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Pentoxifylline Modulates Meningeal Inflammation in Experimental Bacterial Meningitis

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Pentoxifylline has been shown to decrease endotoxin-induced tumor necrosis factor alpha production and reverse the inflammatory actions of interleukin-1 (IL-1) and tumor necrosis factor on leukocyte function. Because of the potential role of this cytokine-leukocyte interaction in the pathogenesis of bacterial meningitis, we investigated the ability of pentoxifylline to modulate meningeal inflammation in the rabbit meningitis model. Pentoxifylline treatment (initially an intravenous injection of 20 mg/kg followed by 6 mg/kg per h) started 20 min before intracisternal injection of 20 ng of *Haemophilus influenzae* type b lipooligosaccharide (endotoxin) reduced significantly concentrations in cerebrospinal fluid of leukocytes ($P < 0.0001$), protein ($P < 0.001$), and lactate ($P < 0.001$) during the 9-h infusion compared with values in intravenous-saline-treated rabbits. When pentoxifylline was given 1 h after *H. influenzae* type b endotoxin, the mean peak lactate and leukocyte concentrations in cerebrospinal fluid were significantly lower than those in control animals. Pentoxifylline also significantly decreased lactate and protein concentrations ($P < 0.05$) and tended to diminish leukocyte counts ($P = 0.08$) compared with results in control animals after antibiotic-induced release of endotoxin in animals with *H. influenzae* meningitis. In this regard, dexamethasone was superior to pentoxifylline and no synergism was observed when the drugs were combined. Additionally, pentoxifylline attenuated meningeal inflammatory changes induced by intracisternal inoculation of 10 ng of rabbit recombinant IL-1β compared with results in either dexamethasone- or saline-treated animals. We conclude that pentoxifylline is effective in this animal model in modulating the meningeal inflammatory response following intracisternal inoculation of *H. influenzae* type b endotoxin or organisms or rabbit recombinant IL-1β.

Significant advances in antimicrobial therapy of bacterial meningitis have been made in recent years; however, the morbidity and mortality rates attributable to this infectious disease remain high. An area of intense interest is assessment of new therapeutical modalities that regulate the inflammatory response in acute bacterial meningitis. It has been speculated that the inflammatory response does little to control the infection and likely has an adverse effect on the central nervous system (4, 9). One of the principal protagonists of this inflammatory response is the polymorphonuclear leukocyte. Several hours after meningeal infection an intense influx of leukocytes occurs. Bacterial cell wall fragments and endotoxin have been shown to induce production of inflammatory mediators, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), by macrophages and other cells (6, 25, 28; B. Wispelwey, W. J. Long, J. M. Castracane, and W. M. Scheld, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 873, 1988). These cytokines stimulate the function of neutrophils and provoke degranulation, superoxide production, and increased leukocyte adherence to endothelium (1). The hydroxyl radicals and several enzymes released by neutrophils after activation are thought to have deleterious effects on brain tissue (5). Agents that can reduce leukocyte influx into cerebrospinal fluid (CSF) and/or modify their inflammatory activities could be beneficial in preventing brain damage.

Because of its fibrinolytic activity and ability to inhibit platelet aggregation, pentoxifylline [1-(5-oxo-hexyl)-3,7-dimethylxanthine] has been used for several years for treatment of patients with intermittent claudication resulting from occlusive arterial disease (27). Recently, it has been found that pentoxifylline can improve survival rates and decrease organ damage in different animal models of bacterial infection (3, 13, 31, 34, 40; H. S. Bjornson, C. Cave, and A. B. Bjornson, 25th ICAAC, abstr. no. 674, 1985). Moreover, pentoxifylline can reverse or counteract many of the effects of endotoxin and endotoxin-induced cytokines on leukocyte function (33). For example, neutrophils that have been activated by inflammatory cytokines and incubated with pentoxifylline had decreased superoxide production, decreased degranulation, and decreased adherence to endothelial cells. In addition, recent evidence indicates that pentoxifylline can also depress in vitro production of TNF by macrophages (32).

In view of these properties, we investigated the efficacy of pentoxifylline in modulating the meningeal inflammatory response in rabbits that occurs after intracisternal inoculation of *Haemophilus influenzae* type b or administration of *H. influenzae* type b lipooligosaccharide (endotoxin) or rabbit recombinant IL-1 beta (rrIL-1β).

MATERIALS AND METHODS

Bacterial strain. *H. influenzae* type b strain DL42 is a non-β-lactamase-producing organism isolated from the CSF of a child with meningitis. This strain is fully virulent in the infant rat model for invasive *H. influenzae* type b disease and belongs to lipooligosaccharide antigenic group 2, which is the predominant endotoxin antigenic type among invasive strains of this pathogen (11).

Preparation of live *H. influenzae* type b inoculum for intracisternal inoculation. *H. influenzae* type b strain DL42 was grown in brain heart infusion broth supplemented with

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NAD and hemin (10 μg of each per ml). After overnight incubation, the culture was centrifuged at 300 × *g* at 4°C for 20 min. The supernatant was removed and the cells were suspended in phosphate-saline buffer (pH 7.2) at a final inoculum of 1×10^5 to 2×10^5 colonies per ml.

