THE EFFECTS OF PENTOXIFYLLINE ON LIPOPOLYSACCHARIDE (LPS) FEVER, PLASMA INTERLEUKIN 6 (IL 6), AND TUMOR NECROSIS FACTOR (TNF) IN THE RAT

Lin G. LeMay, Arthur J. Vander, Matthew J. Kluger

The purpose of these studies was to test whether pentoxifylline, a drug that can inhibit the production and action of cytokines hypothesized to be endogenous pyrogens (for example, interleukin 1 and tumor necrosis factor [TNF]), is antipyretic. We also tested the effects of pentoxifylline on plasma activities of interleukin 6 (IL 6) and TNF in response to an injection of a fever-inducing dose of lipopolysaccharide (LPS). Our results showed that a high dose of pentoxifylline (200 mg/kg) caused hypothermia in control rats and blocked LPS fever, while a low dose (50 mg/kg) did not have these effects. Injection of the high dose of pentoxifylline in control rats caused a rise in plasma IL 6 but not in plasma TNF. However, the peak levels of plasma IL 6 and TNF activities following an injection of LPS were significantly reduced by pretreatment with pentoxifylline. Overall, the data are consistent with the hypothesis that pentoxifylline is an antipyretic drug, which may act at least in part by inhibiting the secretion of pyrogenic cytokines.

Pentoxifylline is a methyl xanthine derivative that has been used in the clinical treatment of vascular disorders to reduce blood viscosity and to increase erythrocyte deformability.[1,2] Pentoxifylline also has been shown to suppress the production of lipopolysaccharide (LPS) induced biological[3] and immunological[4] tumor necrosis factor (TNF) activities and to inhibit the inflammatory action of interleukin 1 (IL 1) and TNF on neutrophil function.[5] TNF has many biological effects, including causing hemorrhagic necrosis of tumors[6,7] and cachexia during infection and cancer,[8-11] and inducing septic shock.[9,12,13] Injection of TNF results in fever, and many review articles include TNF as an endogenous mediator of fever (i.e. an endogenous pyrogen).[14,15] However, it has been shown that injection of antiserum to TNF enhances LPS fever rather then suppressing it in the rat,[16] which indicates that TNF may be an endogenous cryogen or antipyretic rather than a pyrogen in LPS fever in this species. In the rabbit, anti-TNF has been reported both to block LPS fever[17] and to leave it unaltered.[18] It has been reported that TNF can stimulate the release of interleukin 6 (IL 6), a cytokine that not only plays an important role in production of antibodies[19,20] and acute phase proteins,[21,22] but is also another putative endogenous pyrogen.[23-25] IL 6 causes prostaglandin-dependent fever upon injection into rats, and IL 6 concentration increases markedly in the plasma and cerebrospinal fluid (CSF) of LPS-injected rats.[25]

Based on the previously reported effects of pentoxifylline on the production and action of cytokines thought to be endogenous pyrogens, we hypothesized that pentoxifylline would have antipyretic properties, attenuating LPS fever in the rat. In this study, we tested this hypothesis and, in addition, determined the effects of pentoxifylline on plasma activities of IL 6 and TNF in response to the injection of fever-inducing doses of LPS.

RESULTS

Effects of Pentoxifylline on Body Temperature and LPS Fever

The purpose of this experiment was to assess the antipyretic properties of pentoxifylline. Rats were injected intraperitoneally (IP) at time 0 with saline or one of two doses of pentoxifylline (50 mg/kg or 200 mg/kg). At time 60 min post-injection the rats were then injected IP with saline (Fig. 1) or 10 μg LPS/kg (Fig. 2). The initial temperature increases during the first hour in the saline/saline and saline/LPS groups are a nonspecific effect usually seen following injection of any substance

Department of Physiology, The University of Michigan Medical School, Ann Arbor, MI 48109.

