Modulating Effects of Pentoxifylline on Cytokine Release Syndromes

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Introduction

Tumor necrosis factor-α (TNF) has been identified as the most important mediator of septic shock and cachexia in animals and humans. Moreover, TNF plays a major role in the pathophysiological events of many other infectious and immunological diseases. Therefore, drugs interfering with the formation of TNF may have beneficial effects in these clinical circumstances.

Among the various compounds elaborated by macrophages in response to endotoxins, prostaglandins have been recognized to contribute to the regulation of cytokine biosynthesis [1, 2]. TNF synthesis is negatively controlled by prostaglandin E (PGE), most probably by increases in intracellular cAMP. This finding has led to the hypothesis that endogenously formed PGE limits the extent of TNF synthesis by macrophages. In agreement with this, it has been found that inhibition of prostaglandin synthesis by indomethacin or aspirin results in increased TNF production [3]. Based on the above results, the assumption seemed reasonable that intracellular increases in cAMP by inhibition of phosphodiesterases (PDEs) would also lead to attenuation of TNF synthesis and, hence, should be of benefit in the syndromes described above. In our laboratories and clinic, a PDE inhibitor which is clinically in wide use, pentoxifylline (3,7-dimethyl-1-(5-oxo-hexyl)-xanthine, POF), has been tested with regard to its effects on cytokine production and endotoxin lethality. Pentoxifylline is a drug of known hemorrheological activity. Its effects were supposed to be based on its ability to increase erythrocyte flexibility, to reduce blood viscosity and filterability, and to increase capillary flow in several diseases. It has been used clinically for therapy of patients with various types of vascular insufficiency particularly intermittent claudication [4].

Pentoxifylline Inhibits Tumor Necrosis Factor Synthesis in Macrophage Cultures and in Lipopolysaccharide-Treated Mice

The putative TNF inhibitory activity of POF was first tested in macrophage cultures stimulated with endotoxin (lipopolysaccharide, LPS; 100 ng/ml) in the presence of POF. The results shown in Fig. 1 provide evidence that POF, over the whole concentration range of 3.1–50 μg/ml, provided significant in-

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hibition on the formation of TNF by macrophages [5]. This finding is in agreement with the reported evidence of others that POF inhibited the formation of TNF mRNA in mouse peritoneal macrophages [6] and TNF released by human monocytes [7]. In order to further characterize the action of POF on TNF synthesis, its inhibitory activity was studied in mice [5]. TNF determinations were done in sera of D-galactosamine-sensitized mice challenged with endotoxin (LPS; 10 μg/animal). The sera were sampled 1 h after LPS injection and TNF was quantified in an L-cell cytolysis assay. The values for TNF activity in serum are shown in Fig. 2. POF, at a dose of 50 mg/kg suppressed TNF formation completely and 10 mg/kg had pronounced inhibitory effects on the appearance of TNF in serum.

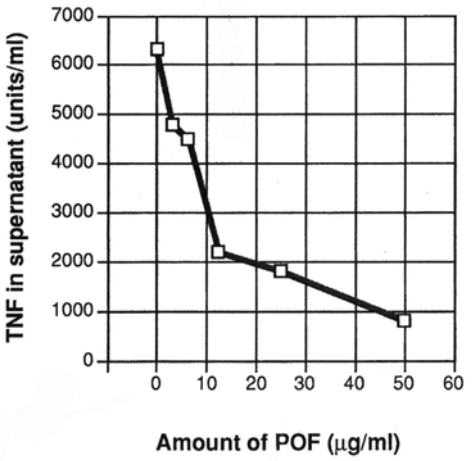

Fig. 1. Inhibition of endotoxin (LPS)-induced tumor necrosis factor (*TNF*) synthesis in mouse peritoneal macrophages by pentoxifylline (*POF*). Values obtained with more than 10 μg/ml POF were significantly ($p<0.05$) different from controls without POF