Preparation of *H. influenzae* type b endotoxin. Endotoxin was purified from cells of *H. influenzae* type b strain DL42 by using the hot phenol-water method described by Westphal and Jann (41) as modified by Johnson and Perry (14). The purity of this endotoxin preparation was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by silver staining (37) and Western (immuno-) blot analysis with hyperimmune rat serum to *H. influenzae* type b DL42 (16). It has been shown previously that *H. influenzae* type b endotoxin, in either its purified form or as an integral part of the outer membrane of *H. influenzae*, when injected intracisternally induces meningeal inflammation and alteration of blood-brain barrier permeability (26, 35). Intracisternal inoculation of 20 ng of *H. influenzae* type b endotoxin consistently induced substantial changes in the indices of meningeal inflammation (35); this amount of endotoxin is contained in approximately 2×10^6 *H. influenzae* type b cells. Because CSF samples from patients with *H. influenzae* type b meningitis contain from 10^4 to 10^7 colonies per ml, 20 ng of *H. influenzae* type b endotoxin was chosen as an appropriate inoculum to induce meningitis in our studies.

Preparation of rrIL-1β. rrIL-1β was kindly provided by Masaru Yoshinaga, Kumamoto University, Kumamoto, Japan. This molecule was purified from *Escherichia coli* HB101 strains previously transformed with an rrIL-1β-containing plasmid (21). Twenty picograms of rrIL-1β corresponds to 1 U when estimated by thymocyte comitogenic assay. The amount of endotoxin contained in this preparation is approximately 1 pg/ml, as measured by *Limulus* assay.

Cytolytic assay for TNF. TNF activity was determined by using a L929 cell line assay previously described by Flick and Figgord (10) with some modifications (25). The degree of cytotoxicity was quantified spectrophotometrically (490 nm) by using a computerized automated enzyme-linked immunosorbent assay plate reader (model 2550 enzyme immunoassay reader; Bio-Rad Laboratories, Hercules, Calif.). Equivalent concentrations of recombinant human TNF were determined for experimental samples by interpolation of the recombinant human TNF standard curve run simultaneously (0.1 pg/ml to 1 μg/ml). The lower limit of detectability of this assay was 10 pg/ml. Samples were assayed in quadruplicate, and a standard deviation within 10% of the mean was observed. To verify the specificity of the assay, samples were preincubated with polyclonal anti-TNF antibody, which showed complete elimination of the cytotoxicity. TNF is stable for long periods when stored at −70°C.

To rule out the possibility that pentoxifylline was cytotoxic to L929 cells, different amounts of the drug (1 ng to 1 μg) were added directly to these cells and tested for cytolytic activity. No cytolytic activity was observed.

Measurement of lactate and protein in CSF. CSF samples were analyzed for lactate by a kinetic enzymatic method that uses the reaction of lactate with β-NAD^+ in the presence of lactate dehydrogenase to produce NADH and pyruvate. The production of NADH was quantitatively monitored at an A_{340} on a model 2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) with a deuterium source. The precision of the method was tested by running a known standard of 40 mg/dl (UV test 826-UV; Sigma Chemical Co., St. Louis, Mo.) 10 times over the course of 1 day. The coefficient of variation was 2.1%.

Protein concentrations were determined by the turbidity method. Turbidity produced by sulfosalicylic acid when added to a solution containing protein is proportional to the concentration of the protein in the solution. Turbidity was measured with a spectrophotometer set at 420 nm. A protein standard (no. p-7656; Sigma) as a control was assayed with each run.

Experimental drug. Pentoxifylline (Trental) was supplied by Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J., in 5-ml vials containing 20 mg of the drug per ml. Pentoxifylline was administered in an intravenous (i.v.) injection of 20 mg/kg of body weight followed by continuous infusion of 6 mg/kg per h for 6 to 12 h. This dosage was chosen on the basis of data from previous studies in several animal models of infection (3, 13, 31, 34, 40; Bjornson et al., 25th ICAAC).

Meningitis model and in vivo experiments. A model of experimental meningitis originally described by Dacey and Sande (7) was used in a modified form. New Zealand White male rabbits (2 to 3 kg) were anesthetized with intramuscular injections of ketamine (40 mg/kg of body weight) and acepromazine (3 mg/kg) and immobilized in a stereostatic frame. A spinal needle (3.5 inches, 20 gauge) was introduced into the cisterna magna, and 0.2 ml of CSF was withdrawn. The animals were then inoculated intracisternally with 0.2 ml containing 20 ng of *H. influenzae* type b endotoxin, 2×10^4 colonies of live *H. influenzae* type b organisms, or 10 ng of rrIL-1β.

***H. influenzae* type b lipooligosaccharide experiments.** Twenty-seven rabbits received 20 ng of endotoxin intracisternally. Fourteen of those were treated with a continuous i.v. infusion of normal saline (3 ml/kg of body weight per h) starting 20 min before endotoxin inoculation. The other 13 rabbits received a continuous i.v. infusion of pentoxifylline in a regimen (see above) providing the same infusion volume and rate as that employed in controls. Both treatment regimens were maintained for 9 h. CSF samples of 0.2 ml were obtained at 1, 3, 6, and 9 h after endotoxin inoculation and analyzed immediately for leukocyte counts with a Neuber hemacytometer (American Optical Corp., Buffalo, N.Y.).

The efficacy of pentoxifylline was also assessed when administered i.v. 1 h after *H. influenzae* type b endotoxin was given intracisternally. Eight animals were inoculated intracisternally with 20 ng of endotoxin, and treatment with saline or pentoxifylline commenced 1 h later and continued for 8 h.

The effect of CSF sampling on meningeal inflammatory indices is believed to be minimal because the total volume of CSF in a 2- to 3-kg rabbit is 4 to 5 ml and it is possible to withdraw at one time as much as 1.6 to 1.7 ml of CSF from the cisternae magnae of these animals; this was not done in any of our experiments. Immediately after collection, CSF samples were centrifuged (3,500 × *g*, 5 min) and the supernatant was stored at −70°C until assayed for TNF, protein, and lactate concentrations.

