor EL (CrEL)] [4,5]. This review focuses on biological and pharmacological properties of CrEL, the formulation vehicle of various hydrophobic drugs, including the anticancer agent paclitaxel.



B

CH_2-O-(CH_2-CH_2-O)$_x$-CO-O-(CH_2)$_7$-CH=CH-CH_2-CHOH-(CH_2)$_5$-CH_3

HC -O-(CH_2-CH_2-O)$_y$-CO-O-(CH_2)$_7$-CH=CH-CH_2-CHOH-(CH_2)$_5$-CH_3

CH_2-O-(CH_2-CH_2-O)$_z$-CO-O-(CH_2)$_7$-CH=CH-CH_2-CHOH-(CH_2)$_5$-CH_3

(x + y + z ~ 35)

Figure 1 Chemical structures of paclitaxel (A) of CrEL (polyoxyethyleneglycerol triricinoleate 35) (B).

PACLITAXEL FORMULATION

After the identification of paclitaxel as the active ingredient in crude ethanolic extracts of the bark of the Western Yew tree, *Taxus brevifolia*, against several murine tumors [6], the development of the drug was suspended for more than a decade due to problems in drug solubilization. Paclitaxel is insoluble in water (less than 0.03 mg/mL), slightly soluble in octanol, propylene glycol and butanol, soluble in CrEL, ethanol, methanol, chloroform, acetone and ether, and freely soluble in dimethyl acetamide. The formulation approach using 50% CrEL and 50% dehydrated ethanol USP was chosen for further development [7]. The pharmaceutical formulation of paclitaxel (Taxol; Bristol-Myers Squibb) contains 30 mg paclitaxel dissolved in 5 mL of this (1:1, v/v) mixture.

The heterogeneous non-ionic surfactant CrEL is a white to off-white viscous liquid with an approximate molecular weight of ~3 kDa and a specific gravity (25°C/25°C) of 1.05-1.06, and it is produced by the reaction of castor oil with ethylene oxide at a molar ratio of 1:35 [4]. Castor oil is a colorless or pale yellow fixed oil obtained from the seeds of *Ricinus communis*, with an extremely high viscosity, and consists mainly of the glycerides of ricinoleic, isoricinoleic, stearic and dihydroxystearic acids. CrEL is usually of highly variable composition, with the major component identified as oxylated triglycerides of rinoleic acid (i.e. polyoxyethylene glycerol triricinoleate 35) (Fig. 1). Polyvinyl chloride (PVC)-free equipment for CrEL administration is obligatory, since CrEL is known to leach plasticizers from PVC infusion bags and polyethylene-lined tubing sets which can cause severe hepatic toxicity [5].

Table 1 Examples of surfactant systems using CrEL

Agent	Therapeutic class	Amount administered*
Aplidine	antineoplastics	~1.5 ml
C8KC	photosensitizers	5.5 ml
Clanfenur	antineoplastics	10.3 ml
Cyclosporin A	immunosuppressives	3.5 ml
Diazepam	sedatives	1.5 ml
Didemnin B	antineoplastics	2.0 ml
Paclitaxel	antineoplastics	25.8 ml
Propofol	anesthetics	~7.0 ml
Teniposide	antineoplastics	1.5 ml

* For an average patient for a single administration of dose

CrEL is being used as a vehicle for the solubilization of a wide variety of hydrophobic drugs, including anaesthetics, photosensitizers, sedatives, immunosuppressive agents and (experimental) anticancer drugs (Table 1). The amount of CrEL administered with these

drugs averages 5 ml (range, 1.5-10.3 ml), although paclitaxel is an exception as the amount of CrEL is much higher per administration, about 26 mL. Therefore, it is important to understand the biological and pharmacological behaviour of CrEL, especially in the formulation of paclitaxel.

BIOLOGICAL EFFECTS OF CREMOPHOR EL

Anaphylactic hypersensitivity reactions

The most well known biological effect of paclitaxel formulated with CrEL is a clinical acute hypersensitivity reaction, characterized by dyspnea, flushing, rash, chest pain, tachycardia, hypotension, angioedema, and generalized urticaria. Despite premedication, consisting of high-dose corticosteroids, H_1 and H_2 antagonists, minor reactions (flushing and rash) still occur in 41-44% of all patients and major, potentially life-threatening, reactions in 1.5-3% [8-10]. Mostly, the hypersensitivity reaction occurs within the first two courses of paclitaxel and can be prevented by reducing the infusion rate. Various (pre)clinical observations, discussed below, point to CrEL as the main contributor to the hypersensitivity reactions:

(a) Using an elegant series of *in vitro* studies, complement activation by CrEL was found to cause hypersensitivity related to paclitaxel chemotherapy [11], as well as to other drugs using CrEL as a vehicle [12,13]. Recently, it was shown that CrEL-induced complement activation in human serum was clearly concentration dependent with a minimum activating CrEL level in the order of 2 μL/mL, a concentration readily achieved clinically in plasma following standard doses of paclitaxel [14].

(b) Histamine release in dogs by CrEL was mainly caused by one of its (minor) constituents, viz. oleic acid [15], whereas cardiac toxicity attributed to paclitaxel, mainly asymptomatic rhythm disturbances, might also be caused by CrEL through a mechanism of histamine release [16].

(c) Improper mixing of high-dose cyclosporin A infusions caused (nonsolubilized) CrEL to sink to the bottom of vials, producing anaphylactoid responses because of highly concentrated CrEL at initial i.v. bolus [17]. All patients allergic to i.v. cyclosporin A, tolerated the CrEL-free oral formulation [18].

(d) Finally, the fact that CrEL concentrations are lower with prolonged paclitaxel infusion schemes (see below) may be an explanation for the lower incidence of hypersensitivity reactions with these schemes. Collectively, these findings indicate that CrEL plays a crucial role in the occurrence of hypersensitivity reactions of paclitaxel and other drugs using CrEL as a formulation vehicle.

Lipoprotein patterns and hyperlipidemia

Lipoprotein alterations accompanying administration of miconazole formulated with CrEL were reported as early as 1977 by Bagnarello et al [19]. Later, CrEL was found to alter the buoyant density of HDL [20] and shift the electrophoretic and density gradient HDL to LDL [21-23]. These authors showed that paclitaxel had a strong affinity for the serum lipoprotein dissociation products, potentially affecting the biodistribution and clearance of the drug. High

concentrations of CrEL may also cause hyperlipidaemia, possibly resulting in Roulaux formation of erythrocytes and a change in shape of leukocytes in blood smears [24]. This suggests that manual blood-cell count analysis is warranted after administration of preparations containing CrEL. Whether the observed hyperlipidaemia after CrEL administration causes a risk for vascular accidents is as yet unknown.

Neurotoxicity

Axonal degeneration and demyelination, one of the principal side effects of paclitaxel resulting in peripheral neuropathy, is supposedly also a biological effect caused by CrEL. The plasma levels of CrEL achieved after therapeutic doses of paclitaxel and i.v. cyclosporin A (also formulated in CrEL), have been shown to produce axonal swelling, vesicular degeneration and demyelination in rat dorsal ganglion neurons [25]. Interestingly, in rats treated with CrEL-free [^{3}H]paclitaxel, paclitaxel was not detectable in the peripheral nervous system, indicating that the anticancer drug itself might not be responsible for the observed toxicity [26]. The hypothesis of CrEL induced neurotoxicity is further supported by the fact that i.v. cyclosporin A causes neurotoxicity in approximately 25% of patients [27], while this side effect is rarely seen with oral administration. This is also consistent with a previous finding that CrEL is not absorbed when given orally as a result of intestinal degradation [28]. It is also noteworthy that neurological symptoms are 10-times less common after treatment with docetaxel, a semisynthetic taxane chemically similar to paclitaxel, as compared to paclitaxel [29]. Unlike paclitaxel, docetaxel is formulated in Tween 80 (i.e., polyoxyethylenesorbitan monooletate), rendering CrEL again suspected for the clinical observations of neuropathy. Neurotoxic properties of CrEL are most likely induced by residual unsaturated fatty acids, possibly due to the appearance of peroxydation products [30]. Therefore, it is suggested that the ethoxylated derivatives of castor oil account for most of the neuronal damage observed [31].

Reversal of P-glycoprotein activity

In the early 1990's several groups independently observed that CrEL was able *in vitro* to modulate the activity of P-glycoprotein, a drug-transporting membrane protein elevated in tumor cells having a multidrug resistance phenotype [32-35]. More recently, similar phenomena have also been described for various other nonionic surfactants, including Tween 80 [36], Solutol HS 15 [37] and Triton X-100 [38]. Surprisingly, however, multidrug resistance has never been successfully modified *in vivo* by any nonionic surfactant, including CrEL [39-41]. A possible explanation for this lack of *in vivo* efficacy is the extremely low volume of distribution of CrEL, approximately equal to the volume of the blood compartment, suggesting that concentrations necessary to affect reversal of multidrug resistance *in vitro* are probably not attained *in vivo* in (solid) tumors [42]. Recent pharmacokinetic experiments conducted in *mdr1a* P-glycoprotein knockout mice support this lack of efficacy, despite high peak plasma levels of CrEL [28]. In contrast to treatment in solid tumors, the pharmacokinetic selectivity of CrEL for the central blood/bone marrow compartment can be an advantage to

treatment of hematological malignancies, in which the expression of P-glycoprotein is known as a principal factor contributing to resistance to chemotherapy [43].

In vitro cytotoxicity

Cytotoxic properties of CrEL in doxorubicin-resistant human breast-cancer cell lines were first reported by Fjällskog et al [44], and confirmed in various human tumor samples [45,46]. It was postulated that formation of free radicals by peroxidation of polyunsaturated fatty acids and/or a direct perturbing effect in the cell membrane causing fluidity and leakage are possible mechanisms contributing to this type of cytotoxicity [47-49]. Using clonogenic assays, however, it has been demonstrated that CrEL can antagonize the cytotoxicity of paclitaxel by a cell-cycle block that inhibit the process of cytokinesis [50]. Thus, although CrEL in itself might have some potential to affect cell survival, the concentrations required to modulate cell growth will also change paclitaxel-mediated (and overall) cytotoxicity. In addition, the pharmacokinetic selectivity of CrEL most likely precludes any vehicle-mediated change in (solid) tumor cell kill *in vivo*.

PHARMACOKINETICS OF CREL

Analytical methods

In view of the contribution of CrEL to clinically observed effects, and to enable further assessment of the impact of its use on paclitaxel pharmacology, the kinetic behaviour of CrEL has been studied extensively in recent years. For this purpose, a variety of analytical methods has been developed. The first assay developed for measurement of CrEL concentrations in plasma was based on the ability of CrEL to modulate daunorubicin efflux in multi-drug resistant T-cell leukemia VLB_{100} cells [51]. Later, a more sensitive and reliable method was developed, which required only microvolumes (20 μL) of plasma [52]. This method is based on measurement of ricinoleic acid after saponification of CrEL followed by precolumn derivatization and reversed-phase high-performance liquid chromatography. Because of the high costs, and time consuming nature of both assays a new method, based on a selective binding of CrEL to the Coomassie brilliant blue G-250 dye in protein-free extracts, was developed [53,54]. Most recently, an electrochemical detection method was developed [55]. Large-scale pharmacokinetic studies have only recently been possible with the development of these newer methods.

CrEL Disposition

Clinical pharmacokinetic studies with CrEL following 3-h paclitaxel infusions indicate a dose-independent behaviour with a terminal disposition half life of approximately 80 h, with a large range depending on the method used for measurement [56,57]. Interestingly, with prolongation of the infusion duration from 1 to 3 and to 24 h, the CrEL clearance increased from about 160 to 300 to 400 $mL/h/m^2$, respectively [14] (Fig. 2). It thus appears that CrEL shows a linear and dose independent but schedule dependent pharmacokinetic behaviour, possibly related to saturation of serum esterase-mediated metabolic degradation. This

schedule dependency leads to an increase in systemic exposure, and thus an increase of the possible CrEL related biological (side-)effects, with shortening of the infusion duration. An example of this phenomenon is the higher risk of allergic reactions in 1-h versus 3-h or 24-h infusions of paclitaxel.

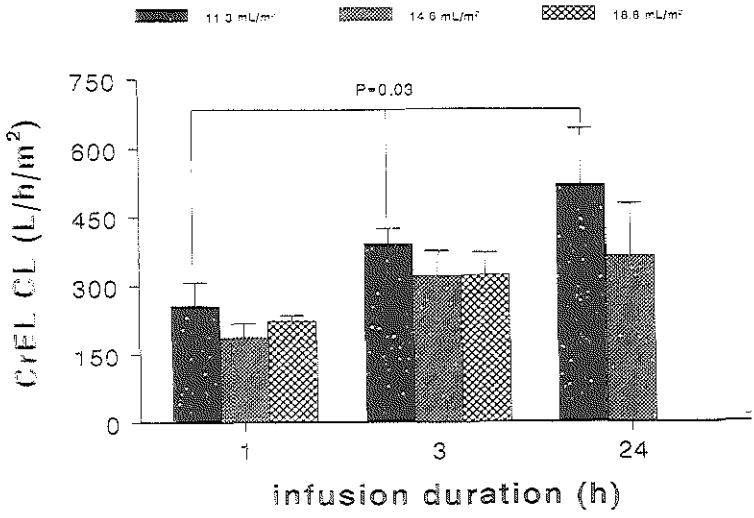


Figure 2 Schedule-dependency in CrEL clearance as a function of infusion duration in cancer patients treated with paclitaxel at 135 (CrEL;11.3 ml/m^2), 175 (CrEL;14.6 ml/m^2) and 225 (CrEL;18.8 m/m^2) mg/m^2 [14].

As mentioned earlier, the volume of distribution of CrEL is extremely low, implying that the tissue (and tumor) delivery of CrEL is probably insignificant. This is in line with observations that CrEL levels in normal and tumor tissue were not detectable in mice [58].

Not much is known about the elimination routes of CrEL. CrEL may be largely degraded in the blood compartment by serum carboxylesterase-induced degradation, similar to that described for Tween 80 [59], causing gradual release of the ricinoleic acid residues attached to the triglyceride structure. It has been shown that hepatobiliary elimination of CrEL is a minor elimination pathway [60]. In addition, the urinary excretion of CrEL accounted for less than 0.1% of the administered dose, in spite of its relatively hydrophilic nature [61].

Effects of CrEL on Drug Disposition

Various studies have shown that CrEL alters the pharmacokinetics of many drugs including cyclosporin A, etoposide, doxorubicin, a number of photosensitizers (e.g., C8KC) and paclitaxel. Initially the effect of CrEL on the disposition of paclitaxel was studied in mice that received the drug by i.v. injection at dose levels of 2, 10 and 20 mg/kg in the presence of various amounts of CrEL [58] (Fig. 3). The paclitaxel clearance of 2.4 L/h/kg at the lowest dose level was reduced to 0.33 and 0.15 L/h/kg at the 10 and 20 mg/kg dose levels.

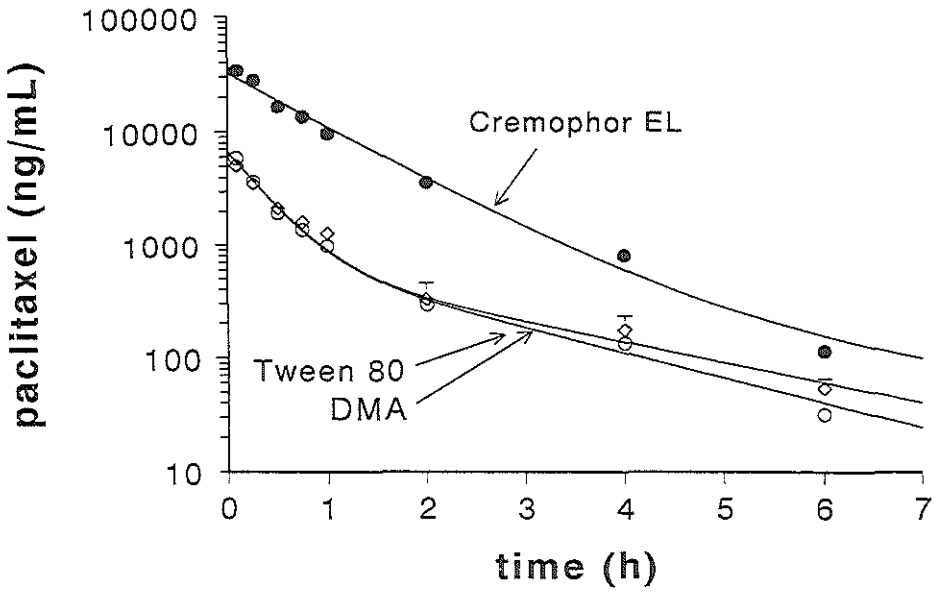


Figure 3 Effect of formulation vehicle [CrEL, Tween 80 or dimethylacetamide (DMA)] on paclitaxel concentration in female FVB mice receiving paclitaxel at a dose of 10 mg/kg [58].

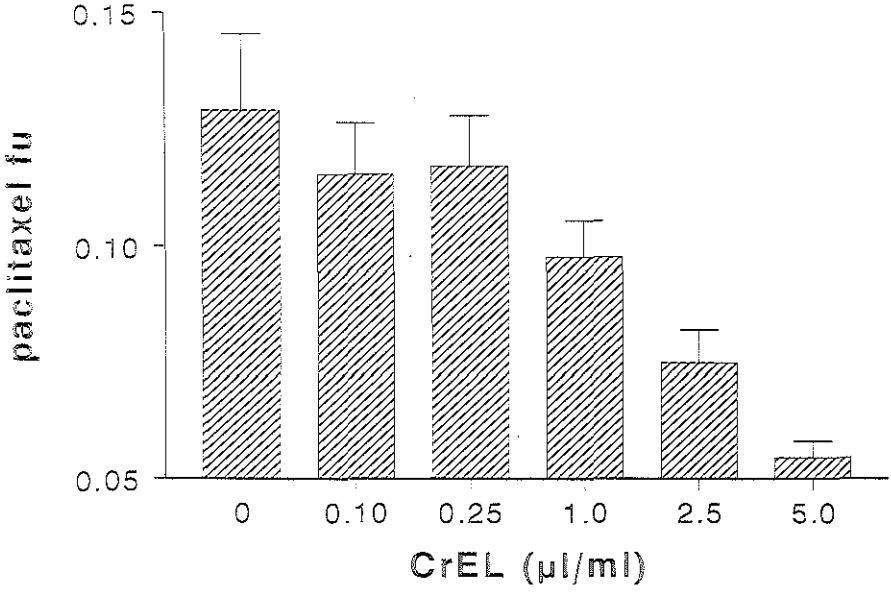


Figure 4 Effect of CrEL concentration on the fraction unbound paclitaxel (fu) in human plasma [56].