1043-4666/90/0204-0012$05.00/0

KEY WORDS: Pentoxifylline/IL 6/TNF/Temperature regulation

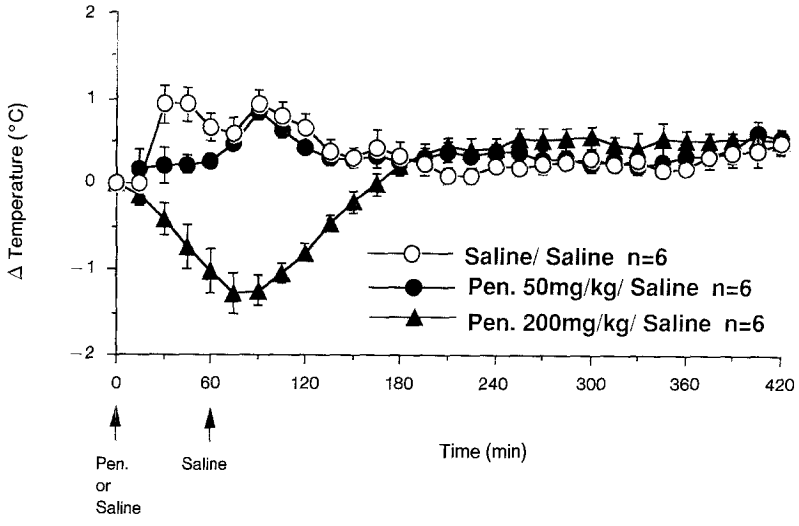

Figure 1. Effects of pentoxifylline on core temperature of rats.

The first IP injection (either saline or pentoxifylline) occurred at time 0 and the second IP injection (saline) occurred at 60 min. Temperatures at time 0 for the three groups were not significantly different from each other and averaged 37.3°C. n, sample size.

IP in the rat. However, in none of the groups injected with pentoxifylline was there an initial rise in body temperature following the injection at time 0. As shown in Fig. 1, the injection of 50 mg/kg pentoxifylline had little effect on normal body temperature, whereas the injection of 200 mg/kg pentoxifylline caused hypothermia (~ −1.3°C) for about 2 hr after the injection. As shown in Fig. 2, in the group injected with saline followed by 10 μg LPS/kg, the temperature began to rise at approximately 150 min, reaching a first peak (~1.4°C) at about 210 min and a second peak (~1.8°C) at 300 to 330 min. In the group injected with 50 mg pentoxifylline/kg followed by LPS, the febrile response to LPS was similar to that seen in the saline/LPS group. In the group injected with 200 mg pentoxifylline/kg followed by LPS, the temperature decreased immediately following the injection of pentoxifylline, reaching a nadir (−1.2°C) at approximately 90 min, returned to the base line at about 210 min, and remained at the base line up to 420 min. There was a significant difference ($p < 0.05$, analysis of variance [ANOVA]) when we compared the mean changes in body temperature at 180 to 420 min in the three groups (designated Saline/LPS, Pen. 50/LPS, and Pen. 200/LPS). A comparison of Pen. 200/LPS group by Fisher's least significant difference method showed that the Pen. 200/LPS group had a significantly lower value ($p < 0.05$) than the other two groups.

In order to separate the effects of pentoxifylline on the LPS fever from the effects of pentoxifylline itself, we generated Fig. 3 by subtracting, for each time, the mean temperatures of the rats injected with saline or pentoxifylline and then saline (data from Fig. 1) from the mean temperatures of rats injected with saline or pentoxifylline and then LPS (data from Fig. 2). Figure 3 shows that the febrile responses after injection of LPS in the rats that were previously treated with saline or 50 mg pentoxifylline/kg were similar. However, despite the fact that the body temperature of the rats injected with 200 mg/kg pentoxifylline had returned to normal by about 180 min following this injection (Fig. 1), there was no febrile response after injection of LPS in the

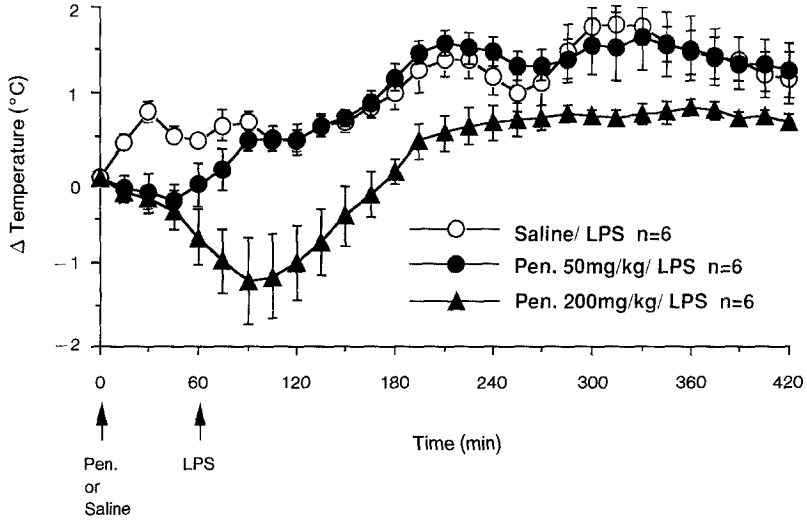

Figure 2. Effects of pentoxifylline on the core temperature response of rats to LPS.