***H. influenzae* type b organism experiments.** Thirty-five rabbits were inoculated intracisternally with an inoculum of 2×10^4 colonies of live *H. influenzae* type b organisms contained in 0.2 ml. At 6 h after inoculation, the animals were treated with the following four regimens.

(i) Eight animals received a continuous i.v. infusion of normal saline (3 ml/kg of body weight per h).

(ii) Eight rabbits were treated with an i.v. injection of 1 mg

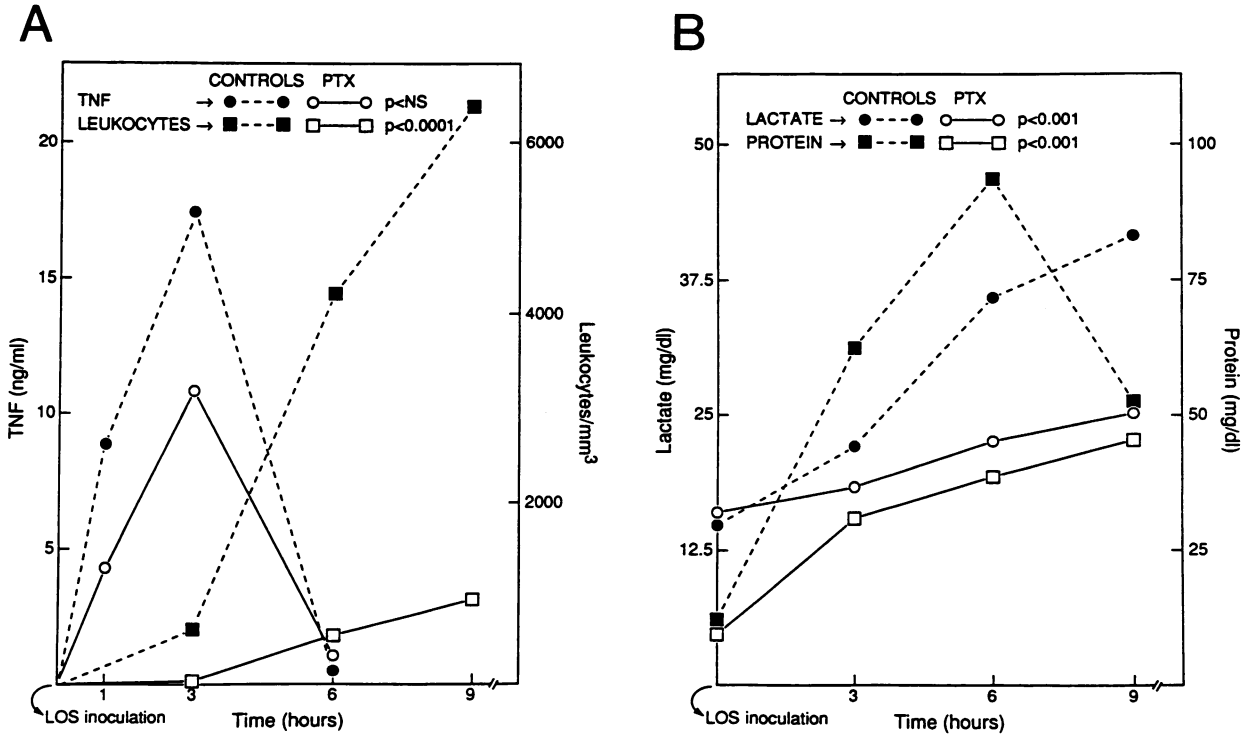

FIG. 1. Modulation of *H. influenzae* lipooligosaccharide (LOS)-induced meningeal inflammation by treatment with pentoxifylline (PTX). Saline (n = 14 rabbits) or pentoxifylline continuous I.V. infusions (n = 13) were started 30 min before intracisternal inoculation of lipooligosaccharide and maintained for 9 h. (A) Leukocyte and TNF concentrations in CSF. NS, Not significant. (B) Lactate and protein concentrations in CSF.

of dexamethasone per kg of body weight 30 min after a saline infusion, as described above, was started.

(iii) Eight animals received an i.v. injection of 20 mg of pentoxifylline per kg of body weight followed by a continuous infusion of 6 mg/kg per h (3 ml/kg per h).

(iv) Eleven rabbits were treated with a combination of regimens ii and iii. All infusions were maintained for a 6-h period. In addition, all animals received ceftriaxone (100 mg/kg i.v.) 30 min after infusions were started. Samples of 0.2 ml of CSF were obtained at 6, 8, 10, and 12 h after *H. influenzae* type b inoculation and processed as described for the endotoxin experiments.

IL-1 experiments. Sixteen rabbits were inoculated intracisternally with 10 ng of rrIL-1β (0.2 ml) 30 min after having undergone one of the three following regimens.

(i) Six animals received a continuous i.v. infusion of normal saline (3 ml/kg per h).

(ii) Six animals were treated with saline as described above and in addition received 1 mg of dexamethasone i.v. per kg.

(iii) Four rabbits received an i.v. injection of pentoxifylline (20 mg/kg) followed by a continuous infusion of 6 mg/kg per h (3 ml/kg per h). All infusions were maintained for 12 h. Two CSF samples were obtained from each animal at 6-h intervals and processed as described above.