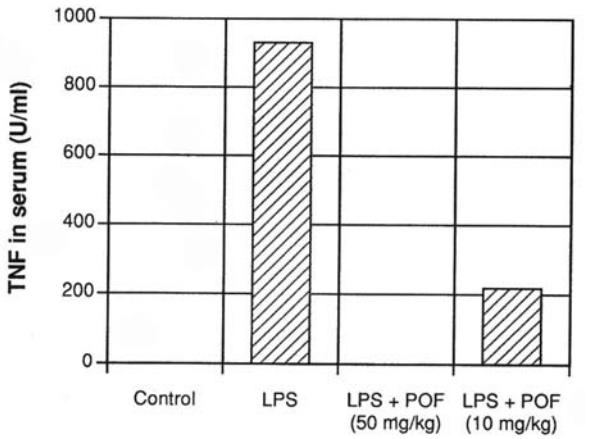

Fig. 2. Inhibition of endotoxin (LPS)-induced tumor necrosis factor (*TNF*) synthesis in mice by pentoxifylline (*POF*)

Effects of Pentoxifylline on Endotoxin Lethal Toxicity

Since the above data showed that POF was able to inhibit TNF synthesis *in vitro* and *in vivo,* it was suggested that xanthine derivatives potentially could be of benefit in endotoxin shock. Therefore, the effect of POF was investigated in several models of endotoxin shock [5, 8–10].

Pretreatment of normal mice with POF (50 mg/kg) 1 h before the challenge with LPS (500 μg/mouse, equivalent to a LD_{50}) led to a significant increase in the survival rate compared to mice challenged with LPS alone. The overall survival rate increased from 50% to 90% (Fig. 3). Mice were rendered hypersensitive against endotoxin by a pretreatment with endotoxin 14 h before the challenge injection. Injection of 200 μg LPS corresponded in LPS-hypersensitive mice to a LD_{80}. Pretreatment of LPS-hypersensitive mice with POF (50 mg/kg) led to a survival rate of 90% (Fig. 3). Mice were rendered tolerant by a pretreatment with endotoxin (80 μg) 4 day prechallenge. When these mice were challenged with a dose of LPS, otherwise corresponding to a LD_{50}, and POF (50 mg/kg), a survival rate of 90% was observed (Fig. 3). As shown in Fig. 3, POF interferes with the lethal effects of LPS in normal, LPS-hypersensitive, and LPS-tolerant mice.

Pentoxifylline Inhibits Endogenous Tumor Necrosis Factor Formation in Endotoxemia in Human Volunteers

Based on the promising findings obtained in experimental animals we sought to approach the question as to the possibility that POF could be of benefit in humans too. A study, therefore, was designed to investigate the effects of

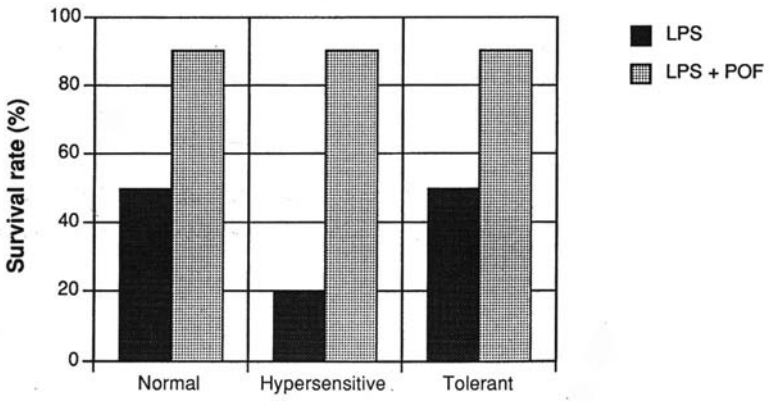

Fig. 3. Protective effect of pentoxifylline (*POF*; 50 mg/kg) on endotoxin (lipopolysaccharide, *LPS*)-induced lethality in normal (15 mg/kg) LPS-hypersensitive (6 mg/kg), and LPS-tolerant (90 mg/kg) mice

POF in human volunteers under conditions of controlled endotoxemia [11].