The first IP injection (either saline or pentoxifylline) occurred at time 0 and the second IP injection (LPS) occurred at 60 min. Temperatures at time 0 for the three groups were not significantly different from each other and averaged 37.3°C. n, sample size.

CYTOKINE, Vol. 2, No. 4 (July 1990: 300-306)

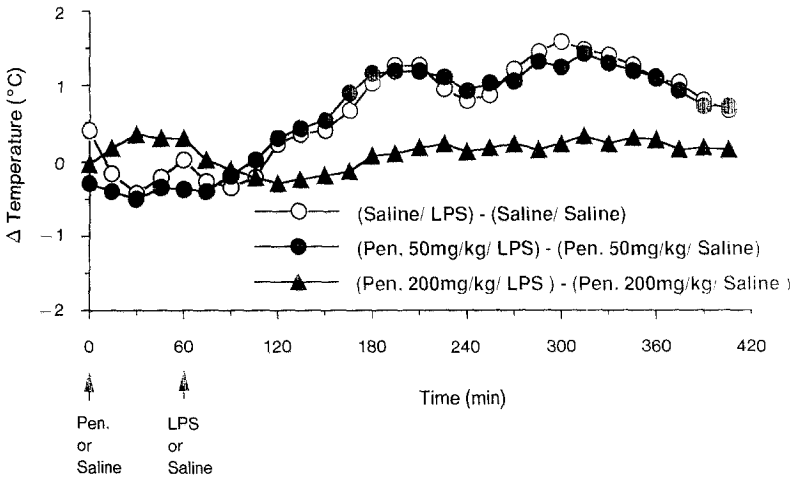

Figure 3. Effects of pentoxifylline on LPS-induced fever independent of the effects of pentoxifylline itself.

The mean temperatures of the rats injected with saline or pentoxifylline and then saline (Fig. 1) were subtracted for each time from the mean temperatures of rats injected with saline or pentoxifylline and then LPS (Fig. 2).

group that had previously been treated with 200 mg pentoxifylline/kg.

Effects of Pentoxifylline on the Plasma IL 6 and TNF Activities During the LPS Fever

In these experiments, rats were injected IP at time 0 with saline or one of two doses of pentoxifylline (50 mg/kg or 200 mg/kg). Sixty minutes later, the rats were injected IP with saline or 10 μg LPS/kg, and at either 120 min or 240 min, blood was removed for measurement of the plasma activities of both IL 6 and TNF.

The 2-hr data for plasma IL 6 activity are shown in Fig. 4. The groups injected with saline/saline or with 50 mg pentoxifylline/kg and saline showed low levels of plasma IL 6 activity (11 ± 5 U/mL and 150 ± 35 U/mL). There was a significant difference ($p < 0.05$, ANOVA) when we compared the plasma IL 6 activities in the three groups (Saline/saline, Pen. 50/saline, and Pen. 200/saline). The plasma IL 6 activity in the group treated with 200 mg pentoxifylline/kg and saline (17,029 ± 8,735 U/mL) was significantly greater ($p < 0.05$, Fisher's test) than the activity in the saline/saline group or the Pen. 50/saline group. LPS caused a large increase in IL 6 in all three groups. Although the level of IL 6 activity (43,759 ± 22,325 U/mL) in the group that was injected with 200 mg pentoxifylline/kg and LPS was nearly twice as great as the level of IL 6 activity in the saline/LPS group (27,248 ± 11,243 U/mL) or in the Pen. 50/LPS group (23,494 ± 11,258 U/mL), there was no statistical difference among these three groups ($p > 0.65$, ANOVA).

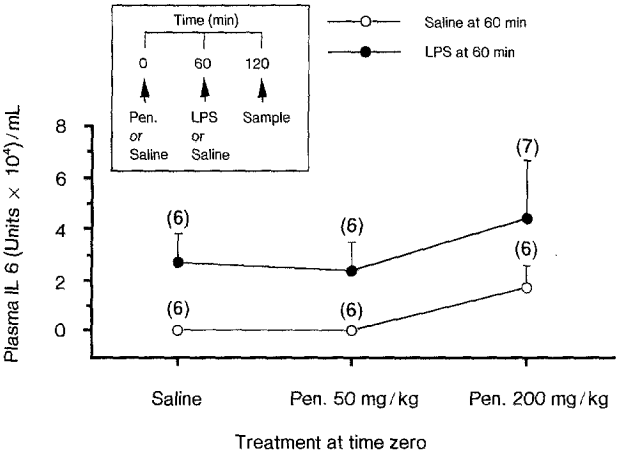

Figure 4. Effects of pentoxifylline alone or pentoxifylline and LPS on plasma IL 6 activity in rats.