The 10 ng of rrIL-1β has been shown to consistently induce CSF pleocytosis when inoculated intracisternally in rabbits (unpublished observations).

Statistical analysis. All data are presented as means ± 1 standard deviation. Two-way analysis of variance was used to assess the effect of independent treatments with time for the following variables: leukocyte, lactate, protein, and TNF concentrations. If the interaction of groups by time was significant at the 0.10 level, then pairwise comparisons were made for groups at each specific time and within a group for different time periods. A Bonferroni approach (20) was employed for the pairwise comparisons, with the 0.05 level considered significant. A chi-square test was used to compare independent proportions at a particular time point, and the two-tailed Student t test was used to compare means at specific points. Values of $P < 0.05$ were considered significant.

RESULTS

Endotoxin experiments. In initial experiments, 14 rabbits were treated with saline and 13 were treated with pentoxifylline. There were significantly lower leukocyte, protein, and lactate concentrations in CSF during the pentoxifylline infusion than during the saline infusion period (Fig. 1). At 3 h after endotoxin inoculation, all saline-treated animals had leukocytes present in CSF, compared with only 4 of 13 (30%) rabbits that received pentoxifylline ($P < 0.001$).

The TNF concentrations were 51 and 38% lower at 1 and 3 h, respectively, after endotoxin intracisternal inoculation in pentoxifylline-treated animals than in controls (P not significant; Fig. 1).

When pentoxifylline was initiated 1 h after endotoxin was given intracisternally, the mean peak concentrations of leukocytes and lactate were significantly lower than those in saline-treated animals (Table 1). The peak concentrations of protein and TNF were not significantly affected by pentoxifylline therapy.

***H. influenzae* type b experiments.** The 35 animals employed in this set of experiments were divided into four treatment groups (saline alone, pentoxifylline alone, dexamethasone

TABLE 1. Mean peak CSF inflammatory indices in rabbits treated 1 h after *H. influenzae* endotoxin administration[a]

Treatment group and *P* value[b]	No. of leukocytes/mm^3	Lactate concn (mg/dl)	Protein concn (mg/dl)	TNF concn (ng/ml)
Saline	7,050 ± 2,620	48.2 ± 6.0	98.3 ± 21.0	21.3 ± 7.4
Pentoxifylline	2,800 ± 1,240	34.0 ± 4.2	85.4 ± 23.2	19.7 ± 8.8
P value	<0.05	<0.01	NS[c]	NS

[a] Values are means ± 1 standard deviation.
[b] $n = 4$ for both groups. *P* from two-tailed Student *t* test.
[c] NS, Not significant.

alone, and pentoxifylline plus dexamethasone). All animals were treated with ceftriaxone either simultaneously with dexamethasone or 30 min after saline or pentoxifylline infusions. Before treatment (6-h values), the four groups of rabbits were comparable with regard to the CSF inflammatory indices (Fig. 2). There were highly significant differences over the 6-h treatment period in the mean leukocyte, lactate, protein, and TNF concentrations in CSF in those rabbits that received dexamethasone alone or dexamethasone plus pentoxifylline compared with the results for saline-treated animals. A significant difference in protein and lactate concentrations ($P < 0.05$) was also observed in those animals that received pentoxifylline alone compared with

