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Pentoxifylline-induced Modulation of Human Leukocyte Function *In Vitro*

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We previously demonstrated that pentoxifylline stimulates leukocyte migration in vitro ***and leukocyte accumulation*** in vivo ***and protects neonatal mice from experimentally induced*** Staphylococcus aureus ***infections. In the present studies we have investigated pentoxifylline's effect on human leukocyte function*** in vitro. ***In these studies we demonstrate that pentoxifylline at low concentrations (ie, 0.01 and 0.1 mg/ml) stimulates both leukocyte migration and microbicidal activity*** in vitro. ***Alternatively, low concentrations (0.001 to 0.1 mg/ml) of pentoxifylline had no significant effect on the binding uptake of*** S. aureus ***by leukocytes, nor did it enhance phagocytic degranulation. At extremely low concentrations (0.001 mg/ml), pentoxifylline enhanced oxygen metabolism by human leukocytes, as reflected by increased H_2O_2 production and chemiluminescence (CL). At higher concentrations (ie, 0.1 to 1 mg/ml), pentoxifylline consistently suppressed these leukocyte functions*** in vitro. ***Thus, this study supports the following hypothesis: 1) the*** in vivo ***effects of pentoxifylline may involve a direct effect on both leukocyte mobilization and microbicidal activity, and 2) the enhanced microbicidal activity induced by pentoxifylline may be a result of enhanced leukocyte oxygen metabolism. In summary, pentoxifylline appears to be an interesting immunomodulator (ie, immunoenhancement and immunosuppression) of leukocyte function*** in vitro, ***but additional studies will be required before the efficacy of pentoxifylline in man can be determined. (Am J Pathol 1990, 136:623–630)***

Despite the continued development of new antimicrobial agents, infections in the compromised host are a widespread and serious problem.[1] A major cause of this problem is often impairment in neutrophil function.[2–7] The use of immunotherapeutic agents to improve neutrophil dysfunction could be very helpful, but studies of several such agents (ie, ascorbic acid, levamisole, and lithium) have not shown consistent efficacy.[8] Recently, studies in our laboratory have suggested that the methylxanthine pentoxifylline may be a useful immunotherapeutic agent. In our original studies, we demonstrated that pentoxifylline enhanced rabbit neutrophil (polymorphonuclear leukocytes [PMN]) chemotaxis *in vitro*.[9] These studies suggested that pentoxifylline might be used to augment host defense in immunocompromised individuals. To investigate this possibility, we recently tested the efficacy of pentoxifylline in a compromised host model using the neonatal mouse. In these studies, pentoxifylline was found to augment mouse PMN chemotaxis *in vitro*, increase leukocyte accumulation *in vivo*, and protect neonatal mice from *Staphylococcus aureus* infection.[6] From this data we hypothesized that the *in vivo* immunopotentiation effect of pentoxifylline might be due in part to enhancement of leukocyte function. In the present study we have extended these previous findings by determining the effect of pentoxifylline on human PMN function *in vitro*. We have found that low levels of pentoxifylline stimulate leukocyte migration, microbicidal activity, H_2O_2 production, and chemiluminescence in human leukocytes. Additionally, we demonstrated that pentoxifylline does not affect phagocytosis or lysosomal enzyme degranulation of human leukocytes. Interestingly, high concentrations of pentoxifylline suppressed leukocyte function *in vitro*. These studies clearly demonstrate that pentoxifylline can modulate leukocyte function *in vitro* and suggest that this agent may be useful in both proinflammatory and anti-inflammatory therapy in humans.

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Materials and Methods

General Reagents

S. aureus 502A was kindly supplied by Dr. Lynn Kates, Department of Pediatrics, Washington University, St. Louis, MO. 5-amino-2, 3-dihydro-1, 4 phthalazinedione (luminol); P-nitrophenyl-N-acetyl-β-glucosaminide; and *Micrococcus lysodeikticus* were purchased from Sigma Chemical Co. (St. Louis, MO). Mueller-Hinton Agar and Trypticase Soy Broth were purchased from Becton Dickinson Co. (Cockeysville, MD). Methyl-^{3}H thymidine was purchased from Amersham Co. (Arlington Heights, IL). Aqueous low-speed centrifugation (LSC) solution was purchased from National Diagnostics (Highland Park, NJ). Pentoxifylline was obtained from Hoechst-Roussel Pharmaceuticals (Somerville, NJ).