Animals were injected with pentoxifylline (either 50 mg/kg or 200 mg/kg) or saline at time 0 and then with either LPS (10 μg/kg) or saline 60 min later. Blood was taken at 120 min. Sample size is indicated in parentheses.

The 4-hr data for plasma IL 6 activity are shown in Fig. 5. The groups injected with saline/saline or with 50 mg pentoxifylline/kg and saline showed low levels of plasma IL 6 activity (91 ± 54 U/mL and 34 ± 17 U/mL). The plasma IL 6 activity in the group treated with 200 mg pentoxifylline/kg and saline (1,387 ± 818 U/mL) tended to be greater than that of the other two groups, but this difference was not statistically significant ($p > 0.09$, ANOVA). In all three groups receiving LPS, there was an increase in plasma IL 6 activity and pentoxifylline tended to cause a dose-dependent attenuation of the IL 6 rise, although there were no significant differences ($p > 0.08$, ANOVA). However, the level of IL 6 activity in the group that was injected with 200 mg pentoxifylline/kg and LPS (98,653 ± 31,038 U/mL) was significantly lower ($p < 0.05$) by Fisher's least significant difference method than the activity in the group injected with saline and LPS (436,175 ± 170,903 U/mL) or the group injected with 50 mg pentoxifylline/kg and LPS (249,385 ± 30,499 U/mL).

The 2-hr and 4-hr data for plasma TNF activity are shown in Fig. 6. Plasma TNF was not detectable in the groups injected with saline and saline, 50 mg pentoxifylline/kg and saline, or 200 mg pentoxifylline/kg and saline at either of these sampling times. In contrast, in all three groups receiving LPS there was a marked increase in TNF activity in the 2-hr plasma sample, but

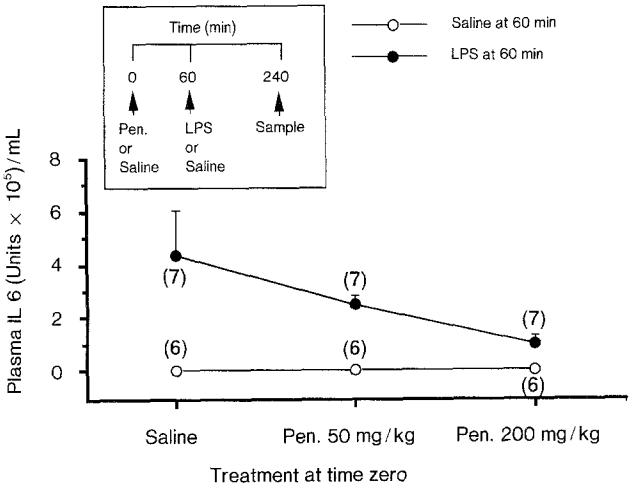

Figure 5. Effects of pentoxifylline alone or pentoxifylline and LPS on plasma IL 6 activity in rats.

Animals were injected with pentoxifylline (either 50 mg/kg or 200 mg/kg) or saline at time 0 and then with LPS (10 μg/kg) or saline 60 min later. Blood was taken at 240 min. Sample size is indicated in parentheses. Note that the scale of the Y axis is tenfold greater than Fig. 4.

pentoxifylline caused a dose-dependent attenuation of the TNF rise ($p < 0.03$, ANOVA). The 2-hr plasma TNF activity in the group treated with 200 mg pentoxifylline/kg and LPS (498 ± 321 U/mL) was significantly lower ($p < 0.05$, Fisher's test) than the activity in the group injected with saline and LPS (5,264 ± 1,325 U/mL) or the group injected with 50 mg pentoxifylline/kg and LPS (2,974 ± 615 U/mL). The 4-hr plasma TNF activities in all groups receiving LPS were much lower than those seen at 2 hr, but even at these very low levels, there was a dose-dependent attenuation of the TNF rise by pentoxifylline ($p < 0.04$, ANOVA), the

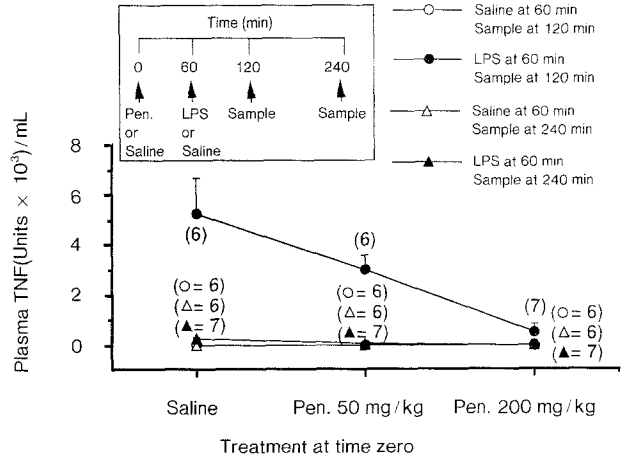

Figure 6. Effects of pentoxifylline alone or pentoxifylline and LPS on plasma TNF activity in rats.