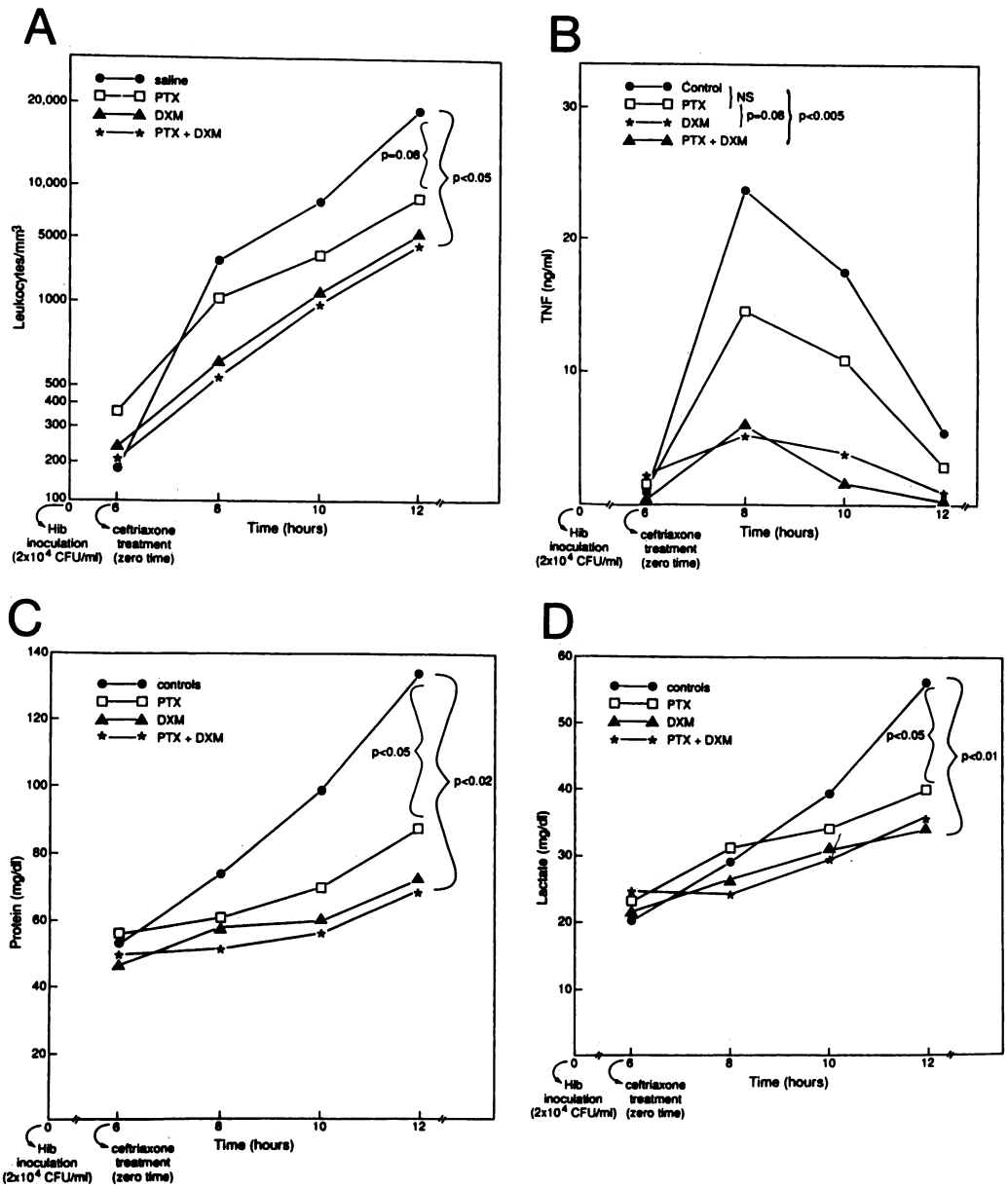

FIG. 2. Modulation of antibiotic-induced meningeal inflammation in rabbits infected with *H. influenzae* type b (Hib) by treatment with various regimens. Continuous i.v. infusions of saline or pentoxifylline (PTX) were started 30 min before ceftriaxone i.v. administration and maintained for 6 h. Dexamethasone (DXM) was given simultaneously with ceftriaxone. (A) Leukocyte counts; (B) TNF concentrations; (C) lactate concentrations; (D) protein concentrations. There were 8 rabbits in each single-treatment group, and 11 animals received combined treatment. NS, Not significant.

TABLE 2. CSF inflammatory indices in rabbits after intracisternal inoculation of 10 ng of rrIL-1β[a]

Treatment group (n)	No. of leukocytes/mm³[b] at:			Lactate concn[c] (mg/dl) at:			Protein concn[d] (mg/dl) at:		
	0 h	6 h	12 h	0 h	6 h	12 h	0 h	6 h	12 h
Control (6)	0	957 ± 521	898 ± 523	17.9 ± 3.4	42.5 ± 11.5	32.4 ± 6.2	33.3 ± 9.4	44.3 ± 17.3	43.8 ± 8.9
Dexamethasone (6)	0	517 ± 400	573 ± 278	16.1 ± 4.6	40.5 ± 10.1	32.1 ± 7.2	32.8 ± 7.4	33.5 ± 8.9	51.1 ± 24.5
Pentoxifylline (4)	0	43 ± 32	125 ± 114	18.9 ± 3.2	23.5 ± 4.0	30.7 ± 7.8	24.5 ± 5.4	17.8 ± 10.8	27.8 ± 21.2

[a] Values are means ± 1 standard deviation.
[b] $P = 0.047$ between controls and dexamethasone-treated rabbits; $P \leq 0.001$ between either controls or dexamethasone-treated rabbits and those that received pentoxifylline (analysis of variance over time).
[c] P not significant between either controls or dexamethasone-treated rabbits and pentoxifylline-treated group.
[d] $P = 0.005$ between either controls or dexamethasone-treated rabbits and pentoxifylline-treated rabbits (over time).

results in saline-treated rabbits (Fig. 2) but not in leukocyte counts ($P = 0.08$) and in TNF concentrations ($P = 0.10$).

IL-1 experiments. A 10-ng amount of rrIL-1β was administered intracisternally to 16 rabbits; six of them received saline, six received dexamethasone, and four were treated with pentoxifylline. Treatments were given 30 min before rrIL-1β inoculation. The mean leukocyte counts were significantly lower in dexamethasone- (analysis of variance, $P = 0.047$) and pentoxifylline-treated animals (analysis of variance, $P = 0.001$) compared with values for control animals (Table 2). Dexamethasone did not significantly affect the protein and lactate concentrations in CSF. By contrast, rabbits that received pentoxifylline had significantly fewer leukocytes and lower protein concentrations in CSF than did the animals in the other two treatment groups. Lactate concentrations were lower at 6 h only in pentoxifylline-treated rabbits compared with the other two rabbit groups, but this difference was not significant. TNF was not detected in any of the CSF samples from these animals.

DISCUSSION

Pentoxifylline is a phosphodiesterase inhibitor and known vasoactive drug with proved clinical efficacy in various circulatory disorders (8). It improves microcirculation as a result of its rheologic effects on erythrocytes, platelets, and plasmatic components, resulting in a decrease of whole-blood viscosity (19, 30). Recently, pentoxifylline was found to be of great benefit in different experimental sepsis models, including sepsis caused by gram-positive and gram-negative bacteria. This drug was shown to increase the survival of mice after i.v., intraperitoneal, or subcutaneous challenge with *Staphylococcus aureus* (17). Neonatal rats with intraperitoneal *E. coli* infections were also protected by pentoxifylline treatment (3). Using mice given i.v. endotoxin, Schade and co-workers (29) found that pentoxifylline dramatically increased survival when administered from 24 h before to 2 h after endotoxin injection. Welsh and colleagues (40) reported that pentoxifylline diminished extravascular protein accumulation and neutrophil sequestration in the lungs of dogs given i.v. endotoxin. Additionally, Ishizaka et al. (13) demonstrated that continuous infusion of pentoxifylline attenuated acute lung injury in septic guinea pigs.