Preparation of Human Leukocytes

Leukocytes used for experiments were obtained from human peripheral blood. Leukocytes were separated from heparinized blood (50 U/ml) by dextran sedimentation, washed twice, and resuspended in Hanks' balanced salt solution (HBSS). Typically, the differential count in cell suspension following dextran sedimentation included 76% neutrophils, 16% lymphocytes, and 6% monocytes. In selected experiments (chemotaxis, chemiluminescence, and H_2O_2), the standard Ficoll-Hypaque sedimentation procedure (Ficoll, Sigma Chemical Co., St. Louis, MO; Hypaque, Winthrop Laboratories, New York, NY) was also employed to separate mononuclear cells from PMNs. Contaminating erythrocytes were removed by hemolysis with 0.87% ammonium chloride for 10 minutes. The leukocyte preparations used in these studies generally were greater than 95% neutrophils.

Leukocyte Migration

Analysis of leukocyte migration was performed with the standard Boyden chamber system with human peripheral PMNs as indicator cells for the assay.[10] Polymorphonuclear leukocytes used for all chemotaxis studies were isolated from human peripheral blood by standard Ficoll-Hypaque procedures. The synthetic chemotactic peptide formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe) was used as the chemoattractant to determine the effect of pentoxifylline on leukocyte chemotaxis. For these studies, varying concentrations of pentoxifylline were placed in the lower compartment of a Boyden chamber in the presence (chemotaxis) or absence (random migration) of a constant concentration of f-met-leu-phe. The Boyden chambers were incubated for 1 hour at 37 C, and the resulting migration was quantitated microscopically.[10] All PMN migration data were expressed as the Chemotactic Index (CI), ie, the summation of the distance traveled by each cell in the filter multiplied by the cell number.[10]

Microbicidal Assay

To determine the effect of pentoxifylline on *in vitro* microbicidal activity in human PMNs, a standard microbicidal assay was used. Initially, an 18-hour trypticase soy broth culture of *S. aureus* 502A was washed twice with sterile distilled water, and the resulting bacterial pellet was resuspended in 5 ml of distilled water. Under these conditions, the suspension contained approximately 2×10^8 *S. aureus* 502A/ml. For these studies, human leukocytes (1×10^7/ml) were pretreated for 20 minutes at 37 C with either HBSS or varying concentrations of pentoxifylline in HBSS. To prepare microbicidal reaction mixtures, 0.5 ml aliquot of bacterial suspension (approximately 2×10^6 bacteria/ml), 0.4 ml of leukocyte solution (containing 1×10^7 cells/ml HBSS), and 0.1 ml of normal human serum were added to a capped plastic tube and incubated under continuous rotation at 37 C for various times. Next, serial 10-fold dilutions of these reaction mixtures were prepared in distilled water and plated for bacterial colony-forming units (CFU) on Mueller-Hinton agar plates. The resulting CFU were quantitated and the data expressed as percent change in *S. aureus* killed.

Binding–Phagocytosis Assays

To determine the effect of pentoxifylline on microbial uptake (ie, binding and phagocytosis) by human leukocytes, radioactive *S. aureus* 502A was used. To prepare radioactive *S. aureus,* the bacteria were grown for 24 hours in trypticase soy broth containing 100 μl of methyl-^{3}H thymidine (5 μCi/mM per 5 ml), washed twice with phosphate-buffered saline (PBS), and resuspended in 5 ml of PBS. For opsonization of the radioactive bacteria, 0.5 ml of the bacterial suspension was diluted with 4 ml of PBS containing 0.5 ml of normal human serum and then incubated in a shaking water bath at 37 C for 5 minutes. After incubation, this solution was centrifuged and resuspended in 5 ml of 0.1% gelatin HBSS. For the binding–phagocytosis assay, 0.2 ml of control or pentoxifylline pretreated (20 minutes/37 C) leukocytes (1×10^7/ml), or 0.2 ml of HBSS, was mixed with 0.2 ml of bacterial suspension in scintillation counting vials and incubated in a shaking bath, and 3 ml PBS (4 C) was added, followed by differential centrifugation. Low-speed centrifugation (LSC) at 100*g* was used to pellet the bacteria–leukocyte complexes. High-

speed centrifugation (HSC) at 900*g* was used to pellet both free bacteria and bacteria–leukocyte complexes. The resulting low-speed or high-speed pellets were resuspended with 3 ml of scintillation fluid (Liquiscint, LSC Aqueous Solution) and counted with the LS-3155P Liquid Scintillation System (Beckman, Irvine, CA). All data were expressed as percent binding/phagocytosis, calculated as follows:

% Binding/phagocytosis:

$$\frac{\{\text{CPM–LSC with leukocytes}\} - \{\text{CPM–LSC without leukocytes}\}}{\{\text{CPM–HSC with leukocytes}\} - \{\text{CPM–HSC without leukocytes}\}} \times 100$$

Phagocytic Degranulation Assay

Leukocyte phagocytosis of opsonized Sephadex (Pharmacia, Bethlehem, PA) beads was used to determine the effect of pentoxifylline on phagocytosis-induced extracellular lysosomal enzyme release. For this assay, Sephadex 300 beads were preopsonized by incubation with fresh normal serum (50 mg beads/ml serum) at 37 C for 30 minutes. The beads were washed twice with 25 ml of HBSS. Leukocytes were pretreated for 20 minutes at 37 C with varying concentrations of pentoxifylline. For the degranulation studies, leukocytes (5×10^6 leukocytes/ml) were suspended for 5 to 10 minutes in HBSS containing Sephadex 300 beads (25 mg/ml). The leukocyte/bead complexes were then removed by centrifugation. The resulting supernatants were assayed for lysosomal enzyme content: ie, lysozyme, N-acetyl-β-glucosaminidase, and lactoferrin. Lysozyme activity was measured using *M. lysodeikticus;*[11] N-acetyl-β-glucosaminidase activity was determined spectrophotometrically using p-nitrophenyl-acetate;[11] and lactoferrin content was determined by enzyme-linked immunosorbent assay (ELISA).[11]

H_2O_2 Assay

To evaluate the effect of pentoxifylline on H_2O_2 production by PMNs, a simple colorimetric assay based on the horseradish peroxidase–mediated oxidation of phenol red by H_2O_2 was used.[12] Generally, for this assay, 0.5 ml of PMNs (2×10^7/ml) was added to various concentrations of pentoxifylline (0.00001 to 1 mg/ml) containing 10 μl of 0.028 mol/l (molar) phenol red (in HBSS; pH 7.0) and 10 μl of horseradish peroxidase (14 mg/ml in HBSS; pH 7.0). The samples were then incubated for 1 hour at 37 C. At the completion of incubation, the samples were centrifuged (600*g*) for 10 minutes, and the resulting cell-free supernatant was transferred to a fresh test tube. Next, 10 μl of 10N NaOH was added to each sample. The resulting reactions were read at 610 nm against a blank that contained 1 ml HBSS (pH 7.0), 10 μl 0.028 mol/l phenol red, 10 μl horseradish peroxidase, and 10 μl 10N NaOH. Standard curves were prepared with known concentrations of H_2O_2 (0.78 to 50 μmol/l). Control reaction mixtures consisted of either untreated PMNs (negative control) or phorbol-12-myristate-13 acetate (2×10^{-7} mol/l) –treated PMNs (positive control). All reactions were performed in duplicate. All data were expressed as percent H_2O_2 production by the PMNs {(Experimental/Control) × 100}.

Chemiluminescence Assays

A standard luminol assay[10] was used to determine the effect of pentoxifylline on chemiluminescence (ie, oxygen metabolism) in human PMNs. For these studies, a PMN suspension was prepared by Ficoll-Hypaque sedimentation techniques from heparinized normal human peripheral blood. Contaminating red cells were lysed by the addition of 0.87% NH_4Cl. The cells were suspended at a concentration of 2×10^6/ml in 0.1% gelatin containing HBSS. The cells were pretreated for 20 minutes at 37 C with varying concentrations of pentoxifylline. Chemiluminescence reaction mixtures were prepared by the addition of 1 ml of pentoxifylline-treated or untreated PMNs to a luminol solution (1 μg/ml). The chemiluminescence response was monitored with an LS-3155P Liquid Scintillation System. All data were calculated by the following equation and expressed as percent non–pentoxifylline-treated control:

$$\%\ \text{Chemiluminescence} = \frac{\text{CPM}_{\text{pentoxifylline}}}{\text{CPM}_{\text{untreated}}} \times 100$$

Data Analysis

The paired *t*-test was used for all statistical analyses of the differences between pentoxifylline-treated leukocytes and untreated cells in all experiments.[13] A *P* value of more than 0.05 was considered statistically significant.