It was also shown that the AUC of paclitaxel is higher when it is formulated in CrEL compared to formulation in Tween 80, suggesting that CrEL is responsible for the nonlinearity of paclitaxel disposition. Despite the fact that much higher plasma levels of paclitaxel are reached when given in the CrEL-containing formulation, the tissue levels of paclitaxel were essentially similar with all tested preparations, indicating that the profound influence of CrEL is only taking place in the central blood compartment. In subsequent years, numerous causes of this apparent nonlinear pharmacokinetic behaviour were proposed. It has been suggested that CrEL might interfere with P-glycoprotein-mediated biliary secretion, thereby reducing paclitaxel elimination [62]. In the isolated perfused rat liver, CrEL inhibited the hepatic elimination of paclitaxel, primarily preventing the drug from reaching the sites of metabolism and excretion [63]. However, recent studies indicate that drug-transporting P-glycoproteins are not essential *per se* for normal hepatobiliary secretion of paclitaxel [28,64], and that, as discussed, the disposition of CrEL itself limits the potential to modulate P-glycoprotein activity *in vivo* [42].

More recently, it has been proposed that the effect of CrEL on paclitaxel pharmacokinetics is associated with encapsulation of the drug within CrEL micelles, causing (concentration-dependent) changes in cellular partitioning and blood:plasma concentration ratios of paclitaxel [64] (Fig. 4). It was shown that the affinity of paclitaxel was (in decreasing order) CrEL>plasma>human serum albumin, with CrEL present above the critical micellar concentration (i.e., ~0.01%). Since this effect was also observed in the absence of plasma proteins, it could not have been caused by altered protein binding or by an increased affinity of paclitaxel for protein dissociation products that are produced by the action of CrEL on native lipoproteins [22]. These findings are consistent with the hypothesis that paclitaxel can be entrapped within micelles (composed primarily of polyethyleneglycerol triricinoleate) and that these micelles act as the principal carrier of paclitaxel in the systemic circulation. The percentage of total paclitaxel trapped in micelles increases disproportionally with higher doses of CrEL administered.

The hypothesis that the nonlinear pharmacokinetics of paclitaxel is related to time-varying CrEL concentrations was recently confirmed in a group of cancer patients all receiving increasing doses of 135, 175 and 225 mg/m^2 [65]. Again, the plasma clearance of paclitaxel turned out to be dose-dependent with the slowest clearance at the highest dose level (Fig. 5). In line with the *in vitro* data the nonlinear disposition of paclitaxel in plasma appeared to be an artifact caused by dose related levels of CrEL in blood [65]. Therefore, the non-micellar bound or unbound fraction of paclitaxel in plasma might be a better pharmacokinetic parameter to predict toxicity and to guide dosing of paclitaxel, since it is generally acknowledged that the unbound fraction of a drug is capable of diffusing across biological barriers and interact with essential structures in (tumour) tissues [66].

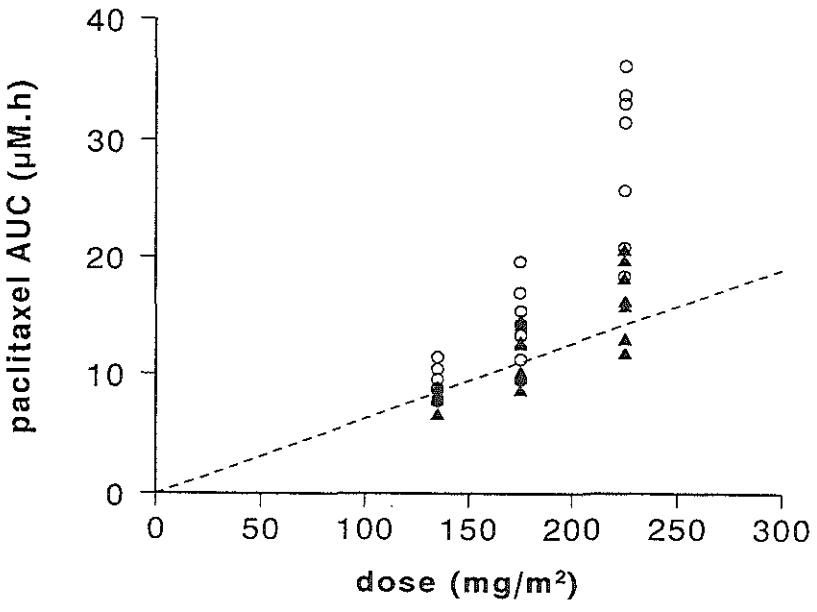


Figure 5 Effect of dose on paclitaxel plasma (circles) and whole blood AUC (triangles) in cancer patients treated with paclitaxel at 3 different dose levels [65].
Please note that: the higher the AUC, the slower the clearance of a drug

Effects of CrEL on Anticancer Drug Pharmacodynamics

Besides its effect on (anticancer) drug disposition, the use of CrEL has also been associated with alterations in pharmacodynamic outcome of a number of drugs co-administered with paclitaxel. For example, clinical combination chemotherapy studies with paclitaxel and cisplatin revealed important sequence-dependent differences in toxicity, with less myelotoxicity when the taxane was given before cisplatin, that could not be linked to consistent changes in pharmacokinetics [67,68]. Interestingly, no toxicity- or pharmacokinetic-sequence interaction was seen in the combination of carboplatin and paclitaxel [69]. One of the mechanisms underlying these clinically important interactions appears to be related to a selective inhibition of cisplatin accumulation in peripheral blood and bone marrow cells by CrEL, without affecting antitumor activity [70-72] (Fig 6). In addition, it has been demonstrated that CrEL markedly reduced the level of serum haematopoietic inhibitory activity, which resulted in a decrease in femoral bone marrow cellularity and upregulated B and T cells, and transiently elevated the incidence of both primitive and committed haematopoietic progenitor cells within several hours after injection [73]. Taken together, these findings suggest that the unusual clinical observations noted with cisplatin-paclitaxel combination therapy are due to a (myelo)protective interaction of cisplatin by CrEL. Since CrEL doses of up to 30 ml/m^2 as a 3-h infusion can be safely administered [74], it is anticipated that an improvement in the therapeutic window for cisplatin could be

obtained by re-formulation of this agent with CrEL. This concept is currently under further investigation (HG, manuscript submitted).

In contrast to the potential benefits obtained from the use of CrEL in the case of cisplatin chemotherapy, combination studies conducted with the combination of paclitaxel and doxorubicin have shown that CrEL can be linked to clinically important kinetic and dynamic interactions that greatly impact on overall toxicity profiles. Two independent studies have shown profound decreases in doxorubicin clearance, particularly when paclitaxel was given prior to the anthracycline [62,75], resulting in substantially worse mucositis and haematological toxicity. Therefore, it is advised to administer doxorubicin first in this combination. In addition, it is expected that even minor modifications in infusion duration or dose may lead to unpredictable pharmacodynamic consequences. Similar interaction phenomena with CrEL have also been described for etoposide, epirubicin, and the irinotecan metabolite SN-38 [76]. Clearly, the magnitude of these interactions depend largely on the combination drug involved, the paclitaxel and CrEL dose administered, and the infusion duration and administration sequence applied for the combined agents.

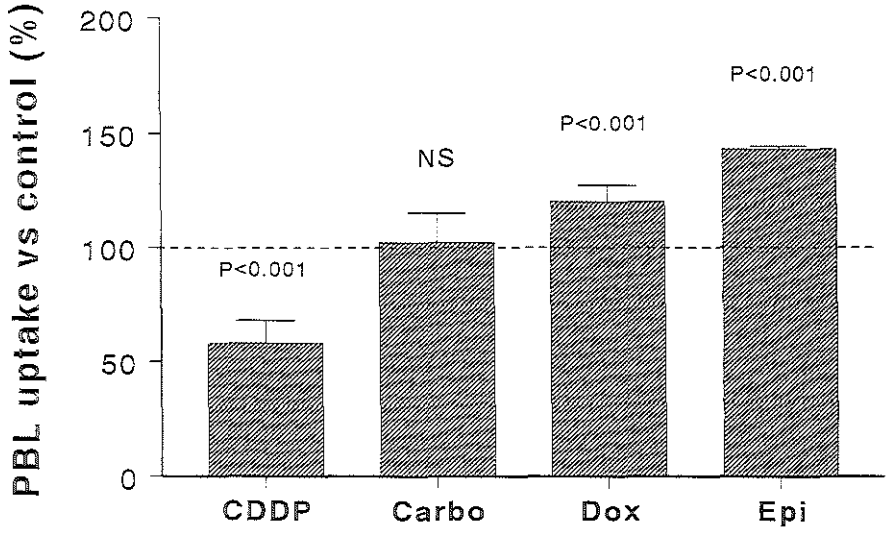


Figure 6 Influence of CrEL on anticancer drug pharmacodynamics. Bars indicate the amount of drug accumulated in peripheral blood leukocytes (expressed as percentage relative to a 100% control) for 2-h incubations with cisplatin (CDDP), carboplatin (Carbo), doxorubicin (dox), and epirubicin (Epi) [(70), unpublished data].

CONCLUSION AND FUTURE PERSPECTIVES

Recent investigations have revealed that CrEL, a widely used formulation vehicle, is a biologically and pharmacologically active ingredient of various commercially available drugs. For example, in case of the anticancer agent paclitaxel, an exceptionally large amount of CrEL is inevitably co-administered with i.v. infusions, causing important biological events that can lead to serious acute hypersensitivity reactions and neurological toxicity, depending on the dose and infusion duration of the administration. In addition, the substantial effects of CrEL on the disposition of other co-administered drugs (e.g. anthracyclines) with a narrow therapeutic window in poly-chemotherapeutic regimens also can be potentially hazardous to the patient. Paradoxically, the *in vitro* and *in vivo* observations of myeloprotective effects related to the use of CrEL in combination with some agents, such as cisplatin, might be exploited further to re-formulate these agents with CrEL in order to achieve an optimisation of their therapeutic window.

In view of inherent problems associated with the use of CrEL, it can be anticipated that therapeutic advantage could be obtained from paclitaxel formulations in which CrEL is absent. Such new formulation approaches should clearly be persued to allow better control of systemic (CrEL-mediated) toxicity and pharmacokinetic interactions observed with numerous agents given in combination with the taxane. Obviously, alternative paclitaxel formulations should allow the drug to be delivered at adequate doses, and the preparation should be stable for several hours to allow handling in the clinical setting. Currently, a large variety of new (CrEL-free) formulation vehicles for paclitaxel are in (pre)clinical development, including co-solvent systems (Tween 80/ethanol/Pluronic L64), water-soluble polymers (e.g., polyethylene glycols), emulsions (e.g., triacetin), liposomes, cyclodextrines, nanocapsules and microspheres (reviewed in [76]). Although one of these preparations might eventually replace the current paclitaxel formulation, for the coming years we will have to cope with the drawbacks of CrEL in the clinical setting. It is also important to keep in mind that CrEL is widely used not only by oncologists, but also by anaesthesiologists and in transplantation medicine, and that few physicians are aware of the biological and pharmacological activity of the compound. For this moment, understanding this activity is one step. On the other hand, it is essential to find other ways to solubilize the drugs formulated in CrEL and, in some cases, to explore potential positive effects of this castor oil derivative. A proper understanding of all biological effects of the formulation would presumably have avoided the major delays in paclitaxel development. This underscores the importance of the choice of a vehicle in drug development in general.

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Chapter 12

Disposition of [G-^{3}H]paclitaxel and Cremophor EL in a patient with severely impaired renal function

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Drug Metab Disposition 27: 1300-1305, 1999

ABSTRACT

In the present work, we studied the pharmacokinetics and metabolic disposition of [G-3H]-paclitaxel in a female patient with recurrent ovarian cancer and severe renal impairment (creatinine clearance: ~20 mL/min) due to chronic hypertension and prior cisplatin treatment. During six 3-weekly courses of paclitaxel at a dose level of 157.5 mg/m^2 (*viz.* a 10% dose reduction), the renal function remained stable. Pharmacokinetic evaluation revealed a reproducible and surprisingly high paclitaxel plasma AUC of 26.0±1.11 μM.h (mean±SD; *n*=6; CV=4.29%), and a terminal disposition half-life of ~29 hrs. Both parameters are substantially increased (~1.5-fold) as compared to kinetic data obtained from patients with normal renal function. The cumulative urinary excretion of the parent drug was consistently low and averaged 1.58±0.417% (±SD) of the dose. Total fecal excretion (measured in 1 course) was 52.9% of the delivered radioactivity, and mainly comprised known mono- and dihydroxylated metabolites, with unchanged paclitaxel accounting for only 6.18%. The plasma AUC of the paclitaxel vehicle Cremophor EL, which can profoundly alter the kinetics of paclitaxel, was 114.9±5.39 μL.h/mL and not different from historic data in patients with normal or mild renal dysfunction. Urinary excretion of Cremophor EL was less than 0.1% of the total amount administered. These data indicate that the substantial increase in systemic exposure of the patient to paclitaxel relates to decreased renal metabolism and/or urinary elimination of polar radioactive species, most likely lacking an intact taxane ring fragment.

INTRODUCTION

The antineoplastic agent paclitaxel has been known as a highly effective chemotherapeutic agent in platinum-refractory ovarian carcinoma since 1989 [1-3]. Fifteen to thirty percent of patients with cisplatin-resistant disease respond to paclitaxel treatment, and in up to 7% of the cases complete remissions can be achieved. These response rates are even higher in patients with tumors still sensitive to platinum-containing chemotherapy. Treatment with paclitaxel at a dose level of 175 mg/m^2 infused over 3 hrs once every 3 weeks is a widely accepted and studied regimen in this situation [2].

The clinical pharmacokinetic behavior of paclitaxel is characterized by a distinct nonlinear disposition profile [4-5], with renal elimination pathways of the parent drug accounting for less than 15% of the dose [6,7]. The primary routes of paclitaxel elimination consist of successive hydroxylation reactions and biliary and intestinal secretion of the parent drug and its metabolic products [8,9]. The major metabolic products identified in humans correspond to 2 monohydroxylated compounds with a hydroxyl function on the α-position at C6 of the taxane ring (6α-hydroxypaclitaxel) or on the *para*-position of the phenyl group at C3' in the C13 side chain (3'-*p*-hydroxypaclitaxel) and 1 dihydroxylated compound (6α,3'-*p*-dihydroxypaclitaxel) [10-12]. The 6α-hydroxylation has been shown to be catalyzed by cytochrome P450 2C8 [13,14], whereas formation of 3'-*p*-hydroxypaclitaxel appears to be dependent on cytochrome P450 3A4 [15,16].

Consistent with the importance of hepatic elimination by the cytochrome P450 family, a recent clinical study with paclitaxel administered to a large group of patients with liver dysfunction showed a substantial increase in the experienced toxicity [17]. In contrast, published pharmacologic data on paclitaxel in adults with renal failure are very limited and available only in abstract form [18-20]. In addition, it is noteworthy that high paclitaxel doses may decrease the creatinine clearance, and that there are no data of patients with sever, predialysis renal impairment treated with paclitaxel. In the present report, we describe the pharmacokinetics of paclitaxel and its formulation vehicle Cremophor EL in a patient with recurrent ovarian carcinoma and severely impaired renal function who was treated with six 3-weekly courses of paclitaxel. In one of the courses, we used [*G*-^{3}H]-paclitaxel, to allow detailed assessment of the elimination routes of paclitaxel and to determine its complete metabolic fate.

PATIENT, MATERIALS AND METHODS

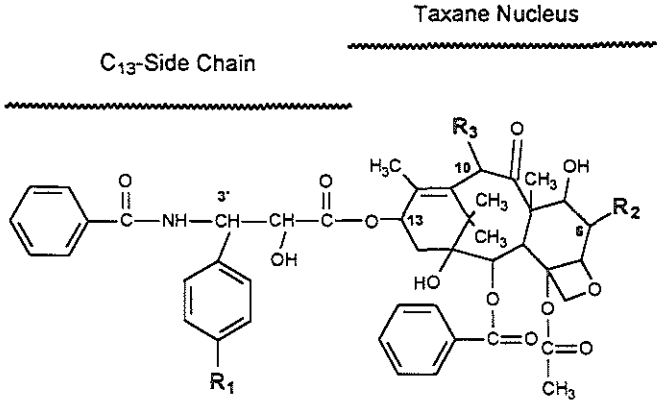
Patient Characteristics and History

The patient studied was a 65-yr old Caucasian female, initially diagnosed at 55 yrs of age with FIGO (i.e. the International Federation of Gynaecology and Obstetrics) stage 3C poorly differentiated serous ovarian carcinoma. She was also known with poorly regulated hypertension and chronic progressive renal insufficiency, presumably due to nephrosclerosis, although a histologic biopsy to prove the diagnosis was never performed. After successful debulking surgery, the patient was treated with 6 cycles of combination chemotherapy consisting of cisplatin and cyclophosphamide. She remained in complete remission for 7 yrs, until December 1996, when there was a local relapse. Second line chemotherapy with carboplatin and cyclophosphamide again induced a complete remission; at that time, the creatinine clearance was 30 mL/min. One and a half years later, the patient again relapsed locally, and was simultaneously diagnosed with a metastasis adjacent to the transverse colon in the upper abdomen. The creatinine clearance was decreased to around 20 mL/min, whereas results for liver function tests were all normal. It was decided to treat the patient with a 3-weekly schedule of paclitaxel at the recommended dose of 175 mg/m^2 *minus* 10% (*viz.* 157.5 mg/m^2), to avoid potential risks related to the critical pre-terminal renal insufficiency. During therapy, the patient did not use any co-medication that might have interfered with paclitaxel disposition. Throughout six courses of treatment, the creatinine clearance remained stable. The courses were very well tolerated without any sign of substantial bone marrow suppression or deterioration of other organ functions. A computer topographic scan performed after three courses showed a partial response, which was sustained after an additional three cycles.

