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Pentoxifylline Modulation of Plasma Membrane Functions in Human Polymorphonuclear Leukocytes

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Pentoxifylline is known to have major effects on cell membrane function in mammalian cells, including human leukocytes. The protective effects of this agent in animal models of infection and inflammation may be due to alterations in phagocyte (neutrophil and macrophage) function. However, the exact mechanism of action of pentoxifylline is unknown. In this study, we evaluated the effect of the drug on several membrane-associated activities in human polymorphonuclear neutrophils and investigated possible mechanisms for the observed changes in neutrophil function. Pentoxifylline inhibited ingestion of microbial particles (*Staphylococcus aureus* and zymosan); decreased superoxide generation activated by zymosan, formyl-methionyl-leucyl-phenylalanine, and concanavalin A (but not phorbol myristate acetate); and decreased uptake (transport) of adenosine stimulated by formyl-methionyl-leucyl-phenylalanine and zymosan. In contrast, pentoxifylline actually increased clindamycin uptake in zymosan-stimulated polymorphonuclear neutrophils. However, pentoxifylline had no effect on uptake of adenosine or clindamycin in unstimulated neutrophils. In comparison with known inhibitors of nucleoside transport (nitrobenzylthioinosine and dipyridamole), the results suggested that pentoxifylline does not bind to membrane nucleoside transport receptors. At concentrations which inhibit neutrophil function, pentoxifylline activity is not mediated through external membrane nucleoside regulatory sites. Thus, pentoxifylline affects the activation signal chain at a point beyond the membrane receptors. Whatever its precise mechanism of action, pentoxifylline has a striking modulatory effect on cell membrane-associated responses in stimulated leukocytes and may prove useful for control of injurious inflammatory states.

Pentoxifylline is a methylxanthine derivative known to alter mammalian cell membranes (13, 27, 34, 36). This drug is used clinically for treatment of certain vascular insufficiency syndromes because of its ability to lower blood viscosity and increase microcirculatory flow (30, 39). The mode of action of pentoxifylline in this setting may be to increase erythrocyte flexibility (deformability). Recently the drug has been shown to have major effects on leukocyte membrane function (2, 14, 19, 24, 26, 34, 36, 42, 43). Thus, pentoxifylline enhances chemotaxis, increases cell deformability, decreases adherence, and blocks the action of inflammatory cytokines on neutrophils. Associated cellular changes include an increase in cyclic AMP levels, decreased free intracellular calcium, and altered phosphatidyl nucleotide metabolism, but the relationship of these findings to the mechanism of action of pentoxifylline is unknown. In addition, there appears to be a protective action of the drug in animal models of bacterial infection and inflammation, perhaps due to alterations in leukocyte (neutrophil and macrophage) function (3, 7, 21, 24, 33, 43, 45–47).

Thus, the evidence indicates that pentoxifylline has a profound influence on leukocyte cell membrane function and structure. In this study, we examined the effects of the drug on several membrane-associated activities in human polymorphonuclear neutrophils (PMN). Specifically, we observed the influence of pentoxifylline on ingestion of microbial particles, oxidative burst activity, and antibiotic and nucleoside uptake (membrane transport) in PMN. We also evaluated possible mechanisms, binding of external membrane nucleoside regulatory or transport receptors, by which pentoxifylline might alter leukocyte function.

MATERIALS AND METHODS

Preparation of human PMN. Peripheral venous blood from normal human volunteers was collected in heparinized syringes. Granulocytes were isolated by dextran sedimentation and Hypaque-Ficoll density gradient centrifugation as previously described (4, 31, 40). When necessary, residual erythrocytes in the granulocyte preparation were lysed with 0.16 M ammonium chloride. The cells were then washed and suspended in Hanks balanced salt sodium (HBSS) (GIBCO, Grand Island, N.Y.).

Phagocytic particles, other activators of PMN oxidative metabolism, pentoxifylline, and nucleosides. A clinical isolate of *Staphylococcus aureus* was stored at −70°C. Organisms were grown overnight in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with or without [^{3}H]thymidine (10 μCi/ml). Zymosan A (Sigma Chemical Co., St. Louis, Mo.) was boiled, washed, opsonized with fresh human serum, stored at −70°C, and then used at a concentration of 0.1 mg/ml in the studies described below. Formyl-methionyl-leucyl-phenylalanine (FMLP; 10^{-7} M), phorbol myristate acetate (PMA; 1 μg/ml), and concanavalin A (ConA; 30 μg/ml) (all from Sigma) were used as soluble membrane-stimulating agents. Pentoxifylline (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.) was stored at −70°C, reconstituted in HBSS, and used for experiments at final concentrations ranging from 10^{-3} to 10^{-6} M. Adenosine, adenosine deaminase, and the nucleoside inhibitors nitrobenzylthioinosine (NBTI) and dipyridamole (Sigma) were used in studies of PMN superoxide production.