By different experimental approaches (12, 29, 39) it has been shown that this drug interferes with augmented granulocyte-endothelium interactions, by modulating intravascular granulocyte hyperreactivity as well as by stimulating antiaggregatory activity of the vessel endothelium. Pentoxifylline has inhibitory effects on neutrophils in vitro, and these effects are most marked when the neutrophils have been preexposed to inflammatory cytokines (33). Prominent among these cytokines are TNF and IL-1, which are produced by macrophages and other cells. These molecules activate neutrophils to increase their stickiness and adherence to endothelial cells, to increase the oxidative burst resulting in the production of superoxide and other active oxygen species, and to increase the rate of granule enzyme release on stimulation (22). In addition, these cytokines promote the entry of leukocytes into injured tissues. Therapy that would inhibit or block the activity of the inflammatory cytokines on neutrophils and endothelial tissue could possibly be beneficial in diminishing tissue damage, believed in part to result from the liberation of harmful products on neutrophil degranulation.

The exact mechanism of the anti-inflammatory activities of pentoxifylline is unknown. Studies attempting to elucidate the mechanism of pentoxifylline on neutrophils showed that intracellular cyclic AMP concentrations were increased and concentrations of intracellular free ionized calcium were decreased (12, 32). These changes could affect the functional activities of neutrophils. Additionally, pentoxifylline appears to inhibit granulocyte function after activation but has a negligible effect on resting neutrophils (12, 32). Whether these effects on neutrophil function along with decreased production or activity of the cytokines explain the modulation of meningeal inflammation observed in this study is unknown at this time.

TNF and IL-1 have been detected in CSF of children with bacterial meningitis, and their presence correlated significantly with the meningeal inflammatory indices (23). In addition, animal studies indicate that both cytokines play a role in mediating the inflammatory cascade that is triggered by *H. influenzae* type b or its lipooligosaccharide (25; Wispelwey et al., 28th ICAAC).

Corticosteroids prevent the production of TNF by macrophages incubated with endotoxin but only if the steroids are present before endotoxin comes in contact with macrophages (2, 15, 18). We demonstrated previously that i.v. administration of dexamethasone simultaneously with or prior to intracisternal inoculation of *H. influenzae* type b endotoxin substantially reduced TNF concentrations in CSF and modulated meningeal inflammation in rabbits, compared with minimal effects when dexamethasone was given 1 h after endotoxin inoculation (25). Additionally, early administration of dexamethasone considerably attenuated the brisk inflammatory changes and reduced significantly TNF concentrations following antibiotic-induced bacterial lysis (24).

Pentoxifylline has been recently shown to suppress endotoxin-induced mononuclear cell-derived TNF by more than 50% by reducing TNF mRNA accumulation and TNF supernatant bioactivity (32). In the present study, peak TNF concentrations in CSF were reduced by more than a third in pentoxifylline-treated animals, but this change was not sta-

tistically significant and is unlikely to be responsible for the marked modulation of meningeal inflammation that occurred in endotoxin-induced meningitis.

The leukocyte margination and adherence to endothelial cells mediated by cytokines in response to microbial products or endotoxin can be inhibited by pentoxifylline (33). These leukocyte properties could be responsible for the complex cascade of events that provokes increased blood-brain barrier permeability and allows entry of leukocytes into CSF. In this regard, at 3 h post-intracisternal endotoxin inoculation only 30% of rabbits receiving pentoxifylline had leukocytes in CSF, compared with all of the animals treated with saline.

Of potential clinical importance, pentoxifylline reduced significantly the peak concentrations of leukocytes and lactate in CSF when administered 1 h after intracisternal inoculation of endotoxin. This effect occurred without a reduction in TNF activity in CSF, suggesting that pentoxifylline attenuated cytokine-induced leukocyte-endothelium interactions. This contention is supported by the observation of High and associates (K. High, B. Wisepelwey, W. J. Long, Jr., E. J. Hansen, and W. M. Scheld, 29th ICAAC, abstr. no. 711, 1989) that pentoxifylline modulated significantly the usual endotoxin-induced increase in blood-brain permeability to protein and leukocytes in experimental meningitis.

The brisk inflammatory response that is produced by antibiotic-induced bacterial lysis has been well described (24, 36, 38). Lysis of *H. influenzae* bacteria involves the explosive release of bacterial cell wall fragments and endotoxin-containing particles into the CSF. Pentoxifylline significantly reduced protein and lactate concentrations in CSF and moderately decreased leukocyte counts. Dexamethasone was superior to pentoxifylline in modulating these inflammatory changes, and no appreciable synergism was observed when dexamethasone and pentoxifylline were used together. We do not know whether other indices of meningeal inflammation, such as intracranial pressure, blood-brain barrier permeability, or arachidonic acid metabolism, are altered by pentoxifylline treatment.

Our knowledge of the interrelationships of the inflammatory pathways in the subarachnoid space is still incomplete. Wispelwey et al. (28th ICAAC) have reported that IL-1 could act as an important mediator in the inflammatory changes observed in bacterial meningitis secondary to intracisternal inoculation of endotoxin or *H. influenzae* type b organisms in rats. rrIL-1β inoculated intracisternally produced an intense inflammatory response in our animal model, and TNF did not participate in that process. Pentoxifylline was found to be superior to dexamethasone in modulating the meningeal inflammatory response after intracisternal inoculation of 10 ng of rrIL-1β. These results suggest that this agent might exert beneficial effects late in the course of an infection, when inflammatory cytokines have already been produced and released.