Animals were injected with pentoxifylline (either 50 mg/kg or 200 mg/kg) or saline at time 0 and then with either LPS (10 μg/kg) or saline 60 min later. Blood was taken at either 120 min or 240 min. Sample size is indicated in parentheses.

values being 264 ± 19 U/mL for the saline group, 67 ± 67 U/mL for the pentoxifylline 50 mg/kg group, and undetectable for the pentoxifylline 200 mg/kg group.

DISCUSSION

These data demonstrate that a high dose of pentoxifylline (200 mg/kg) blocks LPS fever, while a low dose (50 mg/kg) has no effect. However, although the high dose of pentoxifylline completely blocked the rise in body temperature due to LPS, this might be the result of effects of the drug not specifically related to the pathways by which LPS induces fever. For example, it was consistently noted that injection of this dose of pentoxifylline caused the rats to appear dazed or tranquilized. Their eyes were partially closed and inspirations were deeper. The rats would respond to noise or handling, but when returned to their cages showed little exploratory behavior. The rats would remain still, but did not appear to be resting. Their forelimbs were extended and their heads were tilted upwards. This extended posture adopted by the rats might dissipate more heat than the normal huddled posture of rats. In addition, it has been reported that pentoxifylline is a vasodilator,[26] which may also contribute to the hypothermia observed with the high dose. Pentoxifylline has been reported to stimulate the synthesis of prostacyclin; however, it had no effects on the plasma concentration of prostaglandin E_2,[26] a putative neuro-mediator for the regulation of body temperature.

It has been reported that pentoxifylline can also stimulate the release of interferon α,[27] a putative endogenous pyrogen.[28] Since we did not observe a rise in body temperature following the injection of pentoxifylline, we do not think that any possible rise in interferon α activity was high enough to cause fever in our experiments.

Injection of 200 mg/kg pentoxifylline led to an elevation in plasma IL 6 at 2 hr following the injection. The mechanism responsible for this rise is unknown. In a previous study, we showed that simply exposing a rat to the stress of an "open-field" resulted in a rapid rise in plasma IL 6.[29] Possibly the stress associated with this symptom-producing dose of pentoxifylline contributed to the rise in plasma IL 6 observed in our study. Another possibility is that the hypothermia associated with this high dose of pentoxifylline may have stimulated the release of IL 6. The logic behind this suggestion is that if IL 6 is an endogenous pyrogen it might also participate in the regulation of body temperature during non-infectious states. A lowering of body temperature (perhaps below the thermoregulatory set-point) might trigger the release of IL 6, thus helping to restore body temperature to its previous level.

The high dose of pentoxifylline also blocked most of the rise of plasma IL 6 activity that occurred 3 hr after injection of LPS. This suggests to us that pentoxifyl-

CYTOKINE, Vol. 2, No. 4 (July 1990: 300-306)

line's antipyretic effect could be mediated via its effects on reducing plasma IL 6 activity. However, the group injected with the low dose of pentoxifylline and LPS had LPS-induced fever similar to those of rats injected with saline and LPS, but had attenuated increases in IL 6 activity (see Fig. 5). These data therefore pose a problem for the hypothesis that plasma IL 6 is responsible for LPS-induced fever. Either this hypothesis is wrong, or else it may be necessary for plasma IL 6 activity to rise above a certain threshold (for example, >100,000 U/mL) in order for fever to occur. Data obtained in an earlier study from this laboratory support this latter interpretation.[25,30] However, the interpretation of the present experiments solely in terms of IL 6 is made particularly difficult because pentoxifylline not only attenuated the rise in IL 6, a putative endogenous pyrogen, but also attenuated the rise in TNF, a putative endogenous antipyretic or cryogen in some studies[16,18] but a putative endogenous pyrogen in others.[14,15]