Ten male volunteers (26–38 years old), each of whom had given informed consent before joining the study, were treated with endotoxin and examined on two occasions separated by at least 21 days: endotoxin preparation (sodium salt of LPS from *Salmonella abortus equi*) was dissolved in isotonic saline and given as a bolus injection (100 ng) intravenously at 8.00 a.m. An intravenous infusion of isotonic saline (125 ml/h) on the first occasion or POF (500 mg in 500 ml isotonic saline) on the second occasion was started 30 min before endotoxin administration and continued for 4 h.

In nine of ten subjects there was a rise in body temperature of at least 1.0 °C, paralleled by an increase in heart rate. The only subject who did not respond to endotoxin weighed more than 95 kg; the endotoxin dose of 100 ng may not have been high enough to induce the typical symptoms. Eight of the remaining nine subjects responded to endotoxin with symptoms such as myalgia and headache, which started 70–100 min after the injection. Two subjects also had nausea (120 min after endotoxin). Systolic and diastolic blood pressure were not affected. None of the clinical responses to endotoxin were affected by POF.

Circulating TNF levels were determined in sera sampled over short time intervals. It was found that a maximum serum level was reached 2 h postendotoxin (Fig. 4). This increase was statistically significant. At no other time could significant increases in TNF levels be determined. When POF was infused, no increase in TNF levels was observed (Fig. 4). Interleukin-6 (IL-6) serum levels showed a significant increase with its maximum 3 h after endo-

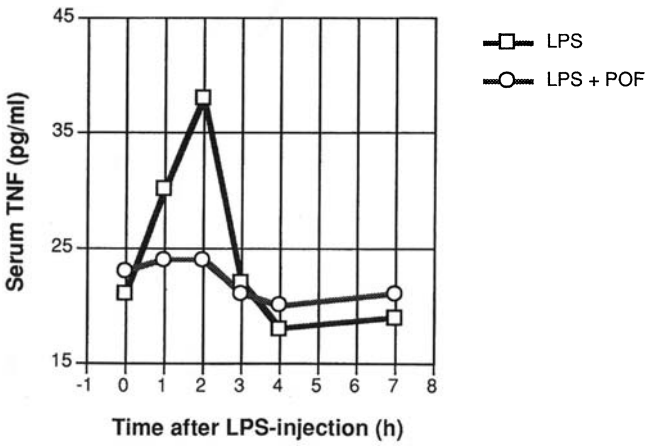

Fig. 4. Effect of pentoxifylline (*POF*; 500 mg/4 h) on circulating tumor necrosis factor (*TNF*) levels in humans after injection of lipopolysaccharide (*LPS*; 100 ng). TNF levels 2 h after LPS administration were significantly different between treatment with and without POF

toxin in comparison to baseline levels. The rise of IL-6 was strictly correlated with the rise of body temperature and appeared 1 h after TNF peak levels. POF treatment did not affect IL-6 levels after endotoxin administration.

In all subjects the cortisol levels before endotoxin injection were in the normal range. In contrast to the expected slight fall due to the known circadian rhythm, cortisol levels 1 h after endotoxin were much lower than baseline. This fall was followed by an apparent rise with a peak 4 h after endotoxin injection, but the levels had reached the normal range of afternoon cortisol levels 7 h after endotoxin injection. POF treatment did not affect the changes in cortisol levels induced by endotoxin.