In summary, findings of the present study indicate that continuous infusion of pentoxifylline ameliorates the inflammatory changes in CSF that occur after *H. influenzae* type b endotoxin exposure either by direct inoculation or following antibiotic-induced bacterial lysis. In addition, pentoxifylline markedly modulated leukocyte counts and lowered protein concentrations after intracisternal inoculation of rrIL-1β. These data provide a paradigm for further studies examining the potential role of pentoxifylline in decreasing meningeal inflammation and/or preventing brain damage caused by cytokine overproduction and leukocyte-endothelium interactions.

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LITERATURE CITED

1. **Bertocchi, F., P. Proserpio, M. G. Lampugnani, and E. Dejana.** 1988. The effect of pentoxifylline in polymorphonuclear cell adhesion to cultured endothelial cells. A preliminary report, p. 68–74. *In* G. L. Mandell and W. J. Novick (ed.), Proceedings of a symposium on pentoxifylline and leukocyte function. Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.
2. **Beutler, B., and A. Cerami.** 1987. Cachectin: more than a tumor necrosis factor. N. Engl. J. Med. **316**:379–385.
3. **Chalkiadakis, G. E., A. Kostakis, P. E. Karayannakos, M. E. Chalkiadakis, S. Sgouromali, H. Giamerellon, and G. D. Skalkeas.** 1985. Pentoxifylline in the treatment of experimental peritonitis in rats. Arch. Surg. **120**:1141–1144.
4. **Chan, P. H., S. Longar, and R. A. Fishman.** 1987. Protective effects of liposome-entrapped superoxide dismutase on posttraumatic brain edema. Ann. Neurol. **21**:540–547.
5. **Chan, P. H., J. W. Schmidley, R. A. Fishman, and S. M. Longar.** 1984. Brain injury, edema, and vascular permeability changes induced by oxygen-derived free radicals. Neurology **34**:315–320.
6. **Cybulsky, M. I., M. K. W. Chan, and H. A. Movat.** 1988. Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor necrosis factor and their implication in gram-negative infection. Lab. Invest. **58**:365–378.
7. **Dacey, R. G., and M. A. Sande.** 1974. Effects of probenecid on cerebrospinal fluid concentrations of penicillin and cephalosporin derivatives. Antimicrob. Agents Chemother. **6**:437–441.
8. **DiPerri, T., O. Carandente, A. Vittoria, M. Guerrini, and G. L. Messa.** 1984. Studies of the clinical pharmacology and therapeutic efficacy of pentoxifylline in peripheral obstructive arterial disease. Angiology **35**:427–435.
9. **Ernst, J. D., J. M. Decazes, and M. A. Sande.** 1983. Experimental pneumococcal meningitis: the role of leukocytes in pathogenesis. Infect. Immun. **41**:275–279.
10. **Flick, D. A., and G. E. Figgord.** 1984. Comparison of in vitro cell cytotoxic assay for tumor necrosis factor. J. Immunol. Methods **68**:167–175.
11. **Gulig, P. A., C. C. Patrick, L. Hermanstorfer, G. H. McCracken, Jr., and E. J. Hansen.** 1987. Conservation of epitopes in the oligosaccharide portion of *Haemophilus influenzae* type b. Infect. Immun. **55**:513–520.
12. **Hammerschmidt, D. E., D. Kotasek, T. McCarthy, P. W. Huh, G. Freyburger, and G. M. Vercelloti.** 1988. Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. J. Lab. Clin. Med. **112**: 254–263.
13. **Ishizaka, A., W. V. Zhaohan, K. E. Stephens, H. Harada, R. S. Hogue, P. T. O'Hanley, and A. Raffin.** 1988. Attenuation of acute lung injury in septic guinea pigs by pentoxifylline. Am. Rev. Respir. Dis. **138**:376–382.
14. **Johnson, K. G., and M. B. Perry.** 1976. Improved techniques for the preparation of bacterial lipopolysaccharides. Can. J. Microbiol. **22**:29–34.
15. **Kern, J. A., R. J. Lamb, J. C. Reed, R. P. Danielle, and P. C. Nowell.** 1988. Dexamethasone inhibition of interleukin-1 beta production by human monocytes. Posttranscriptional mechanisms. J. Clin. Invest. **81**:237–244.
16. **Kimura, A., and E. J. Hansen.** 1986. Antigenic and phenotypic variations of *Haemophilus influenzae* type b lipopolysaccharide and their relationship to virulence. Infect. Immun. **51**:69–79.
17. **Krause, P. J., J. Kristie, W. P. Wang, L. Eisenfeld, V. C. Herson, E. G. Maderazo, K. Josaki, and D. L. Kreutzler.** 1987. Pentoxifylline enhancement of defective neutrophil function and host defense in neonatal mice. Am. J. Pathol. **129**:217–222.
18. **Mandell, G. L.** 1988. ARDS, neutrophils, and pentoxifylline.