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TABLE 1. Effect of pentoxifylline on phagocytosis of *S. aureus* by human PMN

Incubation time (min)	Phagocytosis (% of control) at a pentoxifylline concn of[a]:		
	10^{-3} M	10^{-4} M	10^{-5} M
5	82.1 ± 6.1 (5), $P = 0.029$	106.5 ± 9.5 (5), $P = 0.38$	114.1 ± 4.9 (5), $P = 0.03$
10	72.3 ± 6.5 (8), $P = 0.003$	98.1 ± 6.0 (8), $P = 0.59$	101.7 ± 4.8 (8), $P = 0.57$
15	73.5 ± 5.8 (9), $P = 0.001$	89.9 ± 4.9 (9), $P = 0.02$	89.7 ± 5.2 (7), $P = 0.07$
20	82.0 ± 3.7 (10), $P = 0.0006$	92.9 ± 7.0 (10), $P = 0.26$	94.3 ± 4.1 (10), $P = 0.15$
30	99.5 ± 7.3 (10), $P = 0.77$	103.8 ± 7.8 (10), $P = 0.5$	116.0 ± 6.7 (10), $P = 0.003$

[a] Data are means ± standard errors of the means. The number of experiments is shown in parentheses. *P* values reflect differences between control and pentoxifylline groups.

Phagocytosis of microbial particles by human PMN. (i) *S. aureus*. PMN (10^7) were preincubated with or without pentoxifylline for 30 min in tubes containing 0.9 ml of HBSS–0.1% gelatin. Radiolabeled, opsonized *S. aureus* (10^8 bacteria in 0.1 ml of HBSS) was added to each tube. The tubes were then incubated at 37°C in 95% air–5% CO_2 with rotation (Multi-Purpose Rotator; Scientific Industries Inc., Springfield, Mass). After an ingestion period of 5 to 30 min, lysostaphin (10 U/ml) was added to the assay tubes for 10 min to kill extraphagocytic *S. aureus*. PMN were recovered by centrifugation and washed twice in HBSS. The washed pellet (after cell lysis in distilled water) was appropriately diluted and used for bacterial enumeration and radioactive counting. Specimens were plated on tryptic soy agar for enumeration of viable bacteria or processed for determination of radioactivity in a Beckman LS-3801 liquid scintillation counter. Radioactive counts were expressed as disintegrations per minute.

Calculations of bacterial ingestion at each time point were performed as previously described (16, 18). Phagocytosis of *S. aureus* by PMN was determined in the following manner: number of ingested organisms = (number of viable organisms in bacterial inoculum/^{3}H counts in bacterial inoculum) × intracellular ^{3}H counts.

The consequences of PMN exposure to pentoxifylline for ingestion of *S. aureus* was determined and compared with control values as follows: percent control phagocytosis = (intraphagocytic bacteria-control/intraphagocytic bacteria-pentoxifylline) × 100.

(ii) Zymosan. PMN (10^7 in 1 ml of HBSS) were preincubated with or without pentoxifylline as described above. Opsonized zymosan particles were added to the tubes, followed by incubation with rotation at 37°C for 30 min. Cell pellets were collected by centrifugation, washed, and smeared on glass slides. The slides were fixed with methanol and stained with methylene blue.

On each slide, 500 consecutive nonaggregated PMN were counted in a blinded fashion to determine the percentage of cells which phagocytized zymosan and the total number of particles ingested. The phagocytic index (number of ingested particles/number of cells) was also calculated for each experiment.

Influence of pentoxifylline and nucleosides on PMN superoxide production. Superoxide generation by human PMN was determined as superoxide dismutase-inhibitable reduction of ferricytochrome *c* (8, 16, 23). PMN (2×10^6/3 ml) in HBSS containing 100 μM ferricytochrome *c* were preincubated with or without pentoxifylline, adenosine, or a nucleoside inhibitor for 15 min at 37°C. Cytochalasin b (5 μg/ml) and either a stimulating agent (zymosan, FMLP, PMA, or ConA) or control medium was then added to the incubation tubes. Duplicate samples contained superoxide dismutase (50 μg/ml). After incubation for 30 min, the cells were removed by centrifugation at 4°C. The optical densities of the supernatants were determined at 550 nm in a spectrophotometer (Carey 219). The difference between duplicate samples with and without superoxide dismutase was calculated, and reduced cytochrome *c* was quantitated by using the equation $E_{550} = 2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

