Pentoxifylline Suppresses Irritant and Contact Hypersensitivity Reactions

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Pharmacologic suppression of the effector phase of contact hypersensitivity appears to have major relevance with regard to treatment of type IV reactions like contact dermatitis. Recently, tumor necrosis factor α has been shown to be a critical mediator in hapten-induced irritant and contact hypersensitivity reactions, thus offering new possibilities, for therapeutic intervention. Pentoxifylline, a methylxanthine derivative used in the treatment of vascular disorders, currently has been found to suppress the production of tumor necrosis factor α by human and murine leukocytes. Therefore, the effect of pentoxifylline on the elicitation phase of contact hypersensitivity was studied. Intraperitoneal injection of pentoxifylline into sensitized Balb/c and C3H/HeN mice before application of the challenging hapten dose resulted in a significant reduction of the outcome of the contact hypersensitivity reaction. The suppressive effect of pentoxifylline was dose dependent and maximally pronounced upon injection 3 h before hapten application. In contrast to the effector phase of contact hypersensitivity, induction of contact hypersensitivity was not affected by pentoxifylline when injected into naive mice before performance of sensitization. In addition, irritant dermatitis induced by 1% croton oil or 5% benzalkonium chloride was suppressed by pentoxifylline as well. These data suggest a potential pharmacologic intervention with pentoxifylline as a means to treat contact dermatitis. Key words: contact hypersensitivity/pentoxifylline/tumor necrosis factor α/suppression/irritant reaction. *J Invest Dermatol 101:549–552, 1993*

Contact dermatitis is a complex skin disorder that affects numerous patients. It comprises either an irritant or an allergic mechanism, the latter distinguished by the demonstration of a delayed-type allergy in the affected individual. Besides its remarkable frequency, contact dermatitis can be a disabling disorder, particularly in the chronic stage. Avoidance of the allergen, if detected, is not always practicable; in such cases corticosteroids applied either topically or systemically are the major therapy, bearing the risk, however, of long-term side effects. Therefore, development of new therapeutic strategies influencing contact hypersensitivity (CHS) reactions is of major importance.

Recently, tumor necrosis factor (TNF) α has been identified as a critical mediator in hapten-induced irritant and contact sensitivity reactions [1]. In particular, injection of TNFα antibodies into sensitized mice before application of the challenging dose of the respective hapten abrogated the ear-swelling response significantly. Although these observations contribute to the further understanding of the effector phase of CHS, their therapeutic implications are limited because injection of TNFα antibodies cannot be seriously considered for practical treatment of contact dermatitis in humans at present.

Pentoxifylline (PTX) is a methylxanthine derivative that has been used therapeutically for quite a long time in a huge number of patients suffering from vascular disorders [2]. PTX is thought to reduce the blood viscosity and to increase the filterability of blood cells, which results in a rise in capillary blood flow. Besides its hemorheologic activity, PTX recently has been found to suppress the production of TNFα by murine and human leukocytes [3,4]. In addition, it can counteract TNF stimulation of human granulocytes [5].

According to the observations that TNFα seems to be of importance in the effector phase of CHS [1] and that PTX can antagonize TNFα production and activity, respectively [3–5], PTX may exhibit the capacity to influence contact dermatitis. Therefore, in the present study, we investigated whether PTX is able to suppress the effector phase of the murine CHS model.

MATERIALS AND METHODS

Mice Balb/c and C3H/HeN mice were purchased from the Versuchstierzuchtanstalt Hannover, FRG, or from the Versuchstierzuchtanstalt Himberg, Austria.

Contact Hypersensitivity Mice were sensitized by painting 25 μl of 0.5% 2,4-dinitro-fluorobenzene (DNFB; Sigma Corp., St. Louis, MO) solution (acetone : olive oil, 4 : 1) on the razor-shaved abdomen on days 1 and 2 as reported previously [6]. On day 6 the left ear was challenged by applying 20 μl 0.3% DNFB; the right ear was treated with acetone/olive oil alone. The degree of ear swelling was measured with a spring-loaded micrometer (Mitutoyo, Japan). CHS was determined as the amount of swelling of the hapten-challenged left ear compared to the thickness of the vehicle (acetone : olive oil)-treated right ear in sensitized animals and expressed in cm × 10^{-3} or as percentage increase ear swelling. Negative controls consisted of mice that were ear challenged without prior sensitization. In some experiments, oxazolone (Sigma) was used (25 μl 0.8% for sensitization and 20 μl 0.3% for challenge). Each treatment group consisted of at least seven animals, and each experiment was performed at least three times.