Chemicals

Paclitaxel powder (batch: 484034; purity: 98.3% by reversed-phase HPLC) and commercially available paclitaxel formulated in a mixture of Cremophor EL and dehydrated ethanol USP (1:1, v/v; Taxol) were kindly provided by Bristol-Myers Squibb (Woerden, The

Netherlands). The internal standard for quantitative paclitaxel analysis, docetaxel (batch: 14RPOC92320; purity: 98.0% by reversed-phase HPLC), was obtained from Rhône-Poulenc Rorer (Vitry-sur-Seine Cedex, France). Authentic reference standards for 6α-hydroxypaclitaxel, 3'-*p*-hydroxypaclitaxel, and 6α,3'-*p*-dihydroxypaclitaxel were obtained after isolation and purification of patient fecal samples, as described [11]. Chemical structures of the standards were confirmed by on-line photodiode array detection and fast-atom bombardment ionization/mass spectrometry, with the compounds dissolved in methanol added to a glycerol matrix, using a JMS-SX/SX102A Tandem Mass Spectrometer (Jeol, Tokyo, Japan) with a 6 keV xenon atom beam, and a 10 kV accelerating voltage.



Compound	R_1	R_2	R_3
paclitaxel	**H**	**H**	**Ac**
6α-hydroxypaclitaxel	**H**	**OH**	**Ac**
3'-*p*-hydroxypaclitaxel	**OH**	**H**	**Ac**
6α,3'-*p*-dihydroxypaclitaxel	**OH**	**OH**	**Ac**
baccatin III	**13-OH**	**H**	**H**
10-deacetylbaccatin III	**13-OH**	**H**	**H**

Figure 1. Chemical structures of paclitaxel and its known human metabolites.

Standards of baccatin III (purity: >95.0%) and 10-deacetylbaccatin III (purity: >95.0%) from Taxus baccata were purchased from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands). [*G*-^{3}H]Paclitaxel (batch: 227-163-0024; radiochemical purity: 99.7%) with a specific activity of 2.4 Ci/mmol was supplied by Moravek Biochemicals, Inc. (Brea, CA). The majority of the tritium is in the *m*- and *p*-positions of the aromatic rings, with minor amounts in the 10-, 3'- and 2-position of the taxane ring system (see: Fig. 1). The Cremophor EL reference material was obtained from Sigma Chemical Co. (St. Louis, MO), and Coomassie brilliant blue G-250 was purchased from Bio-Rad Laboratories (Munich, Germany) as a concentrated solution in 85% (w/v) phosphoric acid: 95% (v/v) ethanol (2:1, v/v). All other chemicals and reagents used were of reagent grade or better, and originated from Rathburn (Walkerburn, UK). HPLC-grade water was obtained from a Millipore (Milford, MA) Milli-Q-UF system. Ultima Gold scintillation cocktail was purchased from Packard (Meriden, CT).

Treatment and Sampling Schedule

The patient studied received the courses of paclitaxel at a dose level of 175 mg/m^2 minus 10% (*viz.* 157.5 mg/m^2) by a 3-h i.v. infusion. In the third course, the dosing solution for administration was prepared by adding a stock solution of [*G*-^{3}H]paclitaxel in absolute ethanol USP to unlabeled paclitaxel in Cremophor EL-ethanol (1:1, v/v; 6 mg/mL), and diluting this mixture with an aqueous solution composed of 5.25% (w/v) glucose and 0.9% (w/v) sodium chloride. The final dose solution contained 56.9 ng of [*G*-^{3}H]paclitaxel per mL, 512 μg of unlabeled paclitaxel per mL, and 42.7 μL of Cremophor EL per mL (target dose volume, 308 mL/m^2). Blood samples (~5 mL) for pharmacokinetic studies were obtained during all treatment courses in glass hemogard vacutainer tubes with lyophilized sodium heparin (Becton Dickinson, Meylan, France) as anticoagulant, and were obtained at the following time points: immediately prior to dosing; at 0.5, 1, 1.5, 2, 2.5, and 3 h after start of infusion; and at 5, 15, 30, and 45 min, and 1, 2, 4, 6, 8, 12, and 24 h after end of infusion. Samples were centrifuged at 4000*g* for 5 min (4°C) to yield the plasma fraction, which was stored frozen at -80°C in polypropylene vials (Eppendorf, Hamburg, Germany). Complete urine and feces collections were obtained for up to 5 days, and were stored immediately at -80°C in polystyrene containers. Aliquots of urine samples were diluted in ten volumes of drug-free human plasma to prevent continuing degradation of the analytes [21]. Weighted feces samples were homogenized individually in 10 volumes of water using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of the feces homogenate were diluted with human plasma prior to further sample processing as described above for urine.

Drug Measurement

Paclitaxel concentrations in plasma, urine and feces homogenate were measured by reversed-phase HPLC with UV detection following a single solvent extraction, as described [22]. Radioactivity in urine and triplicate aliquots of feces homogenate was determined by liquid scintillation counting using Ultima Gold scintillation cocktail, with a Wallac System 1400 counter (Turku, Finland). Each sample was pretreated with a 5-fold volume of acetonitrile by

vigorous mixing to remove particulates. Estimates of residual radioactivity in the particulates were determined following digestion with 200 μL sulfuric acid and neutralization of the solubilization mixture with a 25% (v/v) solution of ammonium hydroxide. All samples were counted until a preset time of 20 min was reached, with quench correction performed by external standardization. The analytical procedure for Cremophor EL in plasma was based on a colorimetric binding assay [23], with modifications as described [24], using the Coomassie brilliant blue G-250 dye. Cremophor EL concentrations in urine were determined using a modification of the same assay, using 1-mL samples for clean-up and a calibration curve constructed in drug-free urine over a range of 0.01 to 0.2 μL/mL.

Separation and Identification of Metabolites

Paclitaxel metabolites in unextracted urine (~1 mL) and fecal extracts (corresponding to approximately 100 μg of feces) were separated and quantified by HPLC with UV detection or by liquid scintillation counting of collected fractions. The isocratic HPLC system consisted of a constaMetric 3200 solvent delivery system (LDC Analytical, Riviera Beach, FL), a Waters 717plus autosampling device (Milford, MA), a model SpH99 column oven (Spark Holland, Meppel, The Netherlands), a SpectraPhysics UV-2000 variable wavelength detector (San Jose, CA), and a FRAC-100 fraction collector equipped with a PSV-50 valve (Pharmacia Biotech, Uppsala, Sweden). Analytes were separated on a stainless-steel analytical column (150×4.6 mm, I.D.) packed with a stationary phase of 5 μm Inertsil ODS-80A material (GL Science, Tokyo, Japan), supplied with a Lichrospher 100 PR-18 guard column (4.0×4.0 mm; 5 μm particles). The mobile phase consisted of water-methanol-tetrahydrofuran-ammonium hydroxide (54.5:45:2.5:0.1, v/v/v/v), with the pH adjusted to 6.0 (formic acid). The flow-rate of the mobile phase was set at 1.0 mL/min with detection performed simultaneously at 230 and 254 nm, at a column temperature of 60°C. Effluent fractions (1 mL) were collected, and ^{3}H-labeled metabolites were quantified by liquid scintillation counting. In each case, the recovery of radioactivity from the HPLC column was typically >95%. Mass spectra of isolated compounds were obtained from LC/MS/MS analysis using a Finnigan MAT LCQ™ mass spectrometer (ThermoQuest Co., San Jose, CA) operated with an electrospray ionization (ESI) probe. Samples were introduced into the interface through a heated nebulizer probe (500°C) using nitrogen as nebulizing gas. A discharge voltage of 3.3 kV was applied to the corona discharge needle to produce a discharge current of 5 μA, with a capillary temperature adjusted to 175°C. The tube lens offset voltage was adjusted to +40.00 V to maximize sensitivity by balancing desolvation with fragmentation. MS data were collected over *m/z* 200-1000.

Pharmacokinetic Data Analysis

Plasma concentration *versus* time data were analyzed using the Siphar software package (version 4.0; SIMED, Créteil, France), by determination of slopes and intercepts of the plotted curves with multi-exponential functions. The program determined initial parameter estimates and these were improved using an iterative numerical algorithm based on Powell's method. Model discrimination was assessed by a variety of considerations including visual

inspection of the predicted curves, dispersion of residuals, minimization of the sum of weighted squares residuals, and the Akaike and Schwartz information criteria. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic parameters, including drug disposition half-lives ($t_{1/2}$), area under the plasma concentration-time curve (AUC) from zero to infinity, total plasma clearance (CL) and steady-state volume of distribution ($V_{d,ss}$), using standard equations. The peak plasma concentration (C_{max}) was put on par with the observed drug level at the end of infusion. Statistical evaluation and non-compartmental analysis of Cremophor EL plasma concentration data was performed as described previously [25].

RESULTS

The patient studied in this report was referred to our clinic for treatment of a locally relapsed ovarian carcinoma with a metastasis present adjacent to the transverse colon in the upper abdomen. At the time of study the patient had critical pre-terminal renal insufficiency with a creatinine clearance of around 20 mL/min that remained stable during the subsequent treatment cycles, but had adequate hematopoietic and hepatic function. Clinically, the paclitaxel courses at a reduced dose of 157.5 mg/m^2 were very well tolerated without any sign of substantial bone marrow suppression or deterioration of other organ functions. A computer tomographic scan performed after 3 courses showed a partial response, which was sustained after an additional 3 cycles.

Analytical Methods

To gain a preliminary insight into the composition of the paclitaxel metabolites present in the various biological matrices, samples from the patient were initially analyzed by our HPLC procedure developed for plasma [22]. This method was subsequently modified for analysis in feces homogenates and urine, so that baseline resolution of all the chromatographic peaks observed in samples could be achieved. Using this HPLC system, mean chromatographic run times for known compounds were established using pure reference substances at 3.61 min (10-deacetylbaccatin III), 5.50 min (baccatin III), 15.0 min (6α,3'-*p*-dihydroxypaclitaxel), 18.8 min (3'-*p*-hydroxypaclitaxel), 35.0 min (6α-hydroxypaclitaxel) and 51.8 min (paclitaxel). Structural identification of unknown compounds was based on HPLC data, UV absorption characteristics at 230 nm and mass spectrometry of isolated peaks relative to reference derivatives.

Plasma Disposition

The plasma concentration-time profiles of unchanged paclitaxel were remarkably similar for the 6 consecutive treatment cycles studied. All the profiles were best fitted to a 3-compartmental model after zero-order input using the Powell minimization algorithm and weighted least-squares analysis with a weighting factor of 1/*Y*. The mean plasma pharmacokinetic parameters of paclitaxel, as calculated by this triexponential model are listed in Table 1. Plasma concentrations of paclitaxel decreased rapidly immediately after cessation of the 3-hr infusion (fig. 2A), followed by a more prolonged disposition half-life of

~29 hrs, which is approximately 1.5-fold higher as compared to data reported previously in patients with normal renal function [5]. Similarly, the paclitaxel plasma AUC extrapolated to infinity was very reproducible and achieved surprisingly high values of 26.0±1.11 μM.h (mean±SD).

Table 1. Plasma pharmacokinetic parameters of paclitaxel[a]

Compound	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}	$T_{1/2(z)}$	$V_{d,ss}$	Cl
	μM.h[b]	μM.h[b]	μM[c]	h	liters	liters/h
Paclitaxel						
Mean	20.30	26.18	5.810	23.7	401	11.2
S.D.	1.26	1.25	0.745	5.21	54.5	0.55
c.v.	6.21	4.77	12.8	22.0	13.6	4.91
Cremophor EL						
Mean	56.89	114.9	3.51	27.1	7.07	0.186
S.D.	1.283	5.393	0.170	2.97	0.46	0.009
c.v.	2.25	4.69	4.89	11.0	6.49	4.58

[a] Abbreviations: C_{max}, peak plasma concentration; $t_{1/2(l)}$, half-life of terminal disposition phase; $V_{d,ss}$, steady-state volume of distribution; Cl, total plasma clearance; c.v., coefficient of variation.

[b] μl.h/ml for Cremophor EL

[c] μl/ml for Cremophor EL

Table 2. Elimination kinetics of paclitaxel, total radioactivity and metabolites[a]

Parameter	Course I	Course II	Course III
paclitaxel			
CL_T (L/h)	10.9	10.8	11.8
CL_R (L/h)	0.201	0.214	0.127
CL_{NR} (L/h)	10.7	10.6	11.7
fe_{urine} (%)	1.85	1.79	1.10
fe_{feces} (%)	n.d.	n.d.	6.18
radioactivity			
fe_{urine} (%)	n.d.	n.d.	2.25
fe_{feces} (%)	n.d.	n.d.	52.9
total metabolites			
fe_{urine} (%)	n.d.	n.d.	1.15
fe_{feces} (%)	n.d.	n.d.	46.8

[a] Abbreviations: CL_T, total body clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; *fe*, percent of the absolute paclitaxel dose excreted in urine or feces as unchanged drug, total radioactivity or total paclitaxel metabolites; n.d., not done.

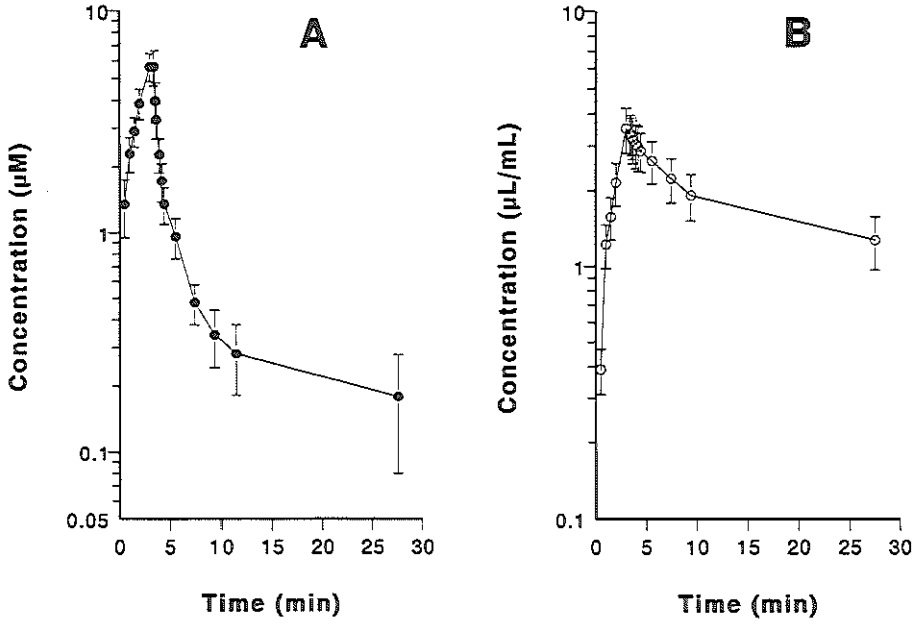


Figure 2. Plasma concentration-time curves of paclitaxel (*panel A*) and of Cremophor EL (*panel B*). Pharmacokinetic data were obtained from a female patient receiving six 3-weekly courses of the drug formulated at 6 mg/mL in a mixture of Cremophor EL-dehydrated ethanol USP (1:1, v/v) at a dose level of 157.5 mg/m^2. Data are presented as mean values (symbols) ± SD (error bars).

Urinary and Fecal Disposition

The urinary excretion pattern, measured in 3 consecutive occasions, was virtually identical in all treatment courses, with 1.58±0.417% of the dose excreted as unchanged drug in the first 24 hrs after drug administration (Table 2). The mean renal clearance of paclitaxel, defined as the product of the dose-fraction excreted unchanged and total body clearance, was 0.181±0.047 L/h, indicating that as much as 98% of the overall clearance could be attributed to nonrenal processes. The total cumulative urinary excretion of radiolabeled compounds afer [G-^{3}H]paclitaxel administration accounted only for 2.25% of the dose, of which 1.15% constituted metabolic products. Reversed-phase HPLC tracings with UV detection and scintillation detection of a urine extract from a urine sample collected during the first 3 hrs after dosing are presented in fig. 3. In addition to the parent drug, trace levels of 10-deacetylbaccatin (MH^+ ion at *m/z* 545) and baccatin III (MH^+ ion at *m/z* 587) could be detected (both accounting to less than 0.01% of the dose), and an unknown prominent radioactive peak early in the solvent front that was reported previously [7,9].

Data of fecal elimination, obtained only during the third treatment course, indicated that 52.9% of the delivered radioactivity was excreted in the first 24 hrs, with unchanged

paclitaxel accounting for only 6.18%. In fecal extracts, 6α-hydroxypaclitaxel (MH^+ ion at *m/z* 870; taxane fragment ion at *m/z* 525) could clearly be distinguished as the predominant species (see fig. 2A). Using reference derivatives, 2 of the additional paclitaxel metabolites could be identified as 6α,3'-*p*-dihydroxypaclitaxel (MH^+ ion at *m/z* 886) and 3'-*p*-hydroxypaclitaxel (MH^+ ion at *m/z* 870; taxane fragment ion at *m/z* 509). The peak labeled 1 in fig. 2A showed a molecular ion at *m/z* 870 and a fragment ion at *m/z* 509, suggesting an unknown metabolite(s) resulting from a single hydroxylation reaction in the C13 side chain. A second unidentified peak (labeled 4 in fig. 2A) had an abundant ion at *m/z* 286 (unmodified C13 side chain) and other characteristic fragments at *m/z* 509, 525, 792 and 810. This metabolite is most likely either 4-deacetylpaclitaxel or 10-deacetylpaclitaxel, resulting from a loss of the acetyl moiety on C4 or C10, respectively, of the taxane nucleus [26,27].

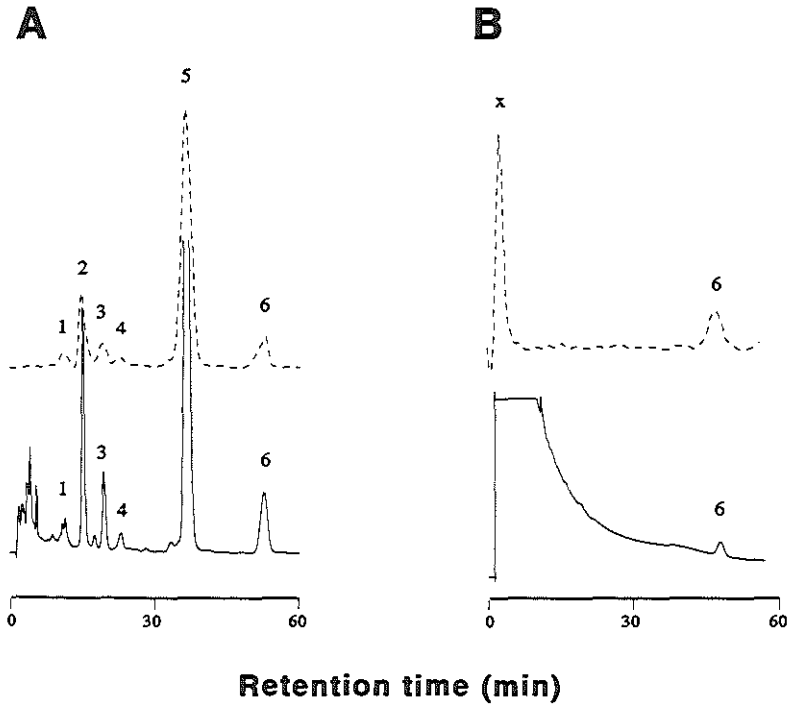


Figure 3. Reversed-phase chromatographic tracing with UV detection (solid lines) and scintillation detection (dashed lines) of a fecal extract (*panel A*) and a urine extract (*panel B*) taken from a samples collected during the first 3 hours after i.v. infusion of paclitaxel. Chromatographic peaks represent: **1**, unknown metabolite; **2**, 6α,3'-*p*-dihydroxypaclitaxel; **3**, 3'-*p*-hydroxy-paclitaxel; **4**, unknown metabolite; **5**, 6α-hydroxypaclitaxel; **6**, paclitaxel (unchanged parent drug).