The mechanism by which the plasma concentrations of IL 6 and TNF are decreased in LPS fever is not known. It has been reported that pentoxifylline suppresses the production of LPS-induced biological[3] and immunological[4] TNF activities, which may explain the low level of these cytokines during LPS fever in rats previously treated with pentoxifylline. In addition, it is possible that pentoxifylline may increase the clearance of these cytokines from the circulation since it is a vasodilator and can increase arterial blood flow.[26]

Although many studies have indicated that TNF is a strong stimulator of the release of IL 6, Zabel et al.[4] have recently shown that administration of pentoxifylline to human subjects abolished the endotoxin-induced rise in TNF (as measured by immunoassay) without affecting the rise of serum IL 6. In the discussion section of that paper, it is stated that there was no attenuation in the endotoxin-induced fever. It is possible that the failure to find any decrease in serum IL 6 or reduction in fever might be the result of the relatively low dose of pentoxifylline used in this human study (7-8 mg/kg).

Overall, our data show that pentoxifylline, at least at a dose that appears to exert some toxicity, lowers normal body temperature, attenuates LPS-induced fever, and attenuates the rise in both TNF and IL 6 in response to LPS. The lower dose of pentoxifylline does not appear to be toxic and has no detectable effect on either normal body temperature or the fever caused by LPS, but does tend to attenuate both the plasma TNF and IL 6 rises caused by LPS.

MATERIALS AND METHODS

Animals

One hundred twelve specific-pathogen–free male Sprague-Dawley rats weighing 250-300 g each were obtained from Charles River (Portage, MI). Rats were housed at 23-25°C with a 12/12 hr light-dark cycle and given ad libitum tap water and rodent chow.

Measurement of Body Temperature

Core temperature was measured by biotelemetry with the use of transmitters implanted intraperitoneally (Mini Mitter, Inc., Sunriver, OR).[31] The transmitters were implanted at least 4 days before experiments. Each transmitter was calibrated prior to implantation. Output (frequency in Hz) was monitored by a mounted antenna placed under each rat's cage and the data fed into a peripheral processor (Dataquest III system, Mini Mitter, Inc.) connected to an IBM PC. Temperatures were recorded at 5 min intervals. To avoid any circadian variation in body temperature or plasma IL 6 activity, all injections of LPS and pentoxifylline were made between 8:00 AM and 10:00 AM.

Bioassay for IL 6

Plasma IL 6 activity was measured using the IL 6–dependent B-9 hybridoma cell line.[32-34] The B-9 cells were cultured in Iscove's modified Dulbecco's medium (IMDM; Life Technology, Inc.) supplemented with human recombinant IL 6 (rIL6) (10 U/mL obtained from L. Aarden), 20 uM 2-mercaptoethanol, 10% heat-inactivated fetal calf serum (FCS), 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells were washed once in the above medium without added IL 6 before the addition of the plasma samples or known amounts of human rIL 6.

To run the IL 6 assay, a 5 μl sample to be assayed was combined with 5000 B9 cells in 195 μl IMDM/5% FCS in a flat-bottom microtiter plate (Corning) for a final volume of 200 μl. All samples were run in duplicate. The control medium, which contains no IL 6, was run in quadruplicate. In addition to the undiluted plasma samples, a serial dilution of each sample was assayed. Serial dilution was performed because our standard curve indicated that high levels of IL 6 inhibit growth of the B-9 cells. Each sample was serially diluted initially to a maximum of 1:62,500. If IL 6 activity was present at the 1:62,500 dilution, the sample was then further serially diluted to 1:1,562,500.

Cells were pulsed at 68 to 72 hr with 0.5 μCi of [^{3}H]-thymidine and harvested onto glass fiber filter strips (Cambridge Technology, Inc.); the radioactivity incorporated into DNA was counted by a β-scintillation counter (Packard Instrument Company). For each assay, a standard curve was run with recombinant human IL 6. One unit of IL 6 is equal to the amount that caused half-maximum proliferation in the standard curve. From this standard curve, a best-fit regression was calculated for the rising portion of the curve in the linear range. The equation for this best fit line was used to calculate IL 6 activity in plasma samples.

All samples were serially diluted until they become undetectable. All counts per minute (cpm) that fell within 2 standard deviations of the baseline cpm were excluded to reduce the potential error resulting when converting from cpm to units of IL 6 by multiplying by the dilution factor. The largest calculated IL 6 activity that fell outside the 2 standard deviations of the baseline and fell well below the peak was taken as the IL 6 value. This ensured that all data used fell in

the steep linear portion of the standard curve, which increased the accuracy of the values.