In conclusion, we have confirmed the findings of Michie et al. [12] that endotoxin injection in humans leads to a short-term rise in circulating TNF levels. Infusion of POF totally abolished the endotoxin-induced rise in TNF levels. We conclude, therefore, that the lower levels of TNF are due to suppression of its formation by POF. This inhibition was selective for TNF without affecting IL-6 levels. This finding supports the hypothesis that endotoxin-induced IL-6 synthesis occurs independently from the preceding TNF synthesis. Also, clinical signs of endotoxemia, such as fever and myalgia as well as high cortisol levels, which are all thought to be provoked by interleukins [13], are not affected by POF treatment. This result suggested distinct regulatory pathways for different cytokines. It is also in agreement with the earlier finding that increases in cellular cAMP selectively suppressed endotoxin-induced gene expression in macrophages [14]. This study showed that the positive effects of POF determined in animal models may also apply to the human situation. Although controlled endotoxemia in volunteers differs from septic shock and related clinical conditions, our data provide evidence that POF may also have beneficial effects in these clinical situations.

Pentoxifylline Suppresses OKT3-Induced Tumor Necrosis Factor Formation in Renal Transplant Recipients

Treatment of allograft transplant recipients with the murine anti-CD3 monoclonal antibody OKT3 leads to a systemic reaction, characterized by chills, fever, nausea, vomiting, diarrhea and sometimes lung edema. Several reports strongly suggested that these side effects are related to the release of cytokines. In particular, TNF seems to play a pivotal role in the pathophysiology of OKT3-induced systemic reactions. It was demonstrated that passive immunization against TNF attenuated the severe side effects of OKT3 in experimental mice [15]. Furthermore, a strong correlation between OKT3 first dose reaction and serum levels of TNF was shown in humans [16]. We, therefore, investigated the efficacy of POF in inhibiting TNF formation and, thus, preventing the severe side effects of OKT3 first dose administration in renal transplant recipients [17].

We studied 16 recipients of a renal allograft during steroid-resistent rejec-

tion episodes. The diagnosis of rejection was based on clinical and laboratory data and histological findings from fine-needle biopsy. Acute rejection episodes were initially treated with methylprednisolone (MP): 500 mg/day on 4 consecutive days. In the cases where treatment with corticosteroids failed, OKT3 was administered as a daily bolus injection of 5 mg intravenously on at least 5 consecutive days. As recommended by the manufacturer, a single dose of hydrocortisone (100 mg) was administered immediately after the OKT3 first dose injection. Eight patients (controls) were treated with MP (1 mg/kg) 30 min prior to OKT3. Another group of eight patients (POF group) received an intravenous infusion of POF (400 mg in 500 ml isotonic saline) which was started 30 min before the OKT3 first dose and continued for 3 h.

TNF serum levels increases significantly 2 h after OKT3 administration in the controls as compared to baseline levels and POF treatment suppressed OKT3-induced TNF formation (Fig. 5). A significant increase of IL-6 levels

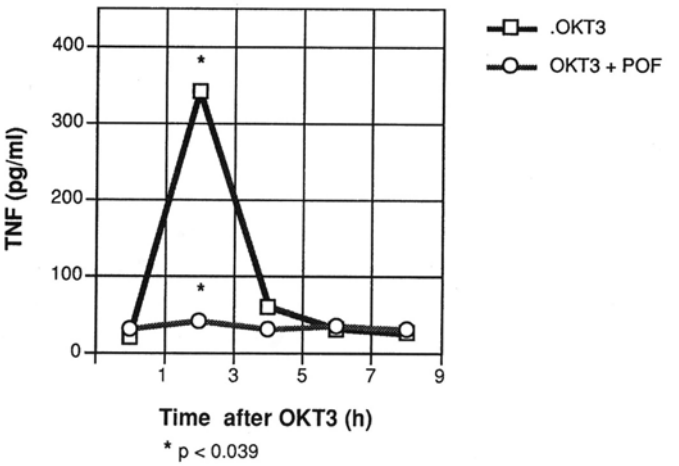

Fig. 5. Effect of pentoxifylline (*POF*; 1.2 g/day) on endogenous tumor necrosis factor (*TNF*) formation in renal transplant recipients after OKT3 first dose injection (5 mg)

Table 1. Clinical response to OKT3 first dose administration in renal transplant recipients: effect of pentoxifylline (POF)