Cremophor EL Kinetics

Disappearance of the paclitaxel formulation vehicle Cremophor EL from the plasma compartment was characterized by elimination in an apparent biexponential manner. The peak plasma concentrations and AUC values of Cremophor EL in the 3 subsequent cycles, shown in Table 1, were 3.51±0.17 μL/mL (mean±SD) and 114.9±5.39 μL.h/mL, respectively, and are consistent with earlier findings obtained from a large cohort of patients treated with paclitaxel at a similar dose of 150 mg/m^2 [25]. The cumulative urinary excretion of Cremophor EL was very low and accounted for 0.08±0.02% (mean±SD) of the administered dose.

DISCUSSION

The administration of paclitaxel to patients with renal insufficiency has been reported previously in five cases and in all, patients were on (long-term) hemodialysis [18-20,28,29]. Although paclitaxel pharmacokinetics was determined in some of these patients, the lack of fecal and urinary data precluded a complete analysis of paclitaxel disposition. In contrast to these previous investigations, we evaluated paclitaxel plasma pharmacokinetics during 6 sequential evaluated courses. Our patient exhibited a quantitatively distinct kinetic profile of paclitaxel, with paclitaxel AUC values and disposition half-lives in plasma approximately 1.5 to 2-fold higher as compared to those reported in patients with normal renal function [5,6]. This high paclitaxel AUC value, which was sustained over the 6 consecutive courses, justifies a dose reduction of paclitaxel in patients with severe predialysis renal impairment, although, surprisingly, no major (hematological) toxicity was observed in this patient other than mild fatigue.

We have recently shown that Cremophor EL, the formulation vehicle used for i.v. paclitaxel administration, causes a profound concentration-dependent alteration of drug accumulation in erythrocytes by reducing the free drug fraction available for cellular partitioning [30]. This phenomenon is caused by micellar incorporation of paclitaxel in the systemic circulation and results in increased plasma concentrations and 'artificial' nonlinear disposition [31]. Since no data were available of Cremophor EL kinetics in patients with renal failure, we speculated that the increased exposure of our patient to paclitaxel, expressed as the AUC in plasma, might have been caused by alteration of Cremophor EL disposition and elimination. However, involvement of Cremophor EL in the observed kinetic behavior of paclitaxel could eventually be ruled out as the plasma clearance and AUC were comparable with those reported previously in a historic control group of patients with normal renal function on a similar treatment schedule [25]. Consistent with this observation, we found that urinary excretion of Cremophor EL, despite its relatively hydrophilic nature, accounted for only less than 0.1% of the delivered dose in this patient. This suggests that renal excretion of intact Cremophor EL and its major constituent polyoxyethyleneglycerol triricinoleate is not important in the overall elimination of this vehicle substance.

Alternatively, we investigated the possibility that metabolic routes and excretion pathways of paclitaxel itself might have been altered due to the patient's disease state. This was

achieved by the use of radiolabeled paclitaxel in the third treatment course. As demonstrated previously, fecal excretion constituted the main route of excretion, with 52.9% of the administered radioactivity recovered in a 24-h feces collection period. This is in excellent agreement with earlier data of Walle et al [7], who reported that 59.1±7.3% of the total dose was excreted as extractable radioactivity in 5 patients with normal organ functions. In line with this study and with our own work characterizing the main hepatic metabolites in patient feces samples [11], only approximately 6% of the fecal radioactivity was excreted as unchanged paclitaxel. 6α-Hydroxypaclitaxel constituted the major metabolite, with 3'-*p*-hydroxy-paclitaxel and 6α,3'-*p*-dihydroxypaclitaxel both present as minor biotransformation products in addition to 2 unknown compounds. In contrast to fecal data, the cumulative urinary excretion of radiolabeled paclitaxel was significantly different from other published data [7]; the total urinary excretion of ^{3}H-labeled paclitaxel and metabolites accounted for 14.3±1.4% (range, 11.0-18.7%) of the dose, with the parent drug representing 4.5±0.5% (range, 3.3-6.2%) *versus* 2.25% (total radioactivity) and 1.58±0.42% (paclitaxel), respectively, in our patient. These comparative data seem to indicate that renal elimination of paclitaxel and its metabolites, particularly the large unknown polar constituents, which may represent (part of) the C13 side chain, is markedly impaired, and this may have contributed to the altered pharmacokinetic profile observed in plasma.

In conclusion, we have shown altered plasma pharmacokinetics of paclitaxel in a patient with severely impaired renal function, treated at a 10%-reduced dose during 6 consecutive courses. Our findings indicate that the substantial increase in systemic exposure of our patient to paclitaxel most likely relates to decreased renal metabolism and/or urinary excretion of unchanged drug or polar radioactive species. These data point to a more prominent role of the kidneys in paclitaxel disposition than previously thought and suggest that additional studies are required to fully appreciate to what extent renal dysfunction can affect paclitaxel pharmacokinetics and pharmacodynamics.

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Chapter 13

Influence of Cremophor EL on the bioavailability of intraperitoneal paclitaxel

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Clinical Cancer Research, submitted

ABSTRACT

It has been hypothesized that the paclitaxel vehicle Cremophor EL (CrEL) is responsible for nonlinear drug disposition by micellar entrapment. In order to gain further insight into the role of CrEL in taxane pharmacology, we studied paclitaxel pharmacokinetics in the presence and absence of CrEL after i.p. and i.v. dosing. Patients received an i.p. tracer dose of [*G*-^{3}H]paclitaxel in ethanol without CrEL (100 µCi diluted further in isotonic saline) on day 1, i.p. paclitaxel formulated in CrEL (Taxol) (125 mg/m^2) on day 4, and i.v. tracer of [*G*-^{3}H]paclitaxel on day 22, and i.v. Taxol (175 mg/m^2) on day 24. Four patients (age, 54-74 years) were studied, and serial plasma samples up to 72 hours were obtained and analyzed for total radioactivity, paclitaxel and CrEL. In the presence of CrEL, i.v. paclitaxel clearance was 10.2±3.76 L/h/m^2, consistent with previous findings. The terminal disposition half-life was substantially prolonged after i.p. dosing [17.0±11.3 (mean±SD) vs 28.7±8.72 hours], as was the mean residence time (7.28±2.76 vs 40.7±13.8 hours). The bioavailability of paclitaxel was 31.4±5.18%, indicating insignificant systemic concentrations after i.p. treatment. CrEL levels were undetectable after i.p. dosing (<0.05 µL/mL), whereas after i.v. dosing, the mean clearance was 159±58.4 mL/h/m^2, in line with earlier observations. In the absence of CrEL, the bioavailability and systemic concentrations of i.p. paclitaxel were significantly increased. This finding is consistent with the postulated concept that CrEL is largely responsible for the pharmacokinetic advantage for peritoneal cavity exposure to paclitaxel, compared to systemic delivery.

INTRODUCTION

The role of i.p. chemotherapy for tumors principally confined to the peritoneal cavity has been extensively studied [1]. Although it has proven to be a safe procedure with encouraging results, it has not yet become a standard therapeutic procedure [1,2]. The major goal of this therapeutic strategy is to expose tumors within the peritoneal cavity to higher concentrations of antineoplastic agents for longer periods of time than can be achieved with systemic drug administration. I.p. treatment with paclitaxel is particularly attractive in patients with ovarian cancer [3-6], since i.v. paclitaxel has proven single agent activity in ovarian cancer [7,8], and potentially also in other tumor types confined to the abdominal cavity such as peritoneal mesothelioma [9,10]. The antitumor effect is dependent on duration of exposure and on the drug concentration in contact with the tumor, and, in the case of paclitaxel, on the unique pharmacokinetic profile of paclitaxel and its solvent vehicle CrEL^3, a nonionic Castor oil derivative.

It has been demonstrated that at high local concentrations, which can be reached by i.v. and especially by i.p. administration, paclitaxel is entrapped in CrEL micelles [11]. Our hypothesis is that this phenomenon accounts for the prolonged (peritoneal) activity at high concentrations as reported for intravesical treatment [12], and that, hence, paclitaxel distribution will depend on CrEL pharmacokinetics. In order to gain further insight into the role of CrEL in the pharmacokinetic behavior of paclitaxel, we studied paclitaxel disposition in

cancer patients with the drug formulated in the presence and absence of CrEL after i.p. and i.v. dosing.

MATERIALS AND METHODS

Materials and Chemicals

[G-^{3}H]Paclitaxel with a specific activity of 2.4 Ci/mmol was obtained from Moravek Biochemicals, Inc. (Brea, CA). The majority of the tritium is in the m- and p-positions of the aromatic rings, with minor amounts in the 10-, 3'- and 2-position of the taxane ring system. The clinical paclitaxel formulation in CrEL and dehydrated ethanol USP (1:1, v/v; Taxol) and paclitaxel powder were purchased from Bristol-Myers Squibb (Woerden, the Netherlands). The CrEL reference material was obtained from Sigma (St. Louis, MO) and Coomassie brilliant blue G-250 from Bio-Rad Laboratories (Munich, Germany) as a concentrated solution in 85% (w/v) phosphoric acid - 95% (v/v) ethanol (2:1, v/v). All other chemicals and reagents used were of reagent grade or better, and originated from Rathburn (Walkerburn, UK). HPLC-grade water was obtained from a Millipore (Milford, MA) Milli-Q-UF system. Ultima Gold scintillation cocktail was purchased from Packard (Meriden, CT). The internal standard for quantitative paclitaxel analysis, docetaxel, was obtained from Aventis (Vitry-sur-Seine Cedex, France).

Patients

Eligible patients had a histological or cytological verified cancer with tumor morbidity due to localization in the abdominal cavity, for which treatment with paclitaxel was a reasonable option or for which no effective therapy was available. Other inclusion criteria included (i) age at least 18 years; (ii) WHO performance status $\leq$2; (iii) no previous chemotherapy and/or radiotherapy in the past 4 weeks; (iv) adequate hematopoietic function (absolute neutrophil count $\geq$1.5x10^9/L and platelet count $\geq$100x10^9/L); and (v) adequate hepatic function (total serum bilirubin level $\leq$1.25x the upper limit of normal). Specific exclusion criteria included (i) signs of bowel obstruction; and (ii) peripheral neuropathy graded >1 (NCI common toxicity criteria). The study protocol was approved by the medical ethical committee of the Rotterdam Cancer Institute, and all patients gave written informed consent before study entry.

Treatment Plan

All patients received one administration of CrEL-free i.p [G-^{3}H]paclitaxel (100 µCi; approximately 0.1 mg) on day 1, one administration of i.p. (unlabeled) paclitaxel in CrEL-ethanol (Taxol) at a dose of 125 mg/m^2 (based on a previous phase I study [3]) on day 4, followed by one administration of CrEL-free i.v. [G-^{3}H]paclitaxel (100 µCi) on day 22, and one i.v. administration of Taxol at a dose of 175 mg/m^2 on day 25. The i.p. administrations were performed by repeated paracenthesis placed under sterile conditions after sonographic localization, which is a safe and practical method for i.p. chemotherapy [13]. All ascites was drained before each i.p. infusion. Paclitaxel for i.p. dosing, either unlabeled or labeled, was administered in 2 liters of isotonic (0.9%, w/v) sodium chloride, preheated to 37°C. The i.p

infusion was administered in 45 minutes and the i.v. administrations in 3 h, also in a volume of 2 liters of isotonic sodium chloride. Polyvinylchloride-containing infusion systems were avoided since CrEL is known to leach plasticizers from these products. Instead, cellulose acetate filters, with 0.22 μm pore size were used for infusions. Prior to i.v. infusion, patients received premedication of ranitidine (50 mg), clemastine (2 mg), and dexamethasone (10 mg), all 30 min before all drug administrations.

The total dose of radioactivity delivered to the patients and the small dose of non-CrEL bound paclitaxel was non-hazardous and no special extra precautions were necessary [14,15]. During the first 2 hours after starting the i.p. administration of paclitaxel, patients were encouraged to change positions every 15 minutes to maintain adequate distribution throughout the peritoneal cavity.

Clinical Assessment

Before therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell count including WBC differential, and serum biochemistry was performed before treatment, as was disease evaluation. During treatment, patients were seen at least weekly by a physician and complete blood cell count and serum chemistry was performed weekly.

Pharmacologic Analysis

Venous blood samples of 5 mL were obtained in the two i.p. cycles at the following time points: before infusion, at 15, 30, and 40 minutes during instillation, and 5, 15, 30 minutes, and 1, 2, 4, 8, 10, 21, 24, 48 and 72 hours post instillation. For the two i.v. cycles, blood samples were collected at 0.5, 1, 1.5, 2, 2.5 and 2.55 hours during infusion, and 5, 15, 30, and 45 minutes, and 1, 2, 4, 8, 10, and 21 hours post infusion. Samples were collected in tubes containing potassium EDTA as anti-coagulant, and centrifuged to obtain the plasma supernatant (3000×*g* for 10 minutes). Duplicate ascitic samples were obtained in one patient following the second i.p. administration immediately following the end of infusion, and 4, 6, and 18 days after the end of infusion, and in one patients following the second i.v. administration at 1.5, and 3 hours after start of infusion. These samples were obtained using a Medicut 16GA cannula (45 × 1.7 mm internal diameter; Sherwood Medical, Tullamore, Ireland), collected in 4.5-mL polypropylene tubes, after discarding the first 10 mL of fluid, and processed as described above for plasma.

Plasma and ascitic fluid samples were analyzed for the presence of paclitaxel and CrEL by HPLC with UV detection [16] and a colorimetric dye-binding microassay [17], respectively. The handling and measurement of [*G*-^{3}H]paclitaxel was performed according to a protocol approved by the local Radiation Approval Committee of the Rotterdam Cancer Institute as described in detail elsewhere [15]. Briefly, radioactivity was determined by liquid scintillation counting following addition of Ultima Gold scintillation cocktail (Turku, Finland). All samples were counted until a preset time of 20 minutes was reached, with quench correction performed by external standardization.

Concentration-time profiles of paclitaxel were analyzed by noncompartmental modeling using Siphar V4 (InnaPhase, Philadelphia, PA). The AUC was calculated by the linear trapezoidal rule with extrapolation to infinity, and the apparent plasma clearance was calculated as the ratio of AUC and dose delivered (in mg/m^2). The bioavailability (F) for i.p. paclitaxel was calculated as: $F=(AUC_{i.p.}/AUC_{i.v.})\times(dose_{i.v.}/dose_{i.p.})\times100\%$. All pharmacokinetic data represent dose-normalized mean values (for i.p. to 125 mg/m^2 and for i.v. to 175 mg/m^2) with SD. Statistical evaluation was performed with the NCSS package V5.X (J. Hintze, East Kaysville, UT, 1992), using the nonparametric Wilcoxon test for matched pairs. The level of significance was set at $P<0.05$.

Table 1 Patient characteristics

Characteristic	Median (range)
Number of patients	4
Age (years)	64 (54-74)
Sex (Female/Male)	4/0
Weight (kg)	66 (55-72)
Height (cm)	168 (163-173)
BSA (m^2)	1.70 (1.50-1.80)
Tumor type	mesothelioma (2), ovarian carcinoma (2)
Toxicity	only mild gastrointestinal and hematological

Abbreviation: BSA, body-surface area.

RESULTS

Patient Characteristics and Toxicity

Out of 6 eligible patients, 2 patients withdrew consent before the second i.p. infusion of paclitaxel due to rapid progressive disease (1 patient) or due to non-related and unexpected occurrence of psychiatric illness (1 patient). Four patients received the 4 planned administrations of paclitaxel and all were pharmacologically evaluable. The patient characteristics are summarized in Table 1. Overall, the treatment was very well tolerated, with no infusion-related complications, and no grade 3-4 hematological and non-hematological toxicity. Disease stabilization was initially obtained in all 4 patients.

Plasma Pharmacokinetics

The pharmacokinetic data of i.p. and i.v. delivered paclitaxel in the presence and absence of CrEL are shown in Table 2. In the presence of CrEL, the terminal disposition half-life of paclitaxel was substantially prolonged after i.p. dosing as compared to i.v. dosing (28.7±8.72 *vs* 17.0±11.3 hours; P=0.006), as was the mean residence time (40.7±13.8 *vs*

7.28±2.76 hours; P=0.005) (Fig. 1), which may be advantageous for local antitumor effects. The bioavailability of i.p. delivered paclitaxel formulated in CrEL was only 31.4±5.18%, indicating low concentrations within the systemic compartment. However, in the absence of CrEL, the bioavailability (98.8±16.6 *vs* 31.4±5.18%; P=0.005) and systemic exposure (7.55±3.38 *vs* 5.04±1.92 μ*M*.h; P=0.064) of i.p. delivered paclitaxel were significantly increased, which may eventually lead to increases in systemic (hematologic) toxicity.

The systemic CrEL levels were undetectable after i.p. dosing of paclitaxel (<0.05 μL/mL), whereas after i.v. dosing, the mean clearance of CrEL was 159±58.4 mL/h/m^2, in line with previous observations [18,19].