Bioassay for TNF

The WEHI 164 subclone 13 (A. Waage, University of Trondheim, Norway) has been shown to be extremely useful in detecting low concentrations of TNF (in the picogram range).[35] WEHI 164 subclone 13 cells were suspended at 5×10^4 in 100 μl per well of RPMI 1640, 10% fetal calf serum, 1 mM L-glutamine, and 0.5 μg/mL actinomycin D in 96-well tissue culture plates. A serial dilution was done for each plasma sample to be assayed, starting from 1:20 to a maximum of 1:40,960 then 100 μl of the diluted sample was added to the cells and incubated for 20 hr at 37°C in 5% CO_2. To measure the percent of viable cells, the 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl-tetrazolium bromide (MTT tetrazolium) cytotoxicity assay was used. Twenty microliters of MTT tetrazolium (5 mg/mL, Sigma) was added to each well and allowed to incubate an additional 4 hr. To dissolve the dark blue tetrazolium crystals, 150 μl of supernatant fluid was removed from each well and 100 μl of isopropranol with 0.04 N HCl was added and allowed to react for approximately 4 hr. The plates were then read in a microELISA reader at 570 nm and units were calculated based on the values obtained with a purified recombinant human TNF standard (Cetus Corp., Emeryville, CA) tested in the same assay. The calculation of TNF activity was done in a manner similar to that for IL 6 (for details please see *Bioassay of IL 6*).

Drugs

Purified LPS (*Escherichia coli* endotoxin 0111:B4, catalog number L 97F-4089, Sigma) was dissolved in 0.9% sterile pyrogen-free sodium chloride solution (saline) and injected IP in doses of 10 μg/kg.

Pentoxifylline (catalog number RC 3795, Hoechst-Roussel Pharmaceuticals, Inc.) was dissolved in saline and injected IP at a dose of 50 mg/kg or 200 mg/kg. The control animals were given the same volume of saline.

Plasma Samples

Rats were anesthetized by intramuscular injection of ketamine hydrochloride and Xylazine (Rompun). Blood was collected within 1.5 min into a heparinized syringe by cardiac puncture. Plasma was separated by centrifugation of the freshly drawn blood and stored at −20°C.

Data Analysis

Data are presented as mean ± SE. Statistical differences among three group means were determined for changes in body temperature, plasma IL 6 activity, and TNF activity using one factor ANOVA, followed by pairwise comparisons tested by Fisher least significant difference.

Acknowledgments

We thank Hoechst-Roussel Pharmaceuticals, Inc. for providing us with pentoxifylline and funds for these studies. We thank L. Aarden for providing us with the B9 cells used in the IL 6 assay and with recombinant IL 6 and A. Waage for providing us with the WEHI cell line used to measure TNF. We thank M.A. Schork, and A. Kshirsagar for their assistance in data analysis.

REFERENCES

1. Antignani PL, Todini AR, Saliceti F, Pacino G, Bartolo M (1987) Results of clinical, laboratory and haemorheological investigations of the use of pentoxifylline in high doses. Pharmatherapeutica 5:50-56.

2. Soliman MH, O'Neal K, Waxman K (1987) Pentoxifylline improves tissue oxygenation following anesthesia and operation. Crit Care Med 15:93-94.

3. Strieter RM, Remick DG, Ward PA, Spengler RN, Lynch III JP, Larrick J, Kunkel SL (1988) Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifylline. Biochem Biophys Res Commun 155:1230-1236.

4. Zabel P, Wolter DT, Schonharting MM, Schade UF (1989) Oxpentifylline in endotoxaemia. Lancet, ii:1474-1477.

5. Sullivan GW, Holliday TC, Novick Jr. WJ, Mandell GL (1988) Inhibition of the inflammatory action of interleukin-1 and tumor necrosis factor (alpha) on neutrophil function by pentoxifylline. Infect Immun 56:1722-1729.

6. O'Malley WE, Achinstein B, Shear MJ (1962) Action of bacterial polysaccharide on tumors. II. Damage of Sarcoma 37 by serum of mice treated with serratia marcescens polysaccharide, and induced tolerance. J Natl Cancer Inst 29:1169-1175.

7. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666-3670.

8. Gerami A, Ikeda Y, Le Trang N, Hotez PJ, Beutler B (1985) Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumor necrosis factor). Immunol Lett 11:173-177.

9. Cerami A, Beutler B (1988) The role of cachectin/TNF in endotoxic shock and cachexia. Immunol Today 9:28-31.

10. Sherry BA, Gelin J, Fong Y, Marano M, Wei H, Cerami A, Lowry S, Lundholm K, Moldawer LL (1989) Anticachectin/tumor necrosis factor-α antibodies attenuate development of cachexia in tumor models. FASEB J 3:1956-1962.