Results

Effect of Pentoxifylline on Leukocyte Migration

We first determined the effect of pentoxifylline on human leukocyte migration using the modified Boyden chamber assay. As can be seen in Figure 1, low concentrations of pentoxifylline (0.047 to 0.37 mg/ml) dramatically stimu-

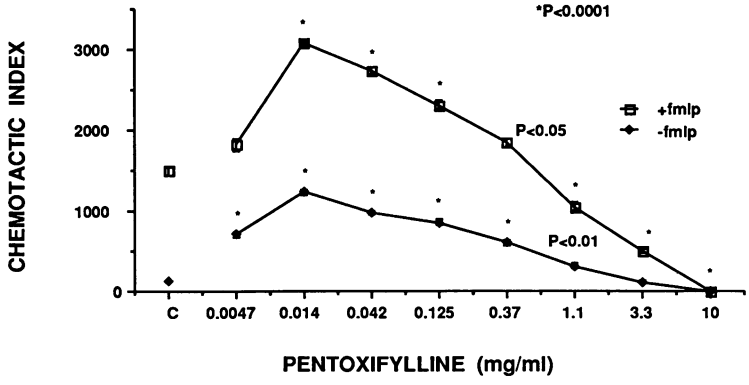

Figure 1. *Effect of pentoxifylline on human PMN chemotaxis. To determine the effect of pentoxifylline on human PMN chemotaxis, PMNs were isolated from human blood and exposed to various concentrations of pentoxifylline (0 mg/ml) using a standard Boyden chamber assay system. The effects of pentoxifylline on both PMN random migration (buffer) and chemotaxis (f-met-leu-phe) were determined, and all data expressed as chemotactic index ± SEM. The positive control value (c) for f-met-leu-phe induced chemotaxis of non-pentoxifylline-treated PMN is indicated by the open square. The control value (c) for untreated PMN random migration is designated by the closed diamond. Using the paired t-test, pentoxifylline induced a significant enhancement of chemotaxis at concentrations between 0.01 and 0.3 mg/ml, and significant suppression of chemotaxis at pentoxifylline concentrations greater than 1 mg/ml. Similar data were obtained for the effect of pentoxifylline for random migration.*

lated chemotaxis when compared with control values (ie, non-pentoxifylline-treated cells). Alternatively, high concentrations of pentoxifylline (ie, >1 mg/ml) suppressed chemotaxis. Pentoxifylline induced a similar pattern of stimulation and suppression in the random migration of leukocytes. These data were consistent with previous studies on the effects of pentoxifylline on rabbit and mouse leukocyte migration.[6,9]

Effect of Pentoxifylline on Microbicidal Activity of Leukocytes

Since the studies described above suggest that low levels of pentoxifylline enhanced motility in the leukocyte, we next determined the direct effect of low concentrations of pentoxifylline on the microbicidal activity of human leukocytes. The results of these experiments (Figure 2) clearly demonstrate that at low concentrations (ie, 0.01 mg/ml), pentoxifylline significantly enhances microbicidal activity of human leukocytes ($P < 0.01$) after 1 hour incubation of the leukocytes with the *Staphylococcus aureus*. Incubation of the pentoxifylline pretreated leukocytes with the *S. aureus* for longer periods of time did not significantly alter the pentoxifylline effect. Interestingly, at higher concentrations (1.0 mg/ml), pentoxifylline suppressed microbicidal activity of human leukocytes by 289 ± 34.78% (N = 17, $P < 0.001$). Thus, these data clearly suggest that pentoxifylline may function *in vivo* as an immunopotentiator by modulating not only leukocyte migration but also microbicidal activity.