Table 2 Plasma pharmacokinetics of i.p. and i.v. paclitaxel

Parameter	+CrEL(Taxol)	-CrEL	P*
i.p. dosing			
C_{max} (μ*M*)	0.14±0.08	0.26±0.07	0.12
AUC (μ*M*.h)	5.04±1.92	7.55±3.38	0.064
MRT (h)	40.7±13.8	7.28±2.76	0.003
F (%)	31.4±5.18	98.8±16.6	0.005
i.v. dosing			
C_{max} (μ*M*)	5.44±1.76	3.63±1.56	0.054
AUC (μ*M*.h)	22.9±10.0	11.4±6.50	0.011
CL (L/h/m^2)	10.2±3.76	22.8±11.7	0.027

* Wilcoxon test for matched pairs.

Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration versus time curve; MRT, mean residence time; F, bioavailability for i.p. administration.

Asctic Fluid Penetration

Given the relatively high total protein content in ascites of the patients (range, 38 to 45 mg/mL), no correction for protein binding was performed. After i.p. administration in the presence of CrEL, the paclitaxel concentration peaked immediately following the end of instillation at levels >1600-fold higher than concomitant plasma concentrations. Paclitaxel elimination from ascites was extremely slow, with detectable levels (~10.2 ng/mL) even at 18 days after dosing and an apparent disappearance half-life of 135.8 hours. In contrast, measurement of paclitaxel in ascites following i.v. administration with CrEL indicated that less than 1.3% of systemic concentrations were present in ascites, indicating lack of a sink effect.

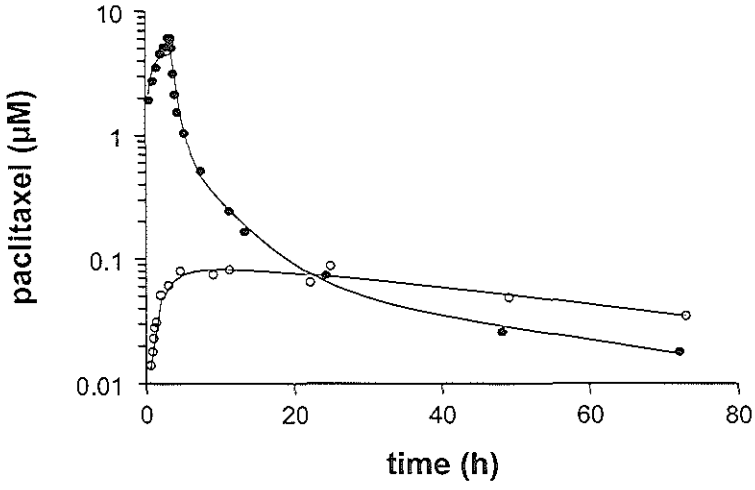

Figure 1 Representative plasma concentration-time profiles of total paclitaxel after i.p. (open symbols) and i.v. (closed symbols) delivery of paclitaxel formulated in a mixture of CrEL-ethanol USP (1:1, v/v).

DISCUSSION

The results of the present study show that the use of CrEL as a formulation vehicle results in a drastic alteration in the pharmacokinetics of paclitaxel after i.p. or i.v. administration. Indeed, the presence of CrEL in the pharmaceutical formulation (Taxol) leads to a substantial decrease in the bioavailability after i.p. drug administration (31.4 *vs* 98.8%). The overall result is a 50% decrease in the paclitaxel plasma AUC after i.p. administration and a 100% increase after i.v. administration.

Previously, the human pharmacokinetics of paclitaxel following i.v. administration has been studied extensively using sensitive and selective HPLC methods [20-22]. Because the normal pharmaceutical formulation already contains significant amounts of CrEL, we have used an i.v. formulation that does not contain this vehicle substance to enable determination of comparative pharmacokinetic profiles. Since paclitaxel without its solvent CrEL is not registered for human use, we were required to use a non-hazardous radiolabeled tracer dose. Thus, we compared therapeutic doses of paclitaxel with CrEL to very small doses of paclitaxel without CrEL and dose-normalized the pharmacokinetic data. Theoretically, this might introduce a methodological flaw, as has been described previously for some agents

[23]. However, the altered disposition of drugs when tracer doses are used as compared to pharmacologic doses has been shown to be caused by the variable degree in saturation of enzymes involved in (hepatic) biotransformation. It has been shown previously, for example, that the use of tracer doses of erythromycin results in aberrant metabolic profiles and therefore in altered drug clearance, and it has been advocated to administer therapeutic doses along with the radiolabeled tracer in those instances [24]. However, it is highly unlikely that this also accounts for the observed differences in paclitaxel pharmacokinetics, because: (*i*) the apparent Michaelis-Menten constant (i.e., K_m) and the maximum-reaction velocity (i.e., V_{max}) values for the principal metabolic route, viz. paclitaxel 6α-hydroxylation, were 5.4±1.0 μM and 30±1.5 nmol/min/nmol cytochrome P-450, respectively, for complementary DNA-expressed cytochrome P-450 isoform 2C8; the values were 4.0±1.0 μM and 0.87±0.06 nmol/min/mg protein, respectively, for human hepatic microsomes [25]. These values are approximately 50 to 100-fold higher than peak concentrations of unbound paclitaxel following therapeutic doses (135 to 225 mg/m^2) in patients receiving 1-, 3-, or 24-hour i.v. infusions [22]; (*ii*) the plasma-protein binding of paclitaxel [26,27], as well as the erythrocyte partitioning of paclitaxel in human samples [11], has been shown previously to be independent of the paclitaxel concentration within the therapeutic range associated with 3-hour i.v. infusions; and (*iii*) no signs of nonlinearity in paclitaxel distribution (unbound [28], whole blood [21], and tissue concentrations [29,30]) and elimination (urine and feces concentrations [21]), have been noted previously.

Following i.p. administration, paclitaxel concentrations in plasma were initially less than those following i.v. administration, and several hours were required for equilibrium to be attained between the peritoneal cavity and the systemic circulation. The limited surface area for paclitaxel diffusion relative to the volumes of fluid, and the fact that the peritoneal fluids are not well stirred, likely contributed to the slow equilibrium kinetics. Nevertheless, concentrations in plasma equivalent to that after i.v. administration were achieved after approximately 20 hours in all patients, and paclitaxel appeared to be more slowly eliminated from the peritoneal cavity than from plasma. This is in keeping with earlier findings, which we now confirm, indicating slow peritoneal clearance of paclitaxel and high peritonea-plasma concentration ratios of >1000 after i.p. drug administration [3]. These authors also documented the persistence of significant peritoneal paclitaxel levels even at 1 week after initial i.p. drug administration, already suggesting very slow peritoneal clearance and continuous exposure of the peritoneal cavity to active concentrations of paclitaxel [4]. Thus, although the described data on paclitaxel accumulation are limited to only four patients, our results are fully in agreement with previous findings and suggest that i.p. administration of paclitaxel can produce significant drug distribution in the peritoneal cavity. In this context, it is of particular interest that cytotoxicity of paclitaxel is a function of the time to drug exposure above a certain threshold concentration [20,31]. The paclitaxel penetration and subsequent accumulation in the peritoneal cavity thus might offer a potential therapeutic advantage in that tumor cells are exposed to high local drug levels for prolonged time periods. In contrast, there appears to be rapid clearance following i.p. paclitaxel administration without CrEL, with bioavailability approaching unity. This suggests a reduced ability of paclitaxel to interact with

tubulin or other essential targets as well as an increased risk of severe (hematologic) toxicity in cancer patients treated with paclitaxel in the absence of CrEL.

We expect that the results presented here for paclitaxel are representative for other poorly water-soluble drugs formulated in this vehicle. This is supported by recent observations that several commonly used anticancer agents, including anthracyclines and epipodophyllotoxins, can be readily incorporated into CrEL micelles [32], thereby strongly affecting the plasma pharmacokinetics [33]. This implies that reformulation of hydrophobic anticancer agents with a vehicle containing CrEL for i.p. treatment might achieve improvement of their therapeutic index.

In conclusion, we have shown that CrEL is mainly responsible for the pharmacokinetic advantage for peritoneal cavity exposure to paclitaxel as compared to systemic delivery and compared to a CrEL-free paclitaxel formulation. These findings provide a rationale for attempts to improve local drug distribution after i.p. administration of other hydrophobic anticancer agents by concomitant administration of CrEL.

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Chapter 14

Comparative pharmacokinetics of unbound paclitaxel during 1- and 3-hour infusions

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SUMMARY

Purpose: The paclitaxel vehicle Cremophor EL (CrEL) profoundly influences the cellular distribution of paclitaxel in human blood *in vitro* by a concentration-dependent reduction of the unbound drug fraction. Since CrEL clearance increases with extending the infusion duration from 3 to 24 hours, we hypothesized that exposure to unbound paclitaxel might also be schedule dependent.

Patients and Methods: CrEL and unbound paclitaxel pharmacokinetics were prospectively analyzed in 29 patients with advanced solid tumors treated with paclitaxel (100 mg/m^2) given as a 1-hour (n=15) or 3-hour (n=14) intravenous infusion.

Results: The systemic exposure (AUC) to CrEL was significantly higher with the 1-hour as compared to the 3-hour schedule (80.2±24.2 *vs* 48.5±24.1 μL.h/mL; P=.002). In contrast, the AUC of unbound paclitaxel was substantially reduced following the 1-hour infusion (0.50±0.10 *vs* 0.62±0.12 μM.h; P=.0085). Similarly, clearance and volume of distribution were significantly dependent on infusion duration (P<.005). A trend was observed toward more severe hematologic toxicity with the 3-hour schedule (P=.053), consistent with increased exposure to unbound drug.

Conclusion: Overall, these findings explain, at least in part, previous observations that short-infusion schedules of paclitaxel lack significant myelotoxicity, whereas potentially CrEL-related side effects, including central neuropathy, are augmented.

INTRODUCTION

Drugs exist in the peripheral circulation either as a free or unbound fraction or bound to plasma proteins, polysaccharides and lipids [1]. It is generally acknowledged that the unbound drug fraction is capable of diffusing across biological barriers and interacts with receptor sites in the circulation or extravascular compartment [2]. Monitoring of unbound drug concentrations (Cu) is particularly useful when the fraction that is bound is not constant, when there is a variation in Cu within the normal physiological range of protein levels, when the Cu helps to predict side effects and efficacy, and when there is a valid method to measure the Cu. Since various antiepileptic drugs meet these criteria, Cu monitoring has been successfully implemented in the clinic for a number of such agents, including phenytoin. In the field of clinical oncology, however, this area has been seriously neglected, with the exception of the epipodophyllotoxins etoposide and teniposide [3,4] for which relationships between exposure to Cu and hematologic toxicity have been established [5,6].

We believe that the anticancer drug paclitaxel also meets the above mentioned criteria for Cu measurement. However, a practical analytical method for paclitaxel Cu only recently became available [7]. This poorly water-soluble drug is currently formulated for clinical use in the polyoxyethylated Castor oil derivative, Cremophor EL (CrEL). It has been shown *in vitro* that CrEL can reduce paclitaxel Cu by trapping the drug in micelles composed primarily of polyoxyethyleneglycerol triricinoleate [8]. Similarly, CrEL has recently also been proposed *in vivo* to cause the nonlinear pharmacokinetic behavior of paclitaxel in plasma [9,10], by

reducing the uptake of drug in red blood cells and tissues, thereby interfering with metabolism and biliary secretion [11,12]. Since the unbound fraction of paclitaxel is the pharmacologically active form, understanding factors influencing paclitaxel Cu might be important in predicting toxicity and antitumor activity. Furthermore, it has been shown that CrEL clearance significantly increases with extension of the infusion duration from 3 to 24 hours [11]. Hence, extension of the paclitaxel administration schedule is expected to be associated with lower CrEL levels and, consequently, increased exposure to paclitaxel Cu. To confirm this hypothesis, we have prospectively analyzed CrEL and paclitaxel Cu in plasma samples of patients treated with paclitaxel administered as a 1- or 3-hour intravenous infusion.

PATIENTS AND METHODS

Eligibility criteria and treatment plan

Patients with a histologically confirmed diagnosis of a malignant solid tumor, for whom paclitaxel as monotherapy was a viable therapeutic option or for whom no other treatment options were available, were candidates for this study. Other patient eligibility criteria included age ≥18 years, WHO performance status ≥2, normal bone marrow function, and adequate renal and hepatic function. All patients were scheduled to receive a total of 6 weekly 1-hour or 3-hour intravenous infusions of paclitaxel.

Drug administration

Vials containing 30 mg of paclitaxel formulated in a mixture of CrEL and ethanol USP (1:1, vol/vol) were provided by Bristol Myers Squibb (München, Germany). Paclitaxel at a dose of 100 mg/m^2 was diluted in 500 mL of 5% (wt/vol) dextrose in water and given to the patient via a peripheral or central venous catheter using a motor-driven programmable infusion pump (model 598, IVAC Corporation, San Diego, CA) over a 1-hour or 3-hour period. Premedication was uniform for all patients and consisted of dexamethasone (20 mg), clemastine (2 mg), and cimethidine (300 mg), all administered intravenously 30 minutes prior to paclitaxel infusion. The protocol was approved by the local ethical committee, and all patients provided written informed consent before entering the study.

Pharmacokinetic study design

Evaluation of paclitaxel and CrEL pharmacokinetics was planned for the first cycle and, in a limited number of patients, third cycle of administration. In each patient a pre-infusion (blank) sample was obtained for evaluation of possible interfering peaks in the reversed-phase high-performance liquid chromatographic (HPLC) analysis, and was drawn before paclitaxel was administered. Blood samples for pharmacokinetic evaluation were obtained at the following time points: immediately before infusion, and 0.5, 1 (end of infusion), 1.5, 2, 3, 4, 12 and 24 h after the start of paclitaxel infusion (1-h schedule) and 1, 2, 3 (end of infusion), 3.5, 4, 5, 6, 12 and 24 after infusion (3-h schedule). All blood samples were collected in 10 mL polypropylene tubes containing 75 IU ammonium-heparinate (Sarstedt

Monovette System, Germany). Plasma was immediately separated by centrifugation at 2000 g for 10 min at 4°C, aliquotted in 1.5-mL fractions in polypropylene vials and stored at –20 °C until analysis.

Drug measurement

Total paclitaxel in plasma (the total of bound and unbound drug) was determined by a previously described validated reversed-phase HPLC procedure with UV detection [13]. Measurement of paclitaxel Cu was performed by equilibrium dialysis using a [G-^{3}H]paclitaxel tracer, as described recently [7]. CrEL analysis, using a colorimetric dye-binding microassay, was achieved as reported in detail elsewhere [14], with modifications [15].

Pharmacologic analysis

Plasma concentration *vs* time profiles of paclitaxel Cu were analyzed by model-independent and compartmental analysis using Siphar V4 (InnaPhase, Philadelphia, PA), by determination of slopes and intercepts of the plotted curves with exponential functions. Initial parameter estimates were obtained by an automated peeling algorithm procedure, with an integrated numerical algorithm based on the Powell method to minimize any objective function. The statistical best fit was determined by application of the Akaike information criterion with the χ^2 test to discriminate between models, and the coefficient of correlation, defined as the ratio of the standard deviation computed using the variance-covariance matrix and the parameter value. Both weighted least-squares and extended least-squares methods were evaluated to estimate model parameters minimizing the sum of squared differences between experimental and computed values and the log-likelihood function. The drug disposition half-lifes and the area under the plasma concentration-time curve (AUC = dose/V $\times \Sigma_i(C_i/L_i)$ was determined based on the best-fitted curve and used for calculation of apparent plasma clearance (CL), defined as the ratio of dose delivered (in mg/m^2) and AUC. The mean residence time (MRT) was calculated as the area under the (first) moment-time curve (AUMC), and the volume of distribution (V_d) as the ratio of (dose × MRT) and AUC. Plasma concentration-time curves of CrEL were analyzed by model-independent computation using the linear trapezoidal rule with extrapolation of the AUC to infinity using the terminal slope factor, as well as by conventional compartmental modeling.

Hematological pharmacodynamics was assessed by calculation of the area over the curve (AOC) of white blood cell (WBC) and absolute neutrophil counts (ANC) following paclitaxel administration, determined on a once-weekly basis (days 1 to 36). The AOC was defined as the area of the WBC or ANC versus time curve below the count immediately paclitaxel infusion was commenced, and was calculated for each patient using a macro in the Excel software package [16]. The relative hematologic toxicity for WBC and ANC was defined as % decrease = [(pretherapy value-nadir value)/(pretherapy value)] × 100%.

Statistical evaluation was performed with the unpaired (two-tailed) Student's t-test and the 95% confidence limits for the mean difference using NCSS V5.X (J. Hintze, East Kaysville, UT; 1992). The level of significance was set at $P<0.05$. All pharmacologic data are expressed as mean values ± standard deviation, unless indicated otherwise.

RESULTS

Patient characteristics

The pharmacokinetics of paclitaxel and CrEL was studied in 29 adult patients with various malignant solid tumors treated with a weekly paclitaxel regimen at dose of 100 mg/m^2 (Table 1). The median age was 56 years (range, 22-71), and the median performance status was 1 (range, 0-2). Paclitaxel was administered as a 1-h infusion in 15 patients and as a 3-h infusion in 14 patients. Patient characteristics and baseline clinical chemistry values were similar between the two groups (Table 1).

Table 1 Patient demographics (median with range)

Characteristic	*1-h infusion*	*3-h infusion*
No. of patients	15	14
Paclitaxel dose (mg)	170 (130-236)	185 (135-220)
Infusion duration (h)	1.00 (0.98-1.22)	3.00 (2.92-3.33)
BSA (m^2)	1.73 (1.31-2.37)	1.85 (1.36-2.18)
Weight (kg)	67.0 (36.6-116)	72.3 (43.0-95.0)
Height (cm)	169 (157-184)	171 (151-184)
Age (years)	54 (22-71)	57 (44-70)
Sex (M/F)	8/7	7/7
Hematocrit (%)	35.4 (29.2-44.0)	38.9 (26.1-43.3)
Serum creatinine (μM)	70.7 (20.0-124)	70.7 (24.0-124)
ASAT (units/L)	11 (6.0-48)	11 (7.0-40)
ALAT (units/L)	13 (3.0-45)	16 (4.0-33)
ALP (units/L)	140 (83-595)	126 (72-363)
gamma-GT (units/L)	50 (9.0-171)	26 (17-98)

Abbreviations: BSA, body-surface area; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; gamma-GT, gamma-glutamyltransferase.