Am. Rev. Respir. Dis. **138**:1103–1115.
19. **Manrique, R. V., and V. Manrique.** 1987. Platelet resistance to prostacyclin. Enhancement of the antiaggregatory effect of prostacyclin by pentoxifylline. Angiology **38**:101–108.
20. **Miller, R. J., Jr.** 1981. Simultaneous statistical inference, 2nd ed., p. 67–78. Springer-Verlag, New York.
21. **Mori, S., F. Goto, K. Goto, S. Ohkawara, S. Maeda, K. Shimada, and M. Yoshinaga.** 1988. Cloning and sequence analysis of a cDNA for lymphocyte proliferation potentiating factor of rabbit polymorphonuclear leukocytes: identification as rabbit interleukin-1 beta. Biochem. Biophys. Res. Commun. **150**: 1237–1243.
22. **Movat, H. Z., M. I. Cybulsky, I. G. Colditz, M. K. W. Chan, and C. A. Dinarello.** 1987. Acute inflammation in gram-negative infection: endotoxin, interleukin-1, tumor necrosis factor, and neutrophils. Fed. Proc. **46**:97–104.
23. **Mustafa, M. M., M. H. Lebel, O. Ramilo, K. D. Olsen, J. S. Reisch, B. Beutler, and G. H. McCracken, Jr.** 1989. Correlation of interleukin-1β and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. J. Pediatr. **115**: 208–213.
24. **Mustafa, M. M., O. Ramilo, J. Mertsola, R. C. Risser, B. Beutler, E. J. Hansen, and G. H. McCracken, Jr.** 1989. Modulation of inflammation and cachectin activity in relation to treatment of experimental *Haemophilus influenzae* type b meningitis. J. Infect. Dis. **160**:818–825.
25. **Mustafa, M. M., O. Ramilo, K. D. Olsen, P. S. Franklin, E. J. Hansen, B. Beutler, and G. H. McCracken, Jr.** 1989. Tumor necrosis factor in mediating experimental *Haemophilus influenzae* type b meningitis. J. Clin. Invest. **84**:1253–1259.
26. **Mustafa, M. M., O. Ramilo, G. A. Syrogiannopoulos, K. D. Olsen, G. H. McCracken, Jr., and E. J. Hansen.** 1989. Induction of meningeal inflammation by outer membrane vesicles of *Haemophilus influenzae* type b. J. Infect. Dis. **159**:917–922.
27. **Porter, J. M., B. S. Cutler, B. Y. Lee, T. Reich, F. A. Reichle, J. T. Scogin, and D. E. Strandness.** 1982. Pentoxifylline efficacy in the treatment of intermittent claudication: multicenter controlled double-blind trial with objective assessment of chronic occlusive arterial disease patients. Am. Heart J. **104**:66–72.
28. **Riesenfeld-Orn, I., S. Wolpe, J. F. Garcia-Bustos, M. K. Hoffmann, and E. Tuomanen.** 1989. Production of interleukin-1 but not tumor necrosis factor by human monocytes stimulated with pneumococcal cell surface components. Infect. Immun. **57**: 1890–1893.
29. **Schade, U. F., J. Van Der Bosch, and M. M. Schonharting.** 1988. Increase of survival rate by pentoxifylline in endotoxic shock, p. 175–183. *In* G. L. Mandell and W. J. Novick (ed.), Proceedings of a symposium on pentoxifylline and leukocyte function. Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.
30. **Schonharting, M., P. Musikic, and P. Muller.** 1988. The haemorheological and antithrombotic potential of pentoxifylline (Trental). A review. Pharmatherapeutica **5**:159–169.
31. **Schonharting, M. M., and U. F. Schade.** 1989. The effect of pentoxifylline in septic shock. New pharmacologic aspects of an established drug. J. Med. (Westbury) **20**:97–105.
32. **Strieter, R. M., D. G. Remick, P. A. Ward, R. N. Spengler, J. P. Lynch, J. Larrick, and S. L. Kunkel.** 1988. Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifylline. Biochem. Biophys. Res. Commun. **155**:1230–1236.
33. **Sullivan, G. W., H. T. Carper, W. J. Novick, Jr., and G. L. Mandell.** 1988. Inhibition of the inflammatory action of interleukin-1 and tumor necrosis factor (alpha) on neutrophil function by pentoxifylline. Infect. Immun. **56**:1722–1729.
34. **Sullivan, G. W., T. N. Patselas, J. A. Redick, and G. L. Mandell.** 1984. Enhancement of chemotaxis and protection of mice from infection. Trans. Assoc. Am. Phys. **97**:337–345.
35. **Syrogiannopoulos, G. A., E. J. Hansen, A. L. Erwin, R. S. Munford, J. Rutledge, J. S. Reisch, and G. H. McCracken, Jr.** 1988. *Haemophilus influenzae* type b lipooligosaccharide induces meningeal inflammation. J. Infect. Dis. **157**:237–244.
36. **Tauber, M. G., A. M. Shibl, C. J. Hackbarth, J. W. Larrick, and M. A. Sande.** 1987. Antibiotic therapy, endotoxin concentration in cerebrospinal fluid, and brain edema in experimental *Escherichia coli* meningitis in rabbits. J. Infect. Dis. **156**:456–462.
37. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver strain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
38. **Tuomanen, E., B. Hengstler, R. Rich, M. A. Bray, and O. Zak.** 1987. Nonsteroidal anti-inflammatory agents in the therapy for experimental pneumococcal meningitis. J. Infect. Dis. **155**: 985–990.
39. **Ward, A., and S. P. Clissold.** 1987. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. Drugs **34**:50–97.
40. **Welsh, C. H., D. Lien, G. S. Worthen, and J. V. Weil.** 1988. Pentoxifylline decreases endotoxin-induced pulmonary neutrophil sequestration and extravascular protein accumulation in the dog. Am. Rev. Respir. Dis. **138**:1106–1114.
41. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–91.