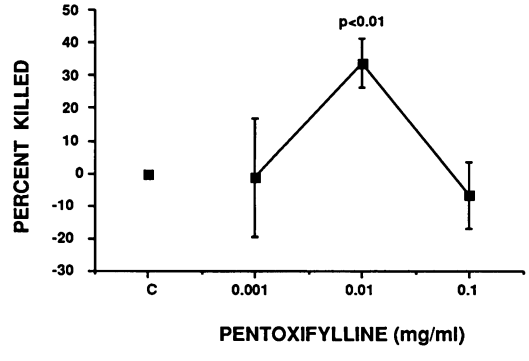

Figure 2. *Effect of pentoxifylline of human leukocyte killing of* S. aureus. *To determine the effect of pentoxifylline on leukocyte microbicidal activity, human leukocytes were preincubated with various concentrations of pentoxifylline (0 mg/ml to 1.0 mg/ml) for 20 minutes at 37 C and then evaluated for microbicidal activity using* S. aureus *and 1-hour incubation. The data presented in Figure 2 represents the mean ± SEM of 17 independent experiments. Leukocyte microbicidal activity values are expressed as %* S. aureus *killed. Pentoxifylline pretreatment at 0.01 mg/ml significantly enhanced leukocyte microbicidal activity (P < 0.01) of the human leukocytes. Pentoxifylline at 0.001 and 0.1 mg/ml had no significant effect on leukocyte killing of* S. aureus. *Higher concentrations of pentoxifylline (ie, 1 mg/ml) significantly depressed microbicidal activity (i.e. −289 ± 34%, P < 0.001).*

Effects of Pentoxifylline on Leukocyte Binding–Phagocytosis

To begin to investigate the possible mechanism(s) of pentoxifylline enhancement of microbicidal activity, we next determined the influence of pentoxifylline on leukocyte binding, phagocytosis, and degranulation. As can be seen in Figure 3, low concentrations of pentoxifylline had no significant effect on binding/phagocytosis of radiolabeled bacteria by leukocytes when compared with control (nontreated) leukocytes. Alternatively, high concentrations of pentoxifylline had a slightly suppressive effect on the binding of radioactive *Staphylococcus aureus*. These data suggest that the pentoxifylline-induced increase in leukocyte microbicidal activity is not likely the result of increases in *S. aureus* uptake by the pentoxifylline-treated leukocytes.

When we investigated the influence of pentoxifylline on phagocytosis-mediated degranulation by leukocytes,

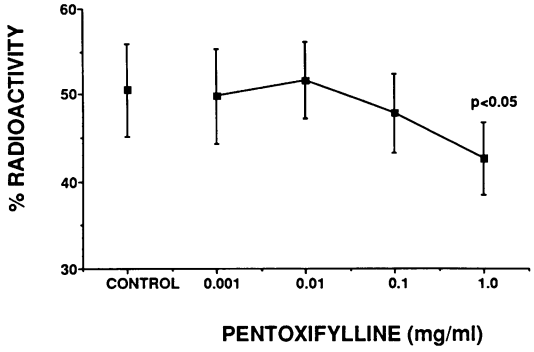

Figure 3. *Effect of pentoxifylline on human leukocyte binding-phagocytosis of* S. aureus. *To determine the effect of pentoxifylline on leukocyte binding-phagocytosis, human leukocytes were incubated with various concentrations of pentoxifylline (0 mg/ml to 1.0 mg/ml) for 20 minutes at 37 C, followed by incubations with radiolabeled* S. aureus. *Leukocyte binding-phagocytosis of opsonized radiolabelled* S. aureus *is expressed as % binding-phagocytosis ± SEM. The data in Figure 3 are expressed as the mean ± SEM of seven independent experiments. Suppression of binding phagocytosis was seen at the 1.0 mg/ml concentration of pentoxifylline (P < 0.05), but no statistical significance was seen at the 0.001–0.1 mg/ml concentration of pentoxifylline.*

we found that low concentrations of pentoxifylline had no significant enhancing effect on β-glucosaminidase or lysozyme release, nor did it enhance lactoferrin release (Figure 4). Generally, higher concentrations of pentoxifylline (ie, ≥ 0.1 mg/ml) consistently suppressed phagocytic degranulation. These data suggest that pentoxifylline-induced stimulation of leukocyte microbicidal activity is not likely the result of enhanced degranulation.