	Controls	POF-treated
Fever >39°C	5/8	2/8
Vomiting/diarrhea	4/8	0/8
Dyspnea	2/8	0/8
Headache	4/8	0/8

as compared to baseline levels was found in all patients 4 and 6 h after OKT3 injection. There were slight but no significant differences in maximal IL-6 values at 6 h post-OKT3 between controls and POF-treated patients. The same was true for circulating interleukin-8 (IL-8) levels, which reached maximal levels 2 h after OKT3 injection in all patients, and which also remained unaffected by POF treatment. With respect to the clinical symptoms (Table 1), POF did not abolish the febrile response, although the number of patients developing high grade temperature seemed to be lower in the POF group. Severe side effects of OKT3 first dose, such as gastrointestinal symptoms, headache and dyspnea, were not observed in any patient in the POF group, whereas nearly all of the controls showed at least one of these symptoms. Most importantly, the OKT3-induced reduction of CD3-positive peripheral blood lymphocytes was not influenced by POF, suggesting that POF did not alter the efficacy of the OKT3 therapy.

In conclusion, this pilot trial demonstrated that POF is of potential benefit in OKT3 treatment based on its ability to suppress TNF formation, thus preventing severe clinical side effects. The data of this trial are encouraging and have been confirmed by others [18, 19].

Effects of Pentoxifylline on Circulating Cytokines in Severe Pulmonary Tuberculosis

Tumor necrosis factor has been identified as an important mediator of chronic cachexia in animals and humans [20]. After having shown that POF can reduce both endotoxin and OKT3-induced endogenous TNF formation in humans, we were also interested in its effects on diseases related to cachexia possibly due to chronic release of TNF. Severe pulmonary tuberculosis is associated with systemic reactions, including cachexia, fever and night sweat. These symptoms may be caused by a chronic cytokine release syndrome, in particular, the release of TNF.

Therefore, circulating cytokines were determined in patients with severe pulmonary tuberculosis and systemic reactions under treatment with tuberculostatic agents, and these patients were treated with POF. Patients with a low disease activity and without systemic symptoms served as controls [21].

As shown in Fig. 6, patients with severe pulmonary tuberculosis including systemic reactions (group 1) showed significantly elevated serum TNF levels in comparison to patients with low disease activity without any systemic reaction (group 2). In patients of group 2 serum TNF levels were found at the detection limit of the assay. TNF levels were strictly correlated with the severity of the disease. POF treatment (1.2 g orally/day) of patients in group 1 resulted in an immediate decrease of circulating TNF levels to baseline inducing well-being and a stop of weight loss. IL-6 levels were also significantly increased in patients of group 1 as compared to patients without clinical symptoms (group 2). Patients of group 2 also showed IL-6 levels at the

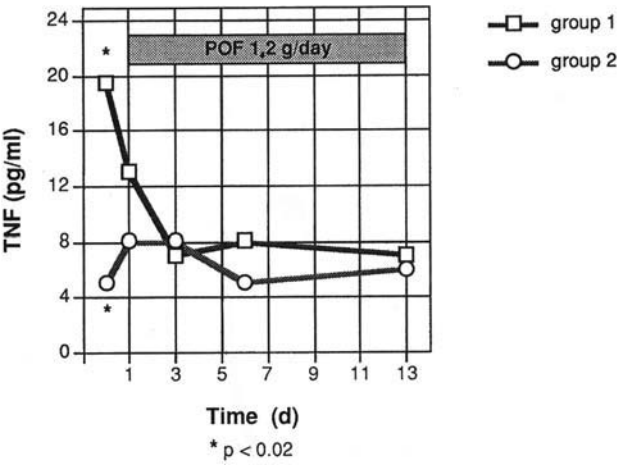

Fig. 6. Modulating effect of pentoxifylline (*POF*; 1.2 g/day) on elevated circulating tumor necrosis factor (*TNF*) levels in patients with severe pulmonary tuberculosis and systemic reactions (group 1; *squares*). Patients with low disease activity without systemic symptoms served as controls (group 2; *circles*)

detection limit of the assay. POF treatment failed to influence the elevated serum IL-6 levels or the febrile response in patients of group 1.