Irritant Dermatitis For induction of irritant dermatitis 20 μl of 1% croton oil (Serva, Heidelberg, FRG) dissolved in acetone were applied on the

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Abbreviations: CHS, contact hypersensitivity; PTX, pentoxifylline.

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Table I. Effect of PTX on the Elicitation Phase of CHS

Mice	Sensitization[a]	Challenge[b]	Treatment[c]	% Ear Swelling[d]
Balb/c		DNFB (0.3%)		2.1 ± 3.7
Balb/c	DNFB (0.5%)	DNFB (0.3%)		51.4 ± 15.7
Balb/c	DNFB (0.5%)	DNFB (0.3%)	PTX	14.1 ± 7.4[e]
Balb/c		Oxazolone (0.3%)		0.5 ± 1.6
Balb/c	Oxazolone (0.8%)	Oxazolone (0.3%)		34.5 ± 11.5
Balb/c	Oxazolone (0.8%)	Oxazolone (0.3%)	PTX	12.7 ± 9.2[e]
C3H/HeN		DNFB (0.3%)		3.3 ± 4.3
C3H/HeN	DNFB (0.5%)	DNFB (0.3%)		29.7 ± 9.2
C3H/HeN	DNFB (0.5%)	DNFB (0.3%)	PTX	8.0 ± 7.2[e]

[a] Hapten was applied on the razor-shaved abdomen on days 1 and 2.
[b] Hapten was applied on the left ear on day 6.
[c] PTX (50 mg/kg body weight) was injected interperitoneally 3 h before performance of challenge.
[d] CHS is expressed as the percentage increase (mean ± SD) of the thickness of the challenged left ear compared to the thickness of the untreated right ear. Ear thickness (mean ± SD) of the untreated control was 19.3 ± 1.1 cm × 10^{-3}.
[e] Significantly different from positive control ($p < 0.001$).

left ear. Ear swelling was measured 8 h after irritant application with a spring-loaded micrometer. Irritant reaction was determined as the amount of swelling of the irritant-treated left ear compared to the thickness of the vehicle (acetone)-treated right ear and expressed in cm × 10^{-3}. As a further irritant, benzalkonium chloride (Sigma) was used dissolved in acetone at a concentration of 5%. Irritant application and measurement was performed as with croton oil.

Injection of PTX PTX (Dr. Rentschler Comp., Laupheim, Germany) dissolved in sodium chloride was injected intraperitoneally, the usual dose being 50 mg/kg body weight unless otherwise stated. Control mice were injected intraperitoneally with the equal volume (100 μl) of physiologic sodium chloride solution, which, however, had absolutely no effect on the outcome of both the sensitization and challenge procedure.

Histology The ears were fixed in buffered formaldehyde, embedded in parafin, sectioned, and stained with hematoxylin-eosin.

Statistical Analysis Statistical analysis was performed by the use of the Student t test. Differences below $p < 0.05$ were regarded as significant.