Pharmacokinetics

The pharmacokinetics of CrEL could be best described by a linear two-compartment model (Fig. 1A), in line with previous findings [17]. Elimination of CrEL was characterized by a decay in an apparent bi-exponential manner, and indicated no significant differences between the two groups with terminal disposition half-lifes of 34.7 and 30.5 hours, respectively. Similarly, infusion duration had no influence on the volume of distribution of CrEL (Table 2). However, the peak concentration (2.91±0.73 *vs* 2.24±0.39 μL/mL; P=.005) and the AUC of CrEL (80.2±24.2 *vs* 48.5±25.1 μL.h/mL; P=.002) were significantly higher with the 1-hour schedule.

Unbound paclitaxel pharmacokinetics could be best described with a linear three-compartment model in both groups (Figs. 1B and 2; r^2 = 0.997±0.002; root mean squared error = 12.7±4.28%), whereas one and two-compartment models were significantly biased (not shown). The AUC of paclitaxel Cu following the 1-hour infusion was significantly reduced as compared to the 3-hour infusion group (0.50±0.10 *vs* 0.62±0.12 μM.h; P=.0085), in spite of higher peak concentrations (0.26±0.07 *vs* 0.15±0.07; P=.002). Similarly, plasma clearance and volume of distribution were significantly dependent on paclitaxel infusion duration (Table 2).

Table 2 Compartmental parameter estimates of Cremophor EL and unbound paclitaxel

Parameter	*1-h inf*	*3-h inf*	P*	Diff (±SE)**	95%C.L.
Cremophor EL					
C_{max} (μL/mL)	2.91±0.73	2.24±0.39	0.005	0.67±0.22	0.22 | 1.12
AUC (μL.h/mL)	80.2±24.2	48.5±25.1	0.002	31.7±9.15	12.9 | 50.4
CL (mL/h)	199±70.0	361±175	0.003	-162±48.9	-262 | -61.6
V_d (L)	5.54±1.32	4.81±1.29	0.14	0.73±0.48	-0.26 | 1.72
Unbound paclitaxel					
C_{max} (μM)	0.26±0.07	0.15±0.07	0.0002	0.11±0.03	0.06 | 0.16
$AUC_{0-\infty}$ (μM.h)	0.50±0.10	0.62±0.12	0.008	-0.12±0.04	-0.20 | -0.03
CL (L/h/m^2)	244±58.8	190±29.1	0.005	53.8±17.4	18.0 | 89.6
V_d [(L/m^2)×10^{-3}]	7.28±1.75	4.55±1.94	0.001	2.73±0.69	1.32 | 4.14
MRT (h)	11.0±2.90	7.50±4.40	0.017	3.30±1.37	0.679 | 6.32

Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration versus time curve; CL, apparent plasma clearance; V_d, volume of distribution; $T_{1/2}$, disposition half-life; MRT, mean residence time.

* Unpaired (two-tailed) Student's t-test; ** mean difference ± standard error with the 95% confidence limits.

Pharmacodynamics

Overall, hematological toxicity was relatively mild, with no grade 4 toxicity (according to NCI-common toxicity criteria) being observed and no febrile neutropenic events. Analysis of hematological pharmacodynamic parameters indicated that infusion duration had no significant effect on the percent decrease in white blood cell count or absolute neutrophil count (Table 3). However, taking into account the entire time course of leukocytes and neutrophils by calculation of AOC values, a trend was observed toward more severe hematologic toxicity with the 3-hour schedule of paclitaxel administration, consistent with the increased exposure to paclitaxel Cu (Table 3). Univariate-regression analysis indicated that the AUC of paclitaxel Cu was correlated with leukocyte AOC (P=.088; Spearman's rho=.39) and neutrophil AOC (P=.070; Spearman's rho=.34).

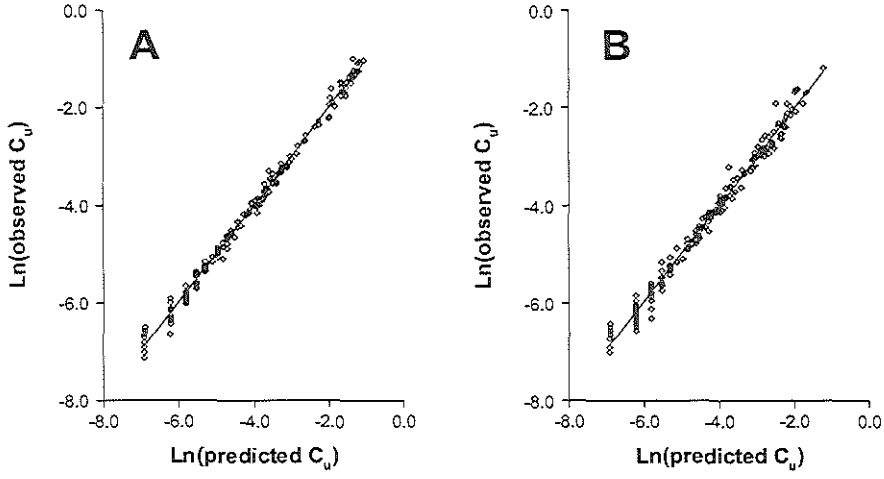

Figure 1 Individual predicted *vs* observed concentrations for unbound paclitaxel in patients receiving a 1-h (*panel* **A**; $y = 0.992847x - 0.027534$, $r^2 = .997$) or 3-h infusion (*panel* **B**; $y = 0.980571x - 0.082332$, $r^2 = .993$) of paclitaxel at a dose of 100 mg/m^2.

Table 3 Hematological pharmacodynamics

Parameter	*1-h inf*	*3-h inf*	P*	Diff (±SE)**	95%C.L.
Leukocytes					
%decrease	47.0±15.5 (17.0-86.1)	45.6±16.7 (13.9-65.4)	0.816	1.41±5.97	-10.8 | 13.7
AOC	62.5±46.0 (1.30-138)	117±93.4 (13.8-299)	0.053	-54.7±27.1	-110 | 0.812
Neutrophils					
%decrease	56.3±14.8 (27.5-90.6)	53.7±18.6 (19.3-83.1)	0.681	2.59±6.22	-10.2 | 15.4
AOC	76.8±54.2 (4.20-172)	129±101 (23.0-308)	0.085	-52.3±29.8	-114 | 8.87

Abbreviation: AOC, area over the blood count versus time curve.

* Unpaired (two-tailed) Student's t-test; ** mean difference ± standard error with the 95% confidence limits.

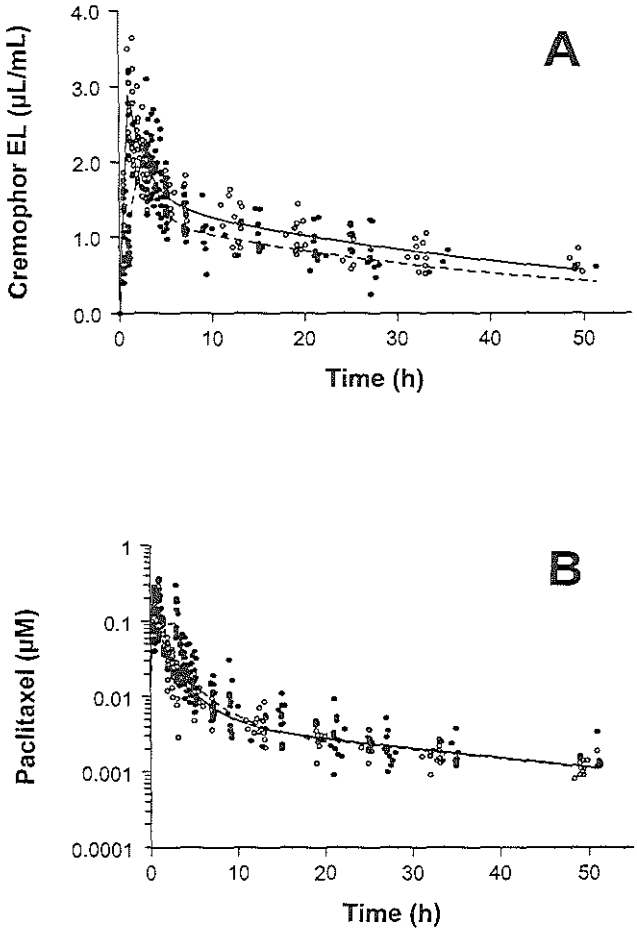

Figure 2 Concentration-time curves of CrEL (*panel* **A**) and unbound paclitaxel (*panel* **B**) in patients receiving a 1-h (open symbols) or 3-h infusion (closed symbols) of paclitaxel at a dose of 100 mg/m^2.

DISCUSSION

In the present study we have described for the first time the human pharmacokinetics of unbound paclitaxel in plasma following 1- and 3-hour infusions. The data complement previous knowledge on the clinical pharmacology of paclitaxel, and may have important practical implications for its optimal use. Previous studies of paclitaxel pharmacokinetics administered either as a 1-h or 3-hour intravenous infusion have consistently focused on measurement of total paclitaxel plasma concentrations, disregarding any effects caused by its formulation vehicle CrEL on unbound drug levels. The use of a recently developed

specific analytical method based on equilibrium dialysis helped to resolve this uncertainty by making it possible to prospectively define unbound paclitaxel pharmacokinetics.

During clinical development of paclitaxel many different infusion durations, ranging from 15 minutes up to 7-weeks, were explored in empirical fashion without a proper pharmacologic rationale [18-25]. The infusion duration was initially extended because of hypersensitivity reactions observed at the shorter administration schedules. Later, proper premedication and physician's experience enabled evaluation of the shorter administration schedules. The problems in paclitaxel development, characterized by many different administration schedules enabled us to learn a lot about the infusion-duration related toxicities and antitumor activity.

Previously, the clearance of CrEL in patients was found to be time-dependent and to increase significantly with extension of the infusion duration from 3 to 24-hours [11]. Our current finding that CrEL disposition is highly dependent on infusion schedule, with disproportional increases in systemic exposure being associated with shortening of infusion from 3 to 1 hour, clearly is in agreement with these prior observations. Our finding that infusion duration has no substantial effect on the terminal disposition half-life suggests that this time-dependent phenomenon is associated with capacity-limited CrEL metabolism within the systemic circulation. Evidence exists that elimination routes of polyoxyethylated surfactants such as CrEL are dictated by serum carboxylesterase-mediated biotransformation, which can be saturated once a certain threshold concentration is exceeded [26]. This phenomenon has significant implications for vehicle-related side effects observed with the use of paclitaxel. It has been established, for example, that acute hypersensitivity reactions associated with paclitaxel chemotherapy are directly attributable to complement activation due to binding of naturally occurring anticholesterol antibodies to the hydroxyl-rich surface of CrEL micelles [27,28]. Thus, the current trend to pursue shorter schedules of paclitaxel administration can have a much greater impact on complement-activating CrEL levels at the site of infusion and on total CrEL exposure than would be expected in case of time-independent pharmacokinetics. It is particularly noteworthy in this context that paclitaxel administration by 30- or 45-minute infusions indicated signs of significant hypersensitivity reactions in all patients even in the presence of standard antiallergenic premedication [18].

A wealth of recent experimental data also shows that CrEL can cause peripheral neuropathy, one of the main side effects reported with paclitaxel chemotherapy [29,30]. Indeed, CrEL concentrations achieved with therapeutic doses of paclitaxel have been shown to produce axonal swelling, vesicular degeneration and demyelination in rat dorsal root ganglion neurons exposed to this vehicle [31], that are similar to data from electrophysiologic studies in patients with neurotoxicity after treatment with paclitaxel [32]. Although the etiology of this side effect has not yet been completely elucidated, it is of particular interest that in a recent comparative trial, there was a trend toward more severe peripheral neuropathy in patients receiving paclitaxel as a 1-hour infusion as compared to a 3-hour infusion [33]. Additional clinical and pharmacological information is currently being collected in ongoing

trials with paclitaxel as well as retrospectively to further explore the relationship between the schedule dependent CrEL disposition and peripheral neurotoxicity.

Of the greatest importance for the antitumor activity of paclitaxel treatment is the disposition of unbound paclitaxel in plasma. The pharmacokinetic model presented here accurately describes the plasma concentration *vs* time profile of unbound paclitaxel, without any signs of nonlinearity for both the 1- and 3-hour schedules. The disappearance of unbound drug from the central plasma compartment was characterized by a terminal disposition half-life of approximately 22 hours. The parallel decline of unbound paclitaxel concentrations between the two groups suggests that the altered clearance is associated with a change in the initial drug distribution immediately following drug administration. This is consistent with the postulated concept that the interference of CrEL with paclitaxel pharmacokinetics is related to a disproportional accumulation process in plasma [12].

Overall, the plasma clearance of unbound paclitaxel was ~30% faster following 1-hour infusion, resulting in significant decreases in AUC of unbound drug as compared to the 3-hour infusion. This finding of decreased systemic exposure to unbound paclitaxel with shortening of infusion duration may have considerable ramification with respect to the clinical use of paclitaxel. Previous studies designed to establish relationships between pharmacokinetics and pharmacodynamic outcome for total paclitaxel concentration have revealed that 11.2 hours above a threshold concentration of 0.1 μM [34] or 17.4 hours above 0.05 μM^9 were predicted to yield a 50% decrease in absolute neutrophil count. However, we have shown recently that when exposure is based on unbound concentrations, AUC could be identified as an important component of the kinetic-dynamic relationship [35]. Our current findings indicate that increased exposure to unbound paclitaxel, as a result of prolonging infusion duration from 1 to 3 hours, indeed results in a trend toward more severe hematologic toxicity. In this study, hematologic toxicity was relatively mild and not clinically relevant due to the low paclitaxel dose, precluding detection of statistically significant differences between both groups. More insight will be provided by ongoing studies comparing 1- and 3-h infusions of paclitaxel administered in a 3-weekly schedule at higher doses of 175-225 mg/m^2 with adequate determination of pharmacokinetic-pharmacodynamic relationships (AS and JV, manuscript in preparation).

Collectively, this study demonstrates that CrEL and unbound paclitaxel disposition are subject to considerable variability depending on the paclitaxel infusion duration. Since the AUC of unbound paclitaxel is related to the neutrophil survival fraction, these findings explain, at least in part, previous observations that short-infusion schedules lack significant myelotoxicity, whereas potentially CrEL-related side effects, including acute hypersensitivity reactions and peripheral neuropathy, are augmented. The current findings, coupled to our recently developed population model for paclitaxel pharmacokinetics-pharmacodynamics [35] provide a possibility to move from empirical-derived observations toward mechanism-based chemotherapy by defining the proper paclitaxel infusion duration.

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Chapter 15

Summary, conclusions and future perspectives

This thesis includes phase I and pharmacological studies on inhibitors of topoisomerase I and pharmacological studies on paclitaxel, with a focus on the role of paclitaxel's solvent Cremophor EL.

In **Chapter 2**, an overview is given on the development of orally administered topoisomerase I inhibitors. Significant levels of anti-tumor activity in human tumor xenografts were seen especially with prolonged duration of exposure. Since oral drug delivery is a more convenient method for prolonged drug administration, and preferred by patients, further development of oral formulations is warranted. Common concerns expressed on the development of oral formulations of drugs for cancer treatment relate to their sometimes low oral bioavailability and frequently large intra- and interpatient variation in systemic exposure. The latter is not dissimilar to intravenous drug administration, which is frequently ignored. Efforts to improve absorption and minimize intestinal metabolism/efflux of the oral chemotherapeutic agent using new formulations might lead to better bioavailability. Pharmacokinetic and pharmacodynamic evaluations have enabled guidance in recommendations of schedules. Given the interpatient variation in exposure it is interesting to note that flat dosing of topotecan resulted in the same systemic exposure compared with the more complex dosing per body surface area. In order to limit the interpatient variation in exposure to 9-AC, a limited sampling model for oral 9-AC was developed, enabling prediction of the systemic exposure and thereby optimizing treatment for any given patient. Drug sequencing plays a key role in the combination topotecan/cisplatin and might be important for combination with other classes of drugs.

Therefore, forthcoming phase I trials on combination therapy with oral topoisomerase I inhibitors should include studies on sequence dependence and pharmacokinetic analyses to evaluate any mutual interaction.

Diflomotecan (BN80915) is an E-ring modified camptothecin analogue topoisomerase I inhibitor which confers greater stability in plasma, a potential advantage for anti-tumor activity. As with other camptothecins oral administration has pharmacological and clinical advantages, and fixed dosing is as accurate as dosing per square meter given the interpatient pharmacokinetic and pharmacodynamic variability. In **chapter 3** a phase I bioavailability study to assess the feasibility of the administration of oral diflomotecan, to determine the maximum-tolerated dosage (MTD) and oral bioavailability and to explore the pharmacokinetics was described.

Diflomotecan was administered orally for 5 days every 3 weeks to adult patients with solid malignant tumors. Four flat oral dose levels were explored. The main toxicity was hematological, and some patients experienced alopecia, mild gastrointestinal toxicity and fatigue. At the 0.35 mg dose 2/4 patients experienced dose limiting toxicity (DLT) with grade 3 thrombocytopenia with epistaxis and febrile neutropenia. Pharmacokinetics of diflomotecan was linear over the dose range studied and showed a moderate degree of interpatient variability. Systemic exposure was correlated to the decrease in white blood cells. The MTD

for phase II studies of 0.27 mg/d p.o. x 5 every 3 weeks is convenient, generally well tolerated and exerts a favorable pharmacokinetic profile.

For the bioavailability study, diflomotecan was administered both i.v. as a 20 min infusion, 2 weeks later followed by daily times 5 p.o administration at 0.27 mg as a drinking solution. Diflomotecan was determined by an LC-MS/MS method using a structural analogue of the drug as internal standard. The mean oral bioavailability (F) was 67.1% at the recommended dose. Compared to other camptothecin analogs, including topotecan (F=30%), irinotecan (F=12-20%) and lurtotecan (F=11.3%), diflomotecan has a higher F, which may be an advantage with potential clinical importance.

This study indicates significant systemic exposure to diflomotecan with a moderate degree of interpatient PK variability. These results strongly support development of diflomotecan for oral administration in cancer therapy.

In **chapter 4** a phase I and pharmacologic study is described, in which the feasibility of the combination of oral topotecan and i.v. cisplatin and the sequence dependent effects are assessed. Oral topotecan was administered for five days in combination with a fixed dose of 75 mg/m^2 i.v. cisplatin, either before topotecan on day 1 (CT) or on day 5 (TC), repeated every 3 weeks. Patients were treated in a randomized cross-over design. The CT sequence induced significantly more myelosuppression than the TC sequence, and resulted in MTD at a topotecan dose of 1.25 mg/m^2/d. In the less toxic TC sequence MTD was established at 2.00 mg/m^2/d. No significant pharmacokinetic interaction was found to explain the difference in toxicity between the sequences. This sequence dependent toxicity may be based on the combination of DNA-damage by cisplatin and interference with DNA-repair by the topoisomerase I inhibitor topotecan.