Effects of Pentoxifylline on Leukocyte Oxygen Metabolism

Since oxygen metabolism clearly plays an important role in microbicidal activity, we next determined the influence of pentoxifylline on oxygen metabolism (H_2O_2 production and chemiluminescence) in human leukocytes. As seen in Figure 5, low levels of pentoxifylline (0.01 to 0.00001 mg/ml) consistently enhanced H_2O_2 production by the human PMNs. At higher concentrations, pentoxifylline (0.1 to 1 mg/ml) suppressed H_2O_2 production by PMNs. Extremely low levels of pentoxifylline (ie, 0.001 mg/ml) consistently enhanced PMN chemiluminescence (Figure 5). Higher concentrations of pentoxifylline consistently suppressed chemiluminescence in human leukocytes (Figure 6). These data suggest that pentoxifylline-induced enhancement of oxygen metabolism may contribute to pentoxifylline-induced enhancement of leukocytes microbicidal activity *in vitro*.

Discussion

Infections are a widespread and serious problem despite the continued development of new antimicrobial agents.[8]

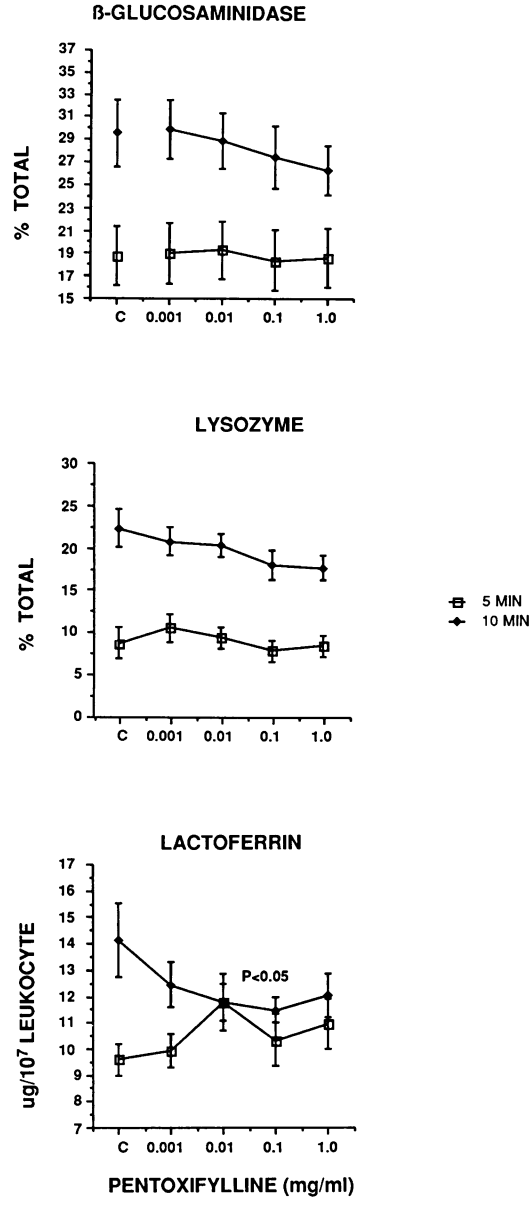

Figure 4. *Effects of pentoxifylline on phagocytic degranulation of human leukocytes. To determine the effect of pentoxifylline on phagocytic degranulation, human leukocytes were preincubated with various concentrations of pentoxifylline (0 mg/ml to 1.0 mg/ml) for 20 minutes at 37 C, and the effects of this pretreatment on lysosomal degranulation was determined using a phagocytosis-based assay (see Methods section). Generally, supernatants from leukocytes phagocytosis (5 or 10 minutes) were evaluated for extracellular lysosomal enzyme releases. Lysozyme activity was measured using* Micrococcus lysodeikticus, *n-acetyl-b-glucosaminidase activity was determined using p-nitrophenyl-acetate, and lactoferrin content was determined by enzyme-linked immunoassay (ELISA). Lysozyme and n-acetyl-b-glucosaminidase releases are expressed as % leukocyte enzyme total and lactoferrin release is expressed as mg/ml of phagocytic supernatant. The results of pentoxifylline pretreatment on treated leukocyte phagocytic degranulation was compared to untreated leukocytes (designated c). The data presented in Figure 4 represent the mean ± SEM of 10 independent experiments. As can be seen in Figure 4, lower concentrations of pentoxifylline had no consistent effect on phagocytic degranulation. In addition, higher concentrations of pentoxifylline (≥0.1 mg/ml) significantly depressed lysosomal enzyme disease.*