RESULTS

Intraperitoneal injection of 50 mg/kg PTX 3 h before DNFB challenge into DNFB-sensitized Balb/c mice resulted in a significant reduction of ear swelling, whereas intraperitoneal application of sodium chloride as a control had no effect (Table I). Application of DNFB to the ears of sensitized mice caused both epidermal and dermal alterations, in particular necrosis of keratinocytes, edema, hemorrhage, and leukocyte infiltration (Fig 1*A*). Administration of PTX prevented both the dermal and epidermal component of the CHS reaction (Fig 1*B*). Similar results were obtained when oxazolone was used instead of DNFB (Table I). The inhibitory effect of PTX on the effector phase of CHS was not strain specific, because comparable data were obtained with C3H/HeN mice (Table I). To determine whether the time lag between PTX injection and performance of ear challenge is of relevance, PTX was injected at 1, 3, 6, 12, or 24 h before challenge. The optimal suppressive effect was observed when PTX was applied 3 h before challenge (Fig 2). The inhibitory activity of PTX was dose dependent, yielding maximum suppression at a concentration of 50 mg/kg body weight (Fig 3). To study whether PTX also affects the induction phase of CHS, PTX was injected intraperitoneally into naive Balb/c mice 3 h before sensitization with 0.5% DNFB and challenge was performed 5 d later. PTX-treated mice, however, did not differ in their ear swelling response from that of positive control mice (Table II), suggesting that under these conditions PTX does not affect the induction phase of CHS. To investigate whether PTX also suppresses irritant reactions, PTX was injected intraperitoneally into Balb/c mice 3 h before application of 1% croton oil on the left ear. In contrast to mice treated with 1% croton oil only, ear swelling was found reduced in PTX-pretreated mice (9.3 ± 3.0 versus 5.2 ± 2.2 cm × 10^{-3}, $p < 0.05$). Similar results were obtained when benzalkonium chloride was used as an irritant (10.2 ± 3.1 versus 6.1 ± 4.4 cm × 10^{-3}, $p < 0.05$).

DISCUSSION

PTX, a methylxanthine known for many years for its hemorheologic properties, recently has been found to prevent multiple organ failure and acute respiratory distress syndrome induced by lipopolysaccharide or recombinant TNFα and IL-2, respectively, in animals [7–9]. PTX also has been shown to reduce the release of TNFα

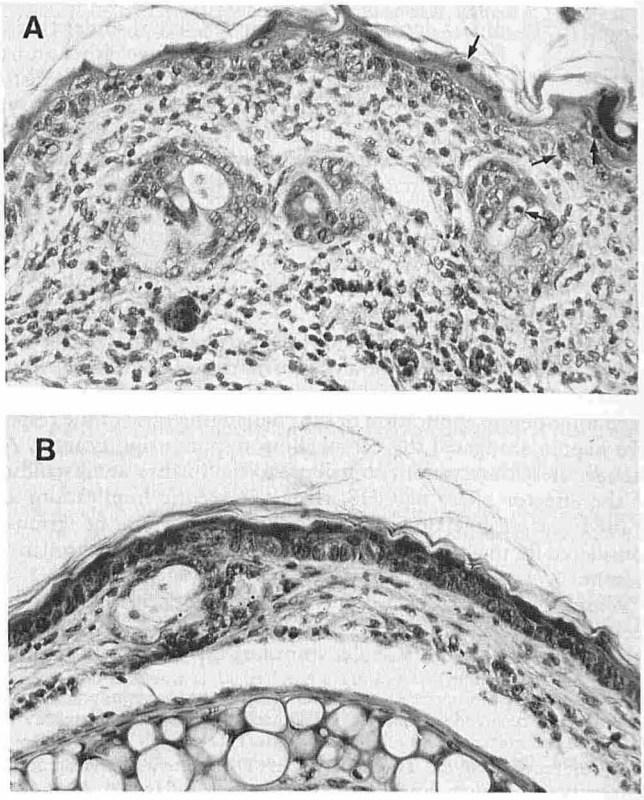

Figure 1. Ear sections from DNFB-sensitized mice (Balb/c) killed 24 h after application of challenging dose of DNFB. Mice were injected with physiologic sodium chloride *(A)* or with PTX intraperitoneally *(B)* 3 h before application of the challenging dose. In *A*, the dermis is edematous and infiltrated with leukocytes. In addition, necrotic cells are detectable within the epidermis and the hair follicles (→). In *B*, no changes are found in the epidermis and only a slight inflammatory infiltrate is detectable within the dermis. *Bar*, 20 μm.