Whether the most toxic sequence is also the most effective should be evaluated in a randomized phase II and/or III study.

Chapter 5 is describing a phase I and pharmacologic study with increased dose topotecan, in comparison with the dosages used in chapter 4, in combination with a lower fixed dose of cisplatin of 50 mg/m^2. This study design was based on the *in vivo* topotecan systemic exposure/antitumor activity relationship. The sequence CT was chosen, since in models it is found to be the most effective sequence. The dose limiting toxicities consisted of myelosuppression and gastrointestinal toxicity at a topotecan dose level of 1.75 mg/m^2/d administered day 1-5 every 3 weeks. The pharmacokinetics of cisplatin and topotecan were similar to earlier data.

We concluded that the MTD of cisplatin 50 mg/m^2 followed by oral topotecan at 1.50 mg/m^2/d x 5 every 3 weeks has no advantage as compared to our recommended schedule of chapter 4, since only a minor dose escalation of topotecan could be achieved by a dose reduction of 33% of cisplatin.

In **chapter 6**, the (non)sense of body-surface area based dosing of orally administered topotecan in adult cancer patients is evaluated, by estimation of the intra- and interpatient

variabilities in topotecan lactone pharmacokinetics of the patients participating in the studies reported in chapter 4 and 5. The intrapatient variability in systemic exposure was 24 ± 13% (median 20%) with a range of 7.6-61%. The interpatient variability in the apparent clearance of topotecan lactone, expressed in L/h/m^2 as well as L/h, was 38 and 42%, respectively. In view of the relatively high intra- and interpatient variabilities in systemic exposure and apparent clearance of topotecan lactone and the relatively small range in observed body-surface area of 12%, oral topotecan can be added to the list of agents where body surface area adjusted dosing does not appear to yield any benefit. Moreover, accurate dosing of oral topotecan based on body surface area lacks precision, since oral topotecan is only supplied as gelatine capsules containing 1.00 or 0.25 mg.

Therefore, we recommend a fixed dose regimen for future use in clinical trials. Studies, designed to improve (pre)treatment estimation of systemic exposure based on individual patient characteristics, will eventually lead to less differences in intra- and interpatient systemic exposure.

Chapter 7 describes the gender dependent differences in topotecan pharmacokinetics in adult cancer patients after oral as well as i.v. administration. A significant 1.3-fold, body surface area corrected, faster apparent clearance of topotecan lactone was found in males as compared to females treated both with oral and i.v. topotecan. A significant correlation between hematocrit and thus with gender, but not with body surface area was found. In *in vitro* studies with artificially altered hematocrit values, a strong significant correlation was found between the hematocrit value and the lactone (the active form) to total topotecan concentrations. In addition, in *in vitro* experiments significantly higher topotecan lactone concentrations were measured in the plasma compartment of the blood samples of the female volunteers. The higher lactone to carboxylate (the inactive form) ratios in blood with lower hematocrit values is most likely caused by the fact that the carboxylate form, which is charged, is not able to pass cell membranes and thus remains in the plasma compartment.

Eventually recognition of the gender/hematocrit-dependency in topotecan pharmacokinetics may lead to more accurate dosing of topotecan in the future.

The influence of third spaces, such as pleural and ascitic fluid, on topotecan pharmacokinetics in 4 patients is described in **chapter 8**. The apparent topotecan clearance, the terminal disposition half-lives and the AUC ratios of lactone to total drug in plasma remained unchanged in the presence or absence of pleural or ascitic fluid. The penetration of topotecan into the pleural or ascitic fluid showed a mean lag time of 1.61 h and ratios with plasma concentration increased with time after dosing in all patient, with a mean ratio of third space total drug AUC to that in plasma of 0.55. These data indicate that topotecan can be safely administered to patients with pleural effusions or ascitic fluid and that there is substantial penetration of topotecan into these third spaces.

These findings are clinically important for lung cancer or ovarian cancer patients with pleural or ascitic fluid, for whom chemotherapy with topotecan often is a reasonable option.

The paclitaxel vehicle Cremophor EL (CrEL) has been shown to selectively inhibit accumulation of cisplatin in peripheral blood leukocytes but not in tumor cells *in vitro*, and we hypothesized that this phenomenon is responsible for the improvement of the therapeutic index of cisplatin observed in combination studies with paclitaxel. In **chapter 9** we report on studies assessing the interaction between CrEL and cisplatin in a murine model, and involving the potential clinical applicability of CrEL as a protector for cisplatin-associated hematologic side effects. In mice, CrEL given in combination with cisplatin, significantly reduced cisplatin-induced hematologic toxicity. Data obtained from cancer patients treated with cisplatin and topotecan preceded by CrEL (12 mL, 3-h i.v.) or without CrEL similarly indicated significant differences in the percent decrease in WBC and platelets between the groups. Pharmacokinetic parameters were not significantly different from historic control values.

Overall, this study provides further evidence on the important role of CrEL in the toxicological profile of cisplatin, and implies that reformulation of cisplatin with CrEL for systemic treatment might achieve improvement of its therapeutic index, particularly in the setting of a weekly dose-dense concept or in combination with radiotherapy.

Chapter 10 describes a phase I study with cisplatin and topotecan ± CrEL in patients with recurrent or progressive ovarian cancer. This study is based on the following facts: both weekly cisplatin chemotherapy and single agent topotecan have proven to be effective in recurrent ovarian cancer, preclinical data show synergism between cisplatin and topotecan, and side effects for this combination are drug sequence dependent and predominantly haematologic. Since preclinical data suggest that CrEL, the formulation vehicle of paclitaxel, has a protective effect on haematological toxicity of cisplatin, CrEL was added to the combination cisplatin and topotecan. In this phase I study, escalating doses of oral topotecan administered on day 1,2,8,9,15,16,29,30,36,37,43,44 were combined with weekly cisplatin 70 mg/m^2/d day 1,8,15,29,36,43 (scheme A) or with the presumably less myelotoxic sequence weekly cisplatin day 2,9,16,30,37,44 (scheme B). In scheme C, CrEL 12 mL was administered prior to cisplatin in the sequence of Scheme A. In scheme A 4/10 patients, all treated with topotecan 0.45 mg/m^2/d, experienced DLT: predominantly hematological. Both patients in scheme B (topotecan 0.45 mg/m^2/d) had DLT due to a delay > 2 weeks because of prolonged haematological toxicity. No DLT was observed in the first 3 patients in scheme C (topotecan 0.45 mg/m^2/d). However, 2 out of 3 patients treated at dose level topotecan 0.60 mg/m^2/d in scheme C experienced DLT due to > 2 weeks delay because of persistent thrombocytopenia or neutropenia.

We concluded that there is a modest clinical effect of CrEL on haematological toxicity for this cisplatin based combination regimen, which seems to reduce these side effects but does not really enable an increase of the oral topotecan dose.

Chapter 11 is a review on CrEL, the formulation vehicle used for various poorly-water soluble drugs, including the anticancer agent paclitaxel (Taxol). In contrast to what it was held for previously, CrEL is not an inert vehicle but it exerts an array of biological effects,

some of which have important clinical implications. Its use has been associated with severe anaphylactoid hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy. The pharmacokinetic behaviour of CrEL is dose independent, although its clearance is highly influenced by infusion duration. This is particularly important since CrEL can affect the disposition of various drugs by changing the unbound drug concentration through micellar encapsulation. In addition, it has been shown that CrEL, as an integral component of paclitaxel chemotherapy, modifies the toxicity profile of certain anticancer agents given concomitantly by mechanisms other than kinetic interference.

A clear understanding of the biological and pharmacological role of CrEL is essential to help oncologists to avoid the pitfalls that come with the use of paclitaxel or other agents using this vehicle substance. With the present development of various new anticancer agents, it is recommended that alternative formulation approaches should be pursued to allow better control of toxicity and pharmacological interactions related to the use of CrEL.

In **chapter 12** we describe the pharmacokinetics and metabolic disposition of [*G*-^{3}H]-paclitaxel in a female patient with recurrent ovarian cancer and severe renal impairment (creatinine clearance: ~20 mL/min) due to chronic hypertension and prior cisplatin treatment. During six 3-weekly courses of paclitaxel at a dose of 157.5 mg/m^2 (*viz.* a 10% dose reduction), the renal function remained stable. Pharmacokinetic evaluation revealed a reproducible and surprisingly high paclitaxel plasma AUC and a terminal disposition half-life of ~29 hrs. Both parameters are substantially increased (~1.5-fold) as compared to kinetic data obtained from patients with normal renal function. The cumulative urinary excretion of the parent drug was consistently low and and total fecal excretion was 52.9% of the delivered radioactivity, and mainly comprising known mono- and dihydroxylated metabolites, with unchanged paclitaxel accounting for only 6.18%. The plasma AUC of the paclitaxel vehicle CrEL, which can profoundly alter the kinetics of paclitaxel, was not different from historical data in patients with normal renal function.

In conclusion, we have shown that the kidneys play a more prominent role in paclitaxel metabolism than previously thought.

In **chapter 13** we report on the first comparative study of paclitaxel pharmacokinetics in the presence and absence of CrEL after i.p. and i.v. dosing. Patients received an i.p. tracer dose of [*G*-^{3}H]paclitaxel in ethanol without CrEL on day 1, i.p. paclitaxel formulated in CrEL (Taxol) on day 4, and i.v. tracer of [*G*-^{3}H]paclitaxel on day 22, and i.v. Taxol on day 24. Four patients were studied, and serial plasma samples were obtained up to 72 hours and analyzed for total radioactivity, paclitaxel and CrEL. The terminal disposition half-life was substantially prolonged after i.p. dosing of Taxol, as was the mean residence time. The bioavailability of Taxol was 31.4±5.18%, indicating insignificant systemic concentrations after i.p. treatment. CrEL levels in plasma were undetectable after i.p. dosing, whereas after i.v. dosing, the mean clearance was in line with earlier observations. In the absence of CrEL, the bioavailability and systemic concentrations of i.p. paclitaxel were significantly higher.

This finding is consistent with the postulated concept that CrEL is mainly responsible for the pharmacokinetic advantage for peritoneal cavity exposure to paclitaxel, compared to systemic delivery.

Chapter 14 describes a study based on the concept that CrEL profoundly influences the cellular distribution of paclitaxel in human blood *in vitro* by a concentration-dependent reduction of the unbound drug fraction. Since CrEL clearance increases with extending the infusion duration from 3 to 24 hours, we hypothesized that exposure to unbound paclitaxel might also be schedule dependent. CrEL and unbound paclitaxel pharmacokinetics were prospectively analyzed in patients with advanced solid tumors treated with paclitaxel given as a 1-hour (n=15) or 3-hour (n=14) intravenous infusion. The systemic exposure (AUC) to CrEL was significantly higher with the 1-hour as compared to the 3-hour schedule, and subsequently the AUC of unbound paclitaxel was substantially reduced following the 1-hour infusion. A trend was observed towards more severe hematological toxicity with the 3-hour schedule (P=.053), consistent with increased exposure to unbound drug.

Overall, these findings explain, at least in part, previous observations that short-infusion schedules of paclitaxel lack significant myelotoxicity, whereas potentially CrEL-related side effects, including central neuropathy, are augmented.

Conclusions

This thesis was originally planned to describe clinical phase I studies with oral topoisomerase I inhibitors and combinations of these agents with other cytotoxic agents. Clinical questions, such as "can I treat this patient with massive ascites with full dose topotecan" and "to what extent will the medication penetrate in the peritoneal cavity?" or "what happens when I treat this patient with severe renal insufficiency with paclitaxel?", could be answered with pharmacological evaluation. From there the fruitful cooperation with the laboratory of experimental pharmacology originated and finally the questions went the other way round: "we know that Cremophor EL selectively protects from cisplatin bone marrow toxicity *in vitro* and *in vivo*, will this also be the case in patients" or "we know about micellar entrapment of paclitaxel at high concentrations, will this be a possible advantage for intraperitoneal administration of paclitaxel?" It is clear that pharmacological evaluation of chemotherapy is an essential part of development of a compound, but to me it is also clear that after registration of a new compound many clinical questions still can be solved by proper clinical and pharmacological research.

Future perspectives

The rationale for studying the clinical pharmacology of anticancer drugs is that the information obtained will result in less toxicity and enhanced antitumor activity for the individual patient. Given the narrow therapeutic window of these agents, efforts to improve individual therapy are warranted. Until now, dosing per square meter, gave us a false feeling of precision in most cases. The interpatient pharmacokinetic variability is related to parameters such as: variability in bioavailability, elimination, and distribution. Current and

future research focuses on these aspects and tries to define more precise dosing schedules and/or to modulate pharmacological aspects in order to improve the clinical efficacy and reduce the toxicity. Eventually this research will lead to more precise population pharmacokinetic dosing models. Currently we are prospectively analyzing such a dosing model intravenous oral topotecan. Serum creatinine and gender add a great deal to the precision of topotecan dosing, but I expect more parameters to be essential. The exact role of erythrocytes is not yet defined and subject to present clinical studies with topotecan.

The growing knowledge on human genetics enables us to identify genetic polymorphisms related to differences in specific drug pharmacokinetics. Polymorphisms in cytochrome P-450 isoenzymes, for example, will in some cases lead to a completely different rate of drug metabolism, with important clinical implications. Another example is the role of Breast Cancer Resistance Protein (BCRP) in the enteral uptake and thus bioavailability of orally administered drugs. Determination of the individual BCRP levels will hopefully enable us to predict bioavailability of BCRP-substrates such as topotecan, and modulation of the drug transporting pumps might eventually lead to a higher and better predictable bioavailability. It is also essential to realize the influence of co-administrated drugs, whether sequence dependent or not, on the pharmacokinetics of anticancer agents. Every possible interaction should be studied, as it may have important clinical implications. Or even, drug interactions can be used on purpose to decrease the interpatient differences in systemic exposure to a specific anticancer drug.

New insights on the role of CrEl, especially in its relation to the unbound fraction of paclitaxel, and determination of the relation between CrEL and unbound paclitaxel and toxicity and efficacy will lead to better defined paclitaxel dosing. This might also be the case for other (anticancer) drugs formulated in CrEL. Next to all the negative aspects of CrEL, more research is needed of two potential pros: its possible selective bone marrow protection from chemo- and radiotherapy and its merits for use as a formulation vehicle for i.p. administration of different anticancer drugs.

Clinical pharmacologic studies will increasingly guide clinicians in their decisions when prescribing antitumor drugs, serving the unique goal of better therapy for the individual cancer patient.

Chapter 15

Samenvatting, conclusies en toekomstperspectieven

Samenvatting

Vele processen zijn betrokken bij de ongeremde celdelingen die leiden tot tumorgroei. Het enzym topoisomerase I en het eiwit tubuline sturen twee van die processen en zijn dus een aangrijpingspunt voor antikanker behandeling. De antikanker middelen behorend tot de groepen van remmers van topoisomerase I en tubuline hebben diverse overeenkomsten. Ze zijn beiden afkomstig uit de natuur, hebben een zeer lang ontwikkelingsproces doorgemaakt, en ze remmen beiden ongebreidelde celdeling, zoals bij kanker wordt gezien. Een nadeel van deze middelen is dat ze bijwerkingen kunnen geven, zoals overgevoeligheidsreacties, misselijkheid, braken, mondslijmvlies ontstekingen, haarverlies, beenmergschade en orgaanfunctie stoornissen. Het blijkt dat het optreden van deze bijwerkingen, maar ook de antikanker werkzaamheid, onder andere afhankelijk is van de dosis, de manier van toediening en het oplosmiddel van de werkzame stof. Vroeg klinisch onderzoek (fase I) met bepaling van bloedspiegels van antikanker middelen (farmacologisch onderzoek) kan leiden tot het juiste individuele toedieningsschema. Doseringsschema's van reeds geregistreerde antikanker middelen kunnen ook door klinisch farmacologisch onderzoek verbeterd worden. In dit proefschrift worden de resultaten beschreven van farmacologische en klinische studies met remmers van topoisomerase I en tubuline, met speciale aandacht voor de invloed van het oplosmiddel Cremophor EL op de farmacologie en de bijwerkingen van deze middelen.

In **hoofdstuk 2** wordt een overzicht gegeven van de ontwikkeling van oraal toegediende remmers van het enzym topoisomerase I. In proefdiermodellen bleek dat de antitumor werking het grootst was bij langdurige blootstelling aan het medicijn. Omdat orale toediening een gemakkelijke manier is van langdurige medicijn toediening, en bovendien door patiënten wordt geprefereerd, is verdere ontwikkeling van orale chemotherapie aantrekkelijk. Problemen bij deze ontwikkeling zijn de soms slechte opname van het medicijn en de grote variatie in blootstelling: zowel variatie tussen de patiënten onderling (interpatiënt) als variatie tussen de verschillende kuren binnen 1 patiënt (intrapatiënt). Deze variatie is zo groot dat geïndividualiseerde dosering per vierkante meter lichaamsoppervlak niet preciezer blijkt te zijn dan gebruik van een vaste dosering voor iedere patiënt. Ontwikkelingen die de opname van de oraal toegediende middelen verbeteren zullen uiteindelijke leiden tot een hogere blootstelling aan het medicijn. Voor bijvoorbeeld de topoisomerase I remmer 9-aminocamptothecine is een model ontwikkeld waarmee met twee bloedafnames de blootstelling aan het medicijn kan worden bepaald, op grond waarvan verdere toedieningen kunnen worden geïndividualiseerd waardoor de eerder genoemde variatie aanzienlijk zal verminderen. Farmacokinetisch (wat is de blootstelling aan het medicijn na toediening) en farmacodynamisch (wat "doet" het medicijn met het lichaam) onderzoek heeft geleid tot aanbevolen schema's. Met name in de veelbelovende combinaties van orale topoisomerase I remmers met cisplatin is dit onderzoek van belang om interacties, en daardoor verhoogde kans op bijwerkingen, uit te sluiten.

Hoofdstuk 3 is een voorbeeld van zo een farmacokinetisch-farmacodynamisch fase I onderzoek met een nieuwe orale topoisomerase I remmer Diflomotecan (BN80915).