Antibiotic and nucleoside uptake by human PMN. Uptake of radiolabeled clindamycin and adenosine by PMN was determined by a velocity gradient centrifugation technique which we have previously described in detail (17, 22, 31, 40). Human PMN (10^7/ml) in tissue culture medium 199 (GIBCO) containing 5% normal human serum were incubated at 37°C with zymosan or FMLP and either [^{3}H]clindamycin hydrochloride (The Upjohn Co., Kalamazoo, Mich.) or [2,8-^{3}H] adenosine (Dupont, NEN Research Products, Boston, Mass.). The concentration of these substances was ~2.5×10^{-5} M (clindamycin, 10 μg/ml; adenosine, 7 μg/ml). In some experiments, pentoxifylline was added to the PMN suspension and preincubated for 20 min before addition of radiolabeled clindamycin or adenosine. At specified times, antibiotic or nucleoside uptake was determined by velocity gradient centifugation in a microcentrifuge. Clindamycin and adenosine uptake was expressed as the ratio of the cellular concentration of antibiotic to the extracellular concentration (*C/E*).

Assay of phagocyte cytoplasmic enzyme release. Release of lactic dehydrogenase (LDH), a cytoplasmic enzyme, was monitored as an indicator of cellular injury. PMN in HBSS (10^7/ml) were preincubated with or without pentoxifylline for 15 min, followed by addition of the stimulating agent (FMLP or zymosan). After additional incubation with rotation for 20 min at 37°C, the cells were recovered by centrifugation, resuspended, and lysed in 0.2% Triton X-100. Enzyme activity was determined in cell pellets, supernatants, and whole-cell suspensions (without centrifugation and after Triton X-100 disruption). LDH was assayed by measuring the increase in A_{340} resulting from reduction of NAD to form NADH (1).

RESULTS

Characteristics of the cell population. The granulocyte population was ≥97% neutrophils. Since ≥90% of these cells were mature polymorphonuclear neutrophilic leukocytes, the cells will be referred to as PMN. Cell viability was >95%, as judged by trypan blue exclusion.

Phagocytosis of microbial particles by huamn PMN. (i) *S. aureus*. Pentoxifylline at 10^{-3} M, but not at lower concentrations of 10^{-4} and 10^{-5} M, produced a significant decrease in phagocytosis of *S. aureus* at periods of 5 to 20 min (Table 1). This reduction in phagocytosis of radiolabeled staphylococci was in the range of 18 to 28%. After 30 min of incubation, the total number of bacteria ingested by control

TABLE 2. Effect of pentoxifylline on phagocytosis of zymosan by human PMN[a]

Experimental group	Phagocytosis (% of control)	Phagocytic index	Phagocytic index (% of control)
Control	100 (11)	201.9 ± 11.2 (11)	100 (11)
Pentoxifylline at:			
10^{-3} M	86.0 ± 3.6 (11), $P = 0.002$	164.5 ± 10.1 (11)	82.6 ± 4.4 (11), $P = 0.002$
10^{-4} M	95.6 ± 3.0 (11), $P = 0.12$	190.5 ± 12.4 (11)	96.3 ± 3.3 (11), $P = 0.22$
10^{-5} M	97.3 ± 1.9 (11), $P = 0.14$	204.8 ± 14.3 (11)	103.3 ± 3.9 (11), $P = 0.32$

[a] Data are means ± standard errors of the means. The number of experiments is shown in parenthesis. P values reflect differences between control and pentoxifylline groups.

and pentoxifylline-exposed PMN had equalized. Thus, the rate of *S. aureus* phagocytosis by pentoxifylline-exposed PMN was slower, but the total number of ingested organisms was the same as with control cells. It is of interest that pentoxifylline at a low concentration of 10^{-5} M actually appeared to stimulate ingestion of *S. aureus* at 5 and 30 min.

(ii) Zymosan. In a similar fashion, pentoxifylline at a concentration of 10^{-3} M inhibited ingestion of opsonized zymosan by human PMN. This decrease in phagocytosis was manifested as a reduction in both the number of cells phagocytizing zymosan and the number of ingested particles per cell (Table 2). Lower concentrations of pentoxifylline (10^{-4} and 10^{-5} M) failed to inhibit phagocytosis.