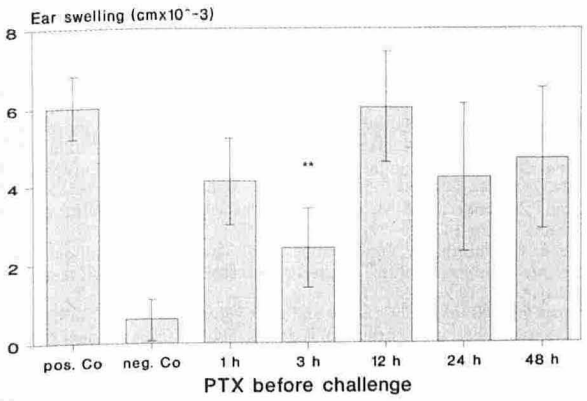

Figure 2. Time kinetics. PTX (50 mg/kg body weight) was injected intraperitoneally into Balb/c mice at the time points indicated before performance of ear challenge. Ear swelling was evaluated 24 h later as the thickness of the left ear minus the thickness of the untreated right ear and expressed as cm $\times$ 10^{-3} (mean $\pm$ SD). Negative control consists of unsensitized mice that were ear challenged only. Experiments were repeated at least three times; results show one representative experiment. **Significantly different from positive control ($p < 0.001$).

after injection of endotoxin to healthy human volunteers or to guinea pigs [10,11]. In the present study, the effect of PTX on the effector phase of CHS was investigated. The rationale for that was based on a recent report showing that TNFα seems to be a critical mediator during this event [1]. The present data provide evidence that PTX indeed is able to prevent elicitation of CHS reaction. The effect of PTX is dose dependent and maximally pronounced upon intraperitoneal injection 3 h before application of the challenging dose. Most experiments were performed with DNFB as the sensitizing agent; however, the suppressive effect of PTX was also observed when oxazolone was used. In addition, the effect of PTX is not strain specific because similar data were obtained when using Balb/c and C3H/HeN mice. PTX had no effect on the outcome of sensitization when injected 3 h before application of the sensitizing dose into naive mice, suggesting that it does not affect the induction phase. However, one cannot exclude a suppressive activity of PTX under other experimental conditions, e.g., injection at other time points or at higher doses.

The inhibitory effect of PTX on the effector phase of CHS,

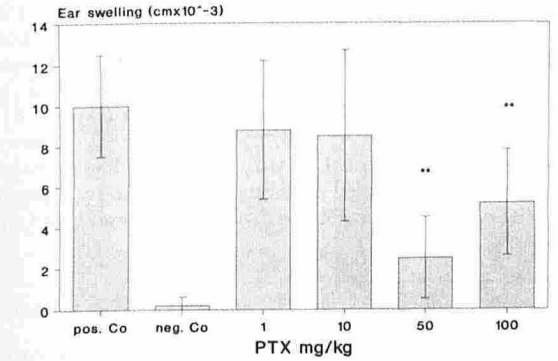

Figure 3. Dose kinetics. PTX was injected intraperitoneally at the concentrations indicated into DNFB-sensitized Balb/c mice. Three hours later challenge was performed on the left ear. Ear swelling was evaluated 24 h later as the thickness of the left ear minus the thickness of the untreated right ear and expressed as cm $\times$ 10^{-3} (mean $\pm$ SD). Negative control consists of unsensitized mice that were ear challenged only. Experiments were repeated at least three times; results show one representative experiment. **Significantly different from positive control ($p < 0.01$).

Table II. Effect of PTX on the Sensitization Phase of CHS

Mice	Sensitization[a]	Challenge[b]	Treatment[c]	% Ear Swelling[d]
Balb/c		DNFB (0.3%)		0.2 $\pm$ 0.4
Balb/c	DNFB (0.5%)	DNFB (0.3%)		60.7 $\pm$ 13.8
Balb/c	DNFB (0.5%)	DNFB (0.3%)	PTX	51.7 $\pm$ 19.8[e]
C3H/HeN		DNFB (0.3%)		0.8 $\pm$ 0.7
C3H/HeN	DNFB (0.5%)	DNFB (0.3%)		27.3 $\pm$ 14.0
C3H/HeN	DNFB (0.5%)	DNFB (0.3%)	PTX	29.0 $\pm$ 7.4[e]