Diflomotecan is een topoisomerase I remmer die door zijn unieke structuur stabieler is in het bloed. Diflomotecan werd dagelijks gedurende 5 dagen iedere 3 weken oraal toegediend aan volwassen kanker patiënten. Vier opklimmende dosisniveaus werden onderzocht. De belangrijkste bijwerkingen waren beenmergdepressie; sommige patiënten hadden last van misselijkheid, braken, haarverlies en vermoeidheid. Het aanbevolen dosisniveau werd vastgesteld op 0.27 mg per dag, hierbij waren de bijwerkingen acceptabel. De gemeten bloedspiegels van diflomotecan waren gerelateerd aan de toegediende orale dosering en er was slechts een kleine variatie tussen de patiënten onderling. Bovendien was de blootstelling in het bloed goed gerelateerd aan de daling van witte bloedcellen. Concluderend kan gesteld worden dat oraal diflomotecan goed verdragen wordt en een gunstig farmacokinetisch profiel heeft.

Voorts wordt een orale biobeschikbaarheidsstudie met diflomotecan beschreven. Na een i.v. toediening, die 2 weken later gevolgd werd door een orale toediening, werd middels seriële bloed afnamen de blootstelling aan het medicijn bepaald. Vergelijking van de blootstelling na i.v. en orale toediening levert de zogenaamde orale biobeschikbaarheid op. Deze blijkt met 67.1% duidelijk beter te zijn dan de orale biobeschikbaarheid van andere orale topoisomerase I remmers zoals topotecan, irinotecan en lurtotecan. De hoge biobeschikbaarheid betekent een potentieel klinisch voordeel van diflomotecan boven andere orale topoisomerase I remmers.

Hoofdstuk 4 beschrijft een fase I en farmacologische studie, waarin opklimmende doseringen oraal topotecan (T) in een 5-daags schema gecombineerd werden met een vaste dosis intraveneus cisplatin (C) van 75 mg/m^2, toegediend in een 3-wekelijks schema. Om het effect van de volgorde van toediening van beide middelen op de bijwerkingen en de farmacokinetiek te onderzoeken, werd cisplatin op de 1ste (volgorde CT) of de 5de dag van de topotecan (TC) toediening gegeven. De volgorde CT gaf aanzienlijk meer beenmergschade dan het omgekeerde TC schema, mogelijk houdt dit verband met de DNA-schade door cisplatin en het verhinderen van de reparatie van die DNA-schade door de topotecan, die aansluitend aan de cisplatin wordt gegeven. Er werd geen farmacokinetische interactie aangetoond. De aanbevolen dagelijkse dosis topotecan in de volgorde TC is 2.0 mg/m^2 en in de volgorde CT 1.25 mg/m^2. Of de meest toxische volgorde van toediening ook de meest effectieve is, zal in een vergelijkende fase II en/of III studie onderzocht moeten worden.

Hoofdstuk 5 beschrijft een fase I en farmacologisch onderzoek met, wederom, cisplatin en oraal topotecan in de volgorde CT, aangezien verwacht wordt dat dit de meest effectieve volgorde is. De dosis cisplatin is verminderd in een poging de topotecan dosering verder te verhogen. De studie opzet was gebaseerd op de, in tumorcelkweken, gevonden relatie tussen topotecan dosering en antitumor activiteit. In deze studie werd opnieuw geen farmacokinetische interactie aangetoond. De aanbevolen dosering van cisplatin 50 mg/m^2, gevolgd door topotecan 1.50 mg/m^2/d x 5 iedere 3 weken, blijkt geen voordeel te hebben ten opzichte van de aanbevolen CT dosering uit hoofdstuk 4, aangezien slechts een kleine dosis verhoging van topotecan ten koste gaat van 33% dosisreductie van de cisplatin.

In een poging de intra- and interpatiënt variatie in blootstelling aan chemotherapeutica te verminderen is het tot dusver gebruikelijk aan de hand van het lichaamsoppervlak van de individuele patiënt te doseren. In **hoofdstuk 6** wordt aangetoond dat dit voor oraal toegediend topotecan, zoals ook voor vele andere cytostatica, niet zinvol is. Dit heeft te maken met het feit dat de intra- en interpatiënt variatie in blootstelling aan topotecan, bij berekening van de dosering per vierkante meter lichaamsoppervlak, veel groter is dan de interpatiënt variatie in lichaamsoppervlak. Bovendien moet de dosis oraal topotecan altijd worden afgerond, aangezien er alleen capsules van 0.25 en 1.00 mg beschikbaar zijn. Het advies is dan ook om de dosis oraal topotecan voor iedere patiënt hetzelfde te houden en niet te individualiseren. Studies, gericht op voorspelling van de blootstelling gebaseerd op individuele patiëntenkarakteristieken, zullen in de toekomst hopelijk leiden tot een vermindering van de inter- en intrapatiënt variatie in blootstelling aan topotecan.

Hoofdstuk 7 beschrijft de geslachts afhankelijke verschillen in topotecan farmacokinetiek. Topotecan kent een zuurgraad afhankelijke omzetting van de actieve in de inactieve vorm. De actieve vorm van topotecan werd door mannen 1.3 maal sneller geklaard dan door vrouwen, ook na correctie voor lichaamsoppervlakte. Aangezien vrouwen een lagere hematocriet (maat voor hoeveelheid rode bloedcellen) hebben, werden er laboratoriumexperimenten uitgevoerd waarbij naar bloed van vrouwelijke vrijwilligers en bloed met kunstmatig verlaagde hematocriet werd gekeken. Het bleek dat na toevoeging van topotecan er een hogere concentratie van de actieve vorm van topotecan in het plasma compartiment van bloed met een lagere hematocriet werd gevonden. Dit zou mogelijk te maken kunnen hebben met het feit dat de inactieve topotecan vorm van topotecan geladen is, en daardoor de celmembraan van de bloedcellen niet kan passeren, waardoor het in het plasma blijft. Erkenning van de geslachts/hematocriet afhankelijke topotecan farmacokinetiek zou in de toekomst mogelijk kunnen leiden tot een exactere dosering van topotecan.

In **hoofdstuk 8** wordt de invloed van derde ruimtes, zoals ascites en pleuravocht, op de plasmakinetiek van topotecan beschreven bij 4 patiënten. De derde ruimtes fungeerden niet als "verzamelplaats" voor topotecan en hadden geen invloed op de farmacokinetiek van het middel. Na 1.61 uur begon de medicatie in de derde ruimte binnen te dringen en de blootstelling bleek uiteindelijk gemiddeld 55% van die in het plasma te bedragen. Uit dit onderzoek kan geconcludeerd worden dat topotecan veilig toegediend kan worden aan patiënten met ascites of pleuravocht en dat er voldoende hoge concentraties gevonden worden ter plaatse. Deze bevindingen zijn klinisch van belang voor long- en eierstokkanker patiënten met pleuravocht of ascites die vaak behandeld worden met topotecan.

Cremophor EL (CrEL), het oplosmiddel van het antikanker middel paclitaxel, blijkt in laboratoriummodellen selectief de witte bloedcellen te beschermen tegen schade aangericht door behandeling met cisplatin. Mogelijk is de beschermende werking reden waarom paclitaxel en cisplatin zo goed gecombineerd kunnen worden. In **hoofdstuk 9** wordt de beschermende werking van CrEL op door cisplatin veroorzaakte beenmergschade

beschreven in achtereenvolgens: een laboratoriummodel, in muizen, en tenslotte in patiënten die behandeld zijn met cisplatin en paclitaxel ± CrEL. De laatste studie toonde een vermindering van het percentage daling van witte bloedcellen en bloedplaatjes wanneer CrEL was toegevoegd aan de behandeling met cisplatin en topotecan. Oplossen van cisplatin in CrEL zou mogelijk van waarde kunnen zijn voor intensieve cisplatin behandelingen, zoals wekelijkse toedieningen of in combinatie met radiotherapie.

Hoofdstuk 10 beschrijft een fase I studie met wekelijks cisplatin en oraal topotecan in verschillende volgordes ± CrEL bij patiënten met uitgezaaide eierstokkanker. Door met name beenmergschade was dit intensieve schema niet haalbaar bij de patiënten die geen CrEL kregen toegediend. De conclusie is dat CrEL wel enige beschermende werking heeft op dit combinatieschema, maar dat verdere dosisverhoging niet mogelijk was. Dit schema is dus niet te prefereren boven andere, minstens zo effectieve, wekelijkse combinatie-behandelingen met cisplatin bij patiënten met uitgezaaide eierstokkanker.

Hoofdstuk 11 is een overzichtsartikel waarin CrEL, het oplosmiddel van slecht-wateroplosbare medicatie, zoals paclitaxel, wordt beschreven. CrEL is geen onschuldig oplosmiddel, maar blijkt een scala aan biologische effecten teweeg te kunnen brengen. Bekende bijwerkingen zijn: ernstige overgevoeligheidsreacties, bloedvervetting, samenklontering van bloedplaatjes en zenuwbaanschade. De klaring van CrEL is afhankelijk van de infusieduur. CrEL kan de vrije (actieve) fractie van geneesmiddelen, die gelijktijdig worden toegediend, beïnvloeden. Het is van belang te beseffen dat gelijktijdige toediening van paclitaxel (en dus ook CrEL) de werking van andere medicatie kan beïnvloeden. Een goed begrip van de biologische en farmacologische rol van CrEL is dus essentieel voor oncologen die paclitaxel voorschrijven.

In **hoofdstuk 12** wordt de farmacologie en de uitscheiding van (radioactief gemerkt) paclitaxel beschreven, toegediend aan een patiënte met een ernstig gestoorde nierfunctie. Tijdens zes 3-wekelijkse kuren paclitaxel, met 10% dosisvermindering, bleef haar nierfunctie stabiel en waren er geen ernstige bijwerkingen. Farmacologisch onderzoek toonde wel een 1.5 maal verhoogde en langduriger blootstelling aan paclitaxel aan. In tegenstelling tot de faeces excretie bleek de urine excretie verlaagd te zijn. Deze gegevens wijzen er op dat de nieren toch een belangrijker rol hebben in de stofwisseling van paclitaxel dan altijd is aangenomen.

Hoofdstuk 13 beschrijft een studie van de paclitaxel farmacokinetiek met en zonder CrEL na intraperitoneale (i.p.) en i.v. toediening. Vier patiënten kregen achtereenvolgens een i.p. dosis radioactief gemerkt paclitaxel zonder CrEL op dag 1, een i.p. dosering paclitaxel in CrEL (Taxol) op dag 4, een i.v. dosis radioactief paclitaxel zonder CrEL op dag 22, en tenslotte i.v. Taxol op dag 24. Bloedmonsters werden tot 72 uur na iedere toediening afgenomen en geanalyseerd op totale radioactiviteit, paclitaxel en CrEL. Na i.p toediening van Taxol werden hoge concentraties in de ascites bereikt met een lange halfwaarde tijd,

terwijl er weinig in de bloedbaan terechtkwam. Zonder CrEL, blijken er wel hoge concentraties in het bloed bereikt te worden na i.p. toediening. Deze bevindingen wijzen er op dat er een voorkeur bestaat voor toediening van paclitaxel met CrEL voor i.p. toediening. Toekomstige studies zouden kunnen aantonen dat toevoeging van CrEL aan ieder i.p. toegediend antikanker middel zinvol kan zijn.

Hoofdstuk 14, tenslotte, beschrijft een vergelijkende studie waarbij paclitaxel door middel van een 1 uurs of 3 uurs infuus werd toegediend. Het bleek dat de klaring van het oplosmiddel CrEL toenam bij langduriger infusie, hierdoor nam de zogenaamde vrije fractie paclitaxel toe bij de 3 uurs ten opzichte van de 1 uurs infusies. Aangezien de vrije fractie de farmacologisch actieve fractie is, was de hypothese dat de hematologische bijwerkingen zouden toenemen bij verlenging van de infusieduur. Er werd inderdaad aangetoond dat de beenmergschade toegenomen was bij de 3 uurs infusies. Overeenkomstig bleek de blootstelling aan CrEL hoger bij de 1 uurs infusies. Een toekomstige studie gericht op de relatie tussen infusieduur en CrEL gerelateerde bijwerkingen zou het klinisch belang van deze bevinding kunnen aantonen.

Conclusies

Dit proefschrift was oorspronkelijk opgezet om klinische en farmacologische studies met orale topoisomerase I remmers te beschrijven. Gaandeweg bleek dat klinische vraagstellingen zoals, "kan ik deze patiënt met massaal buikvocht een volledige dosis topotecan toedienen?", en "hoeveel van die topotecan komt er dan uiteindelijk op de plaats van bestemming, in de buikholte?", of "kan ik deze patiënt met ernstige nierinsufficiëntie ongestraft met paclitaxel behandelen?", beantwoord konden worden met farmacologisch onderzoek. Hieruit ontstond een vruchtbare samenwerking met het laboratorium experimentele farmacologie en uiteindelijk gingen de vragen de andere kant op: "we weten dat CrEL selectief het beenmerg beschermt tegen door cisplatin veroorzaakte schade in laboratoriummodellen en proefdieren, geldt dit ook voor patiënten?", of "we weten nu dat paclitaxel in CrEL micellen gaat zitten bij hoge concentraties, zou dit een potentieel voordeel kunnen betekenen voor i.p. toediening van paclitaxel?". Het is duidelijk dat farmacologisch onderzoek een essentieel onderdeel van de ontwikkeling van nieuwe antikanker middelen is, maar het is mij nu ook duidelijk dat na registratie van een middel veel klinische vragen beantwoord kunnen worden door goed uitgevoerd klinisch farmacologisch onderzoek.

Vooruitzichten

Het doel van klinisch farmacologische studies van antikanker middelen is gegevens te verzamelen die leiden tot het verminderen van de bijwerkingen en het verbeteren van de effectiviteit van deze middelen. In verband met de beperkte therapeutische breedte kan dit onderzoek van grote waarde zijn voor de individuele patiënt. In de meeste gevallen gaf de, tot nu toe veelal gangbare, dosering per vierkante meter lichaamsoppervlak ons een onjuist gevoel van nauwkeurigheid. De interpatiënt farmacologische variabiliteit is gerelateerd aan parameters zoals: biobeschikbaarheid, uitscheiding en verdeling in het lichaam. Huidig en

toekomstig onderzoek richt zich op deze aspecten, in de hoop dat dit zal leiden tot preciezere doseringsschema's en/of verbetering van de effectiviteit en het bijwerkingenprofiel. De nieuwe doseringsschema's zullen veelal gebaseerd zijn op farmacologisch onderzoek in een grotere populatie. Op dit moment toetsen wij een dergelijk populatie farmacokinetiek model voor intraveneus topotecan. De nierfunctie en het geslacht blijken aan dit model bij te dragen, maar ik verwacht dat ander parameters ook een toegevoegde waarde zullen hebben. De rol van rode bloedcellen is nog niet exact bekend en wordt momenteel bestudeerd in een klinische studie.

De toegenomen kennis van menselijke genetica heeft er toe geleid dat er genetische varianten zijn gevonden die gerelateerd zijn aan verschillen in farmacologisch gedrag van medicijnen. Zo blijkt er genetische variatie bestaan in de zogenaamde cytochrome P-450 enzymen. Dit kan bijvoorbeeld leiden tot een groot verschil in de snelheid waarmee medicijnen uit het lichaam worden uitgescheiden. Een andere voorbeeld is de rol van het zogenaamde Breast Cancer Resistance-eiwit (BCR-eiwit) in de opname en dus biobeschikbaarheid van oraal toegediende medicijnen. Bepaling van de individuele BCR-eiwit spiegel zal hopelijk de biobeschikbaarheid van o.a. oraal topotecan helpen voorspellen, en beïnvloeding van dit soort medicijntransporterende eiwitten zal mogelijk tot een hogere en beter voorspelbare biobeschikbaarheid leiden. Het is ten alle tijde van belang de invloed van co-medicatie, en dus ook van andere cytotoxische middelen, en van de volgorde van toediening op de farmacokinetiek van het toegediende antikanker middel te beseffen. Iedere mogelijke farmacokinetische interactie dient uitgezocht te worden, aangezien het belangrijke klinische consequenties kan hebben. Interacties tussen verschillende medicijnen kunnen ook moedwillig worden gebruikt om de variatie in blootstelling aan een specifiek antikanker middel te verkleinen.

Nieuwe inzichten in de rol van CrEL, vooral in relatie met de vrije fractie van Taxol, en bepaling van de relatie tussen CrEL spiegels en de vrije fractie enerzijds en de bijwerkingen en effectiviteit anderzijds, zullen tot beter omschreven doseringsadviezen voor Taxol leiden. Afgezien van de negatieve aspecten van CrEL, is verder onderzoek nodig naar de eventuele positieve aspecten: selectieve beenmerg bescherming tijdens chemo- en radiotherapie en de voorkeur voor CrEL als toevoeging aan intraperitoneale chemotherapie.
Klinisch farmacologisch onderzoek zal de dokter die chemotherapie voorschrijft, in toenemende mate gaan leiden in zijn beslissingen. Uiteindelijk zal dit de individuele kanker patiënt ten goede komen.

DANKWOORD

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CURRICULUM VITAE

Hans Gelderblom werd op 31 oktober 1963 geboren te Den-Helder. In 1982 behaalde hij het diploma VWO, waarna hij naar de Verenigde Staten vertrok en aldaar in 1983 het High School examen behaalde. Vervolgens ging hij geneeskunde studeren aan de Vrije Universiteit te Amsterdam en deed onderzoek op de afdelingen endocrinologie en andrologie. Het artsexamen werd behaald in februari 1991. In de wachttijd voor aanvang van het vervullen van de dienstplicht was hij bedrijfsarts van het GAK te Amsterdam en arts bij de bloedbank Gooi- en Eemland. Bij de Koninklijke Marine was hij arts aan boord van de Hr. Ms. Tydeman en werkzaam als arts-assistent longziekten en interne geneeskunde in het Centraal Militair Hospitaal te Utrecht. Aansluitend startte hij in januari 1993 de opleiding interne geneeskunde, die plaats vond in het Academisch Ziekenhuis Utrecht (Prof. Dr. D.W. Erkelens), Streekziekenhuis Hilversum (Dr. F. van Kersen/Dr. S. Lobatto) en vanaf februari 1998 in de Daniel den Hoed Kliniek (Prof. Dr. G. Stoter). Op diezelfde datum begon de vervolg opleiding interne oncologie, waarna in maart 2000 registratie volgde. Tot zeer recent was hij werkzaam als internist-oncoloog in hetzelfde instituut. Zojuist is hij met zijn werkzaamheden als staflid klinische oncologie in het Leids Universitair Medisch Centrum begonnen.
Zijn belangrijkste hobbies zijn Dop, Bente, Pepijn en hockey.

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