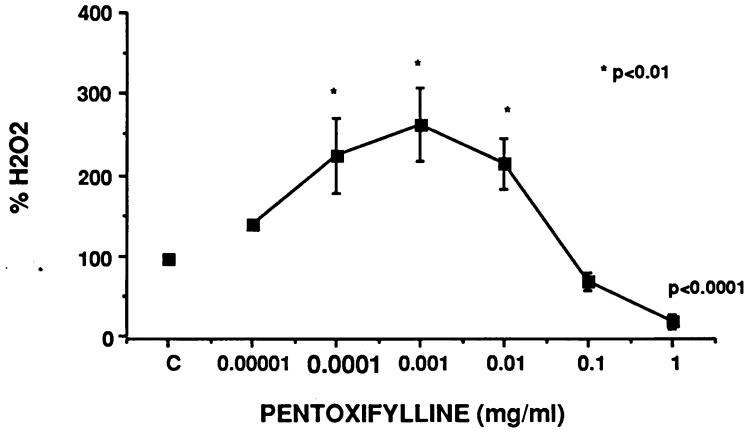

Figure 5. *Effect of pentoxifylline on H_2O_2 production by human PMN. A simple colorimetric assay based on the horseradish peroxidase-mediated oxidation of phenol red by H_2O_2, which results in the formation of a compound demonstrating increased absorbance at 610 nm was used to evaluate the effect of pentoxifylline on H_2O_2 production by human PMN. The data presented here are the mean of eight independent experiments ± SEM. The data are expressed as % H_2O_2 production, ie, (experimental/control) × 100. As compared to control (untreated cells), pentoxifylline at low levels (0.01 to 0.00001 mg/ml) consistently enhanced H_2O_2 production by the PMNs. At higher concentrations (0.1 to 1 mg/ml) pentoxifylline suppressed H_2O_2 by the PMN.*

A major cause of this problem is impaired host defense mechanisms such as diminished levels of complement, decreased amounts of normally functioning antibody, and decreased PMN function.[2-8,14-16] Consequently, there is interest in new therapeutic strategies that will reverse these defects. Until very recently, no drug has been developed that has the potential to significantly enhance PMN function *in vivo*. The development of new pharmacologic agents that directly stimulate PMN function and thus enhance host defense may offer advantages, compared with neutrophil transfusion or administration of type-specific antibody, in regard to a broader spectrum of antimicrobial activity, ease of administration, and side effects.[6-8,17-23] Currently, only a few immunomodulating drugs, such as levamisole and vitamin C, have been investigated, but their use in patients with PMN dysfunction cannot be justified until systematic studies of their efficacy in experimental animals have been carried out.

Our laboratory was the first to study the immunomodulating effects of pentoxifylline.[9] Initially, we demonstrated that pentoxifylline enhanced PMN motility *in vitro*.[9] More recently, we have extended these studies by demonstrating that pentoxifylline decreases the morbidity and mortality from experimentally induced *S. aureus* infection in neonatal mice and that the mechanism for these effects may be due in part to enhanced PMN motility.[6,24] We also found that pentoxifylline increased PMN chemotaxis and accumulation in both neonatal and adult mice.[6] It is therefore likely that the increased survival observed in pentoxifylline-treated neonatal mice[6] was due, at least in part, to the increased delivery of PMNs to the subcutaneous site of *S. aureus* inoculation. Other possible protective mechanisms of pentoxifylline include increased accumulation of other leukocytes (eg, monocytes) at the site of infection, increased blood flow to such reticuloendothelial organs as the liver, or a direct effect of pentoxifylline on multiple PMN functions. Regardless of the mechanism, pentoxifylline appears to be useful in controlling experimental infections in animals. Recent *in vitro* data from our laboratory, as well as others, support the hypothesis that the immunopotentiating effects of pentoxifylline seen *in vivo*[6] are likely a consequence of effects on leukocyte function.[6,9,25-27] Additionally, recent data have suggested that high concentrations of pentoxifylline can suppress inflammation and tissue injury *in vivo*. In fact, *in vitro* studies have suggested that these anti-inflammatory effects of pentoxifyl-