In conclusion, it was found that severe pulmonary tuberculosis is associated with elevated levels of circulating cytokines (TNF and IL-6). Furthermore, POF treatment inhibited chronic TNF formation selectively, and thus reduced TNF-dependent cachexia without affecting chronic IL-6 formation and related symptoms, such as the febrile response and night sweat.

Table 2. Inhibition of endogenous tumor necrosis factor formation by pentoxifylline: new therapeutic strategies

Indication	Efficacy
Transplantation	
Bone marrow transplant	GVH reaction, vascular leakage syndrome, pneumonitis
Renal transplant	OKT3 side effects, acute renal failure
Septic syndrome	ARDS, multiple organ failure
AIDS	Cachexia, virus replication
Tumors	Cachexia
CNS	
Cerebral malaria	Clinical outcome
Multiple sclerosis	Disease activity

Perspectives

As shown above, POF is able to attenuate endogenous TNF formation in humans both in experimental and clinical conditions and thus may improve therapeutic strategies in various diseases in which TNF is identified as a pivotal pathophysiological factor (Table 2). Some of these indications have been examined or will be investigated in further clinical studies.

1) In allogenic bone marrow transplantation increased serum levels of TNF precede major complications such as graft-vs-host (GVH) reaction, vascular leakage syndrome and pneumonitis [22]. Preliminary data of a clinical study performed in Seattle (Washington, USA) in patients undergoing allogenic bone marrow transplantation showed that prophylactic administration of POF can prevent major complications and significantly decreases mortality after 1 year of follow-up [23]. In renal transplantation, POF treatment may improve OKT3 therapy as shown above. Furthermore, there is evidence that POF may prevent hypoxia-related changes in the renal function of transplanted kidneys [24].
2) *In vivo,* POF protected against increased pulmonary vascular permeability and sequestration of neutrophils in the lung of different animal models of acute lung injury [25]. Consequently, as a general outcome, POF was found to improve survival in different models of hemorrhagic [26] and endotoxic shock [8, 27]. Our data show that these findings from animal models may also apply to human beings [28].
3) Increased TNF levels have been observed in cachectic patients with the acquired immunodeficiency syndrome (AIDS), and TNF is known to increase expression of the human immunodeficiency virus type 1 (HIV-1) by acting on its long terminal repeat. Moreover, TNF was found to decrease the therapeutic efficacy of zidovudine (AZT). POF is able to decrease the replication of HIV-1 in human peripheral blood mononuclear cells and in cultured T cells [29]. Thus, patients with AIDS may benefit from POF treatment because it blocks TNF-mediated HIV-1 up-regulation, potentially increases the efficacy of AZT, and may prevent TNF-induced cachexia.
4) TNF has been implicated as mediator of the severe wasting seen in terminal cancer patients, from which its name cachectin is derived [30]. Recently, it was shown that POF-treated cancer patients experienced an improvement in general well-being accompanied by significant decreases in TNF mRNA levels of peripheral blood monocytes [31].
5) In some diseases of the central nervous system such as cerebral malaria [32] and multiple sclerosis [33], elevated TNF levels in the serum and cerebrospinal fluid correlate with fatal outcome and disease progression, respectively. POF was able to prevent cerebral malaria in *Plasmodium berghei*-infected mice [34]. In POF-treated mice, TNF in serum was nondetectable, whereas control mice had high TNF levels on day 6 after infec-

tion. These findings make POF a potential candidate as a supportive agent in human cerebral malaria.

In conclusion, POF is an established drug with no severe side effects and a wide therapeutic range. It may improve therapeutic strategies in cases of acute and chronic cytokine release syndromes by acting as a selective inhibitor of TNF synthesis.

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