11. Mahony SM, Tisdale MJ (1989) Reversal of weight loss induced by tumor necrosis factor-alpha. Cancer Lett 45:167-172.

12. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A (1987) Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330:662-664.

13. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey III TJ, Zentella A, Albert JD, Shires GT, Cerami A (1986) Shock and tissue injury induced by recombinant human cachectin. Science 234:470-474.

14. Dinarello C, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MA, Jr., O'Connor JV (1986) Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin-1. J Exp Med 163:1433-1450.

15. Kettelhut IC, Goldberg A (1988) Tumor necrosis factor can induce fever in rats without activating protein breakdown in muscle or lipolysis in adipose tissue. J Clin Invest 81:1384-1389.

16. Long NC, Kunkel SL, Vander AJ, Kluger MJ (1990) Antiserum against tumor necrosis factors enhances lipopolysaccharide fever in rats. Am J Physiol 258:R332-R337.

17. Nagai M, Saigusa T, Shimada Y, Inagawa H, Oshima H, Iriki M (1988) Antibody to tumor necrosis factor (TNF) reduces endotoxin fever. Experientia 44:606-607.

18. Mathison JC, Wolfson E, Ulevitch RJ (1988) Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Invest 81:1925-1937.

19. Kishimoto T (1989) The biology of interleukin-6. Blood 74:1-10.

20. Takatsuki F, Okano A, Suzuki C, Chieda R, Takahara Y, Hirano T, Kishimoto T, Hamuro J, Akiyama Y (1988) Human recombinant IL-6/B cell stimulatory factor 2 augments murine antigen-specific antibody responses in vitro and in vivo. J Immunol 141:3072-3077.

21. Gauldie J, Richard C, Harnish D, Lansdorp P, Baumann H (1987) Interferon β_2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase response in liver cells. Proc Natl Acad Sci USA 84:7251-7255.

22. Marinkovic S, Jahreis GP, Wong GG, Baumann H (1989) IL-6 modulated the synthesis of a specific set of acute phase plasma proteins in vivo. J Immunol 142:808-812.

23. Helle M, Brakenhoff JPJ, Groot ERD, Aarden LA (1988) Interleukin 6 is involved in interleukin 1-induced activities. Eur J Immunol 18:957-959.

24. Opp M, Obal F Jr, Cady AB, Johannsen L, Krueger JM (1989) Interleukin-6 is pyrogenic but not somnogenic. Physiol Behav 45:1069-1072.

25. LeMay LG, Vander AJ, Kluger MJ (1990) The role of IL-6 in fever in rats. Am J Physiol 258:R798-R803.

26. Poggesi L, Scarti L, Boddi M, Masotti G, Serneri GGN (1985) Pentoxifylline treatment in patients with occlusive peripheral arterial disease. Circulatory changes and effects on prostaglandin synthesis. Angiology 36:626-637.

27. Ikossi MG, Ambrus JL, Chadha KC (1986) Regulation of in vitro human leukocyte interferon production: Effect of prostaglandin synthetase and phosphodiesterase inhibition. Res Comm Chem Pathol Pharmacol 54:379-393.

28. Scott GM, Secher DS, Flowers D, Bate J, Cantell K, Tyrrell DAJ (1981) Toxicity of interferon. Brit Med J 282:1345-1348.

29. LeMay LG, Vander AJ, Kluger MJ (1990) The effects of psychological stress on plasma interleukin-6 activity in rats. Physiol Behav 47:957-961.

30. LeMay DR, LeMay LG, Kluger MJ, D'Alecy LG (1990) Plasma profiles of IL-6 and TNF following fever inducing doses of lipopolysacharride in the dog. Am J Physiol 259:R126-R132.

31. Scales WE, Kluger MJ (1987) Effect of antipyretic drugs on circadian rhythm in body temperature of rats. Am J Physiol 253:R306-R313.

32. Aarden LA, Groot ER DE, Schaap OL, Lansdorp PM (1987) Production of hybridoma growth factors by human monocytes. Eur J Immunol 17:1411-1416.

33. Van Oers MHJ, van der Heyden AA, Aarden LA (1988) Interleukin-6 (IL-6) in serum and urine of renal transplant recipients. Clin Exp Immunol 71:312-318.

34. Aarden LA (1989) Hybridoma growth factor. In Sehgal PB, Grieninger G, Tosato G (eds) Regulation of the Acute Phase and Immune Responses: A New Cytokine, Ann NY Acad Sci 557:192-198.

35. Espevik T, Nissen-Mayer J (1986) A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Methods 95:99-105.