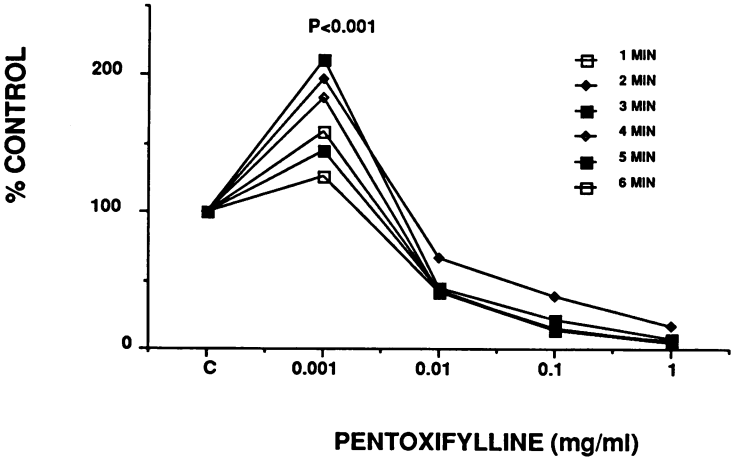

Figure 6. *Effect of pentoxifylline on human PMN chemiluminescence. To evaluate the effect of pentoxifylline pretreatment of leukocytes on oxygen metabolism, PMNs were incubated with various concentrations of pentoxifylline (0 mg/ml to 1.0 mg/ml) for 20 minutes at 37 C and then evaluated in a standard luminol-based chemiluminescence assay (See Materials and Methods). Chemiluminescence values are expressed as % control of untreated PMNs (c) at 2- to 6-minute time intervals. The data present in Figure 4 represent the mean of 10 independent experiments and were evaluated statistically by the paired t-test. Pretreatment of the human PMNs with 0.001 mg/ml of pentoxifylline induced a statistically significant increase ($P < 0.001$) in the baseline (resting) chemiluminescence of the PMNs. Higher concentrations of pentoxifylline induce a significant depression of PMN chemiluminescence.*

line may be regulated through the cytokine system.[28] For example, pentoxifylline has been demonstrated to suppress tumor necrosis factor (TNF) production in monocytes and also to inhibit IL-1 and TNF effects on PMN functions.[28,29] These findings clearly demonstrate that pentoxifylline may be useful *in vivo* as a means of suppressing inflammation and tissue damage caused by PMNs. Clearly, further work will be necessary to determine whether the immunomodulating effects of pentoxifylline will be beneficial in man.

To investigate the potential immunomodulating capacity of pentoxifylline in humans, we evaluated the *in vitro* effects of this drug on human leukocyte function. In the present studies, we demonstrate that pentoxifylline enhances two of the major leukocyte functions involved in host defense—ie, migration and microbicidal activity. This latter effect may be partially due to the stimulation of oxygen metabolism and does not appear to be related to effects on phagocytosis or lysosomal enzyme degranulation. Thus, our present studies suggest that the mechanism by which pentoxifylline enhances host defense *in vivo* may involve not only its ability to stimulate leukocyte migration but also the microbicidal activity at sites of infection. The mechanism for this enhancement is not clear. Some investigations have shown that pentoxifylline elevates intracellular levels of cAMP at high concentrations.[30] This alteration of cAMP levels may be related to inhibitions of chemotaxis and other leukocyte functions seen at high concentrations of this compound.[30] Results of other studies suggest that pentoxifylline causes alterations in the PMN surface membrane that facilitates transduction of an activation signal to the motor apparatus of the cell. Specifically, Hill et al have found that pentoxifylline enhanced the movement of cell surface ConA receptors in human neonatal PMNs after colchicine treatment.[7] The original studies in our laboratory demonstrated that pentoxifylline increased triphosphoinositide levels in leukocytes, and we postulated that these destabilized the membrane skeleton and thereby increased cellular motility.[9] Interestingly, neonatal PMNs have been reported to have a rigid cytoskeleton that may decrease cell surface receptor movement and result in decreased PMN motility. Compared with that of adults, enhancement of membrane skeleton flexibility is an attractive hypothesis for the augmentation of neonatal PMN motility by pentoxifylline. Regardless of the possible mechanism(s) responsible for the *in vivo* and *in vitro* effects of pentoxifylline, our present data support the possible utility of pentoxifylline on both inflammatory and anti-inflammatory therapy in man.

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