[a] Hapten was applied on the razor-shaved abdomen on days 1 and 2.
[b] Hapten was applied on the left ear on day 6.
[c] PTX (50 mg/kg body weight) was injected interperitoneally 3 h before performance of sensitization on days 1 and 2, respectively.
[d] CHS is expressed as the percentage increase (mean $\pm$ SD) of the thickness of the challenged left ear compared to the thickness of the untreated right ear. Ear thickness (mean $\pm$ SD) untreated control was 20.1 $\pm$ 1.6 cm $\times$ 10^{-3}.
[e] Not significantly different from positive control.

however, does not seem to be strictly specific, in that irritant dermatitis induced by 1% croton oil and 5% benzalkonium chloride, respectively, were reduced by PTX as well. This may suggest that similar mechanisms are involved. These data are comparable to findings of Piguet *et al* showing that TNFα is of relevance in primary irritant reactions induced by hapten application [1]. Morphologic similarities between irritant and CHS reactions suggest involvement of the same effector mechanisms, in which TNFα is regarded to play a key role [1]. The same seems to be true also for other conditions, like graft-versus-host disease, cerebral malaria, BCG granuloma formation, and other granulomatous and fibrotic processes [12–16]. Whereas in these reactions T lymphocytes and macrophages are supposed to be the major source of TNFα, in hapten-induced irritant and hypersensitivity reactions keratinocytes are found to express increased amounts of TNFα [1].

PTX is a methylxanthine derivative that functions as a phosphodiesterase inhibitor [17]. PTX is well known for its hemorheologic properties attributed to its influence of red-cell deformability, plasma viscosity, and platelet reactivity [2]. Recently, it was shown that PTX blocks LPS-induced macrophage-derived TNFα at the mRNA and protein level [3]. Because other methylxanthines and dibutyryl cAMP have similar effects on TNF expression, the mechanism of this suppression is supposed to be *via* the generation of intracellular cAMP [3]. In addition, it was recently found that PTX blocks TNF mRNA accumulation in murine macrophages, but, in contrast to dexamethasone, has no effect on the efficacy of translation [18]. Keratinocytes currently have been described to produce TNFα [19] and thus appear to be a likely source of this mediator in irritant and CHS reactions [1]. Therefore, at present we are studying whether the inhibitory effect of PTX on TNFα release is also observed with keratinocytes *in vitro*.

Whether suppression of TNFα by PTX is in fact responsible for the blocking of the effector phase of CHS cannot be answered definitely by the present study. Although our data are in good accordance with those showing TNFα to be a critical mediator during these events [1], other mechanisms may be involved. For example, adhesion molecule expression (CD11a, CD11b, CD11c, CD18) has been found to be downregulated by PTX, resulting in a decreased granulocyte-adhesive function, possibly diminishing the inflammatory response in CHS reaction [20]. This assumption is supported by the observation that antibodies directed against such adhesion structures diminish CHS and irritant reactions [1]. In addition, PTX may directly affect endothelial cells. These alternative mechanisms have to be considered in light of a recent paper showing that TNFα antibodies suppress the induction of CHS while being ineffective when given after sensitization [21]. These data are

in sharp contrast to those by Piguet *et al* [1] and there is no good explanation for this discrepancy at present. However, differences in the dose, preparation, or specificity of the respective antibody may account for the different observations.

In conclusion, the present study demonstrates that PTX can suppress the effector phase of CHS and thus raises new pharmacologic questions about the treatment of CHS reactions. It remains to be determined whether PTX can only function preventatively, or whether it is also curative, i.e., inhibitory when given after administration of the hapten. Although the mechanisms of action involved are not yet clear, it is interesting to speculate on the potential pharmacologic intervention possible with PTX or its derivatives as a means to treat contact dermatitis. This is also supported by the fact that PTX, a drug with well-known pharmakokinetics and safety, has been used therapeutically in humans with vascular disorders for more than a decade, without major side effects.

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