Superoxide generation. When human PMN were exposed to soluble substances (FMLP, ConA, and PMA) or microbial particles (zymosan) which trigger cell membrane activity, the respiratory burst with superoxide production was stimulated. At concentrations from 10^{-3} to 10^{-5} M, pentoxifylline had a marked, concentration-dependent inhibitory effect on superoxide production in PMN stimulated by ConA, FMLP, and zymosan (Table 3). This decrease in superoxide production compared with control cells was in the range of 33% (10^{-4} M) to 77% (10^{-3} M). In contrast, pentoxifylline had only a minor effect on superoxide generation by PMN exposed to PMA.

The activity of adenosine, which is known to modulate stimulus-induced superoxide production in neutrophils (9, 10; W. L. Hand, D. L. Hand, and N. L. King-Thompson, submitted for publication), was evaluated in comparison with pentoxifylline. The nucleoside substantially inhibited PMN superoxide generation stimulated by FMLP, ConA, zymosan, and *S. aureus* (but not PMA) (Table 3). In contrast to pentoxifylline, the inhibitory activity of adenosine was nearly maximal at ~1×10^{-5} to 2.5×10^{-5} M and increased only slightly with higher concentrations. Furthermore, adenosine markedly inhibited superoxide production by PMN stimulated with soluble activators (FMLP and ConA) but had less effect on microbial particle-induced production of superoxide. As noted above, pentoxifylline had a concentration-dependent inhibitory activity which was virtually identical for PMN exposed to FMLP, ConA, or zymosan.

Methylxanthines are antagonists at external membrane nucleoside-binding sites and may block the influence of adenosine on cellular function. Therefore, we studied the effects of adding both pentoxifylline and adenosine to the neutrophil incubation mixture. Not only did pentoxifylline fail to block adenosine activity, but this combination of agents actually had an additive effect (greater inhibition than either substance alone) on FMLP- and zymosan-stimulated PMN (Table 4). As we previously demonstrated (Hand et al., submitted), NBTI and dipyridamole, which bind to cell membrane nucleoside transport receptors and thereby inhibit nucleoside transport (29, 48), also inhibited PMN superoxide production stimulated by FMLP. It is of note that the inhibitory effects of pentoxifylline and NBTI on FMLP-induced superoxide production were additive (Table 4).

Adenosine deaminase blocked the inhibitory effects of adenosine on FMLP- and zymosan-stimulated superoxide production. However, addition of adenosine deaminase caused no alteration in the inhibition of superoxide generation due to pentoxifylline or NBTI.

We considered the possibility that pentoxifylline might directly interfere with reduction of cytochrome *c* rather than

TABLE 3. Influence of pentoxifylline and adenosine on stimulated superoxide production by human PMN

Experimental group	Superoxide generation (% of control) at a drug concn of[a]:					
	10^{-3} M	10^{-4} M	5×10^{-5} M	2.5×10^{-5} M	10^{-5} M	10^{-6} M
Pentoxifylline						
FMLP	25.8 ± 1.5 (5)[b]	60.0 ± 1.9 (11)[b]	73.0 ± 4.1 (4)[b]	76.5 ± 3.0 (4)[b]	83.6 ± 2.7 (4)[b]	
ConA	31.1 ± 12.4 (5)[b]	66.9 ± 5.0 (10)[b]			94.3 ± 5.1 (12)	
Zymosan	23.1 ± 6.4 (4)[b]	66.7 ± 4.8 (15)[b]	77.7 ± 6.7 (6)[b]	95.6 ± 3.6 (6)	98.9 ± 4.8 (16)	102.0 ± 6.8 (7)
PMA	109.4 ± 1.0 (3)[b]	95.6 ± 1.5 (6)[b]			94.3 ± 1.1 (6)[b]	
Adenosine						
FMLP	24.2 ± 9.6 (3)[b]	32.3 ± 4.7 (19)	39.4 ± 8.7 (2)		34.5 ± 10.4 (6)[b]	55.9 ± 12.7 (7)[b]
ConA	33.0 (1)	35.6 ± 3.9 (6)[b]	44.0 ± 4.1 (4)[b]	42.0 ± 7.7 (4)[b]	49.2 ± 5.0 (5)[b]	55.6 (1)
Zymosan		43.4 ± 4.7 (22)[b]	55.2 ± 14.8 (5)[b]	54.1 ± 17.1 (4)[b]	56.9 ± 13.0 (9)[b]	85.3 (1)
S. aureus	59.6 ± 17.3 (5)[b]	74.2 ± 6.1 (15)[b]	74.8 ± 2.8 (9)[b]	77.4 ± 5.0 (6)[b]	89.1 ± 7.0 (16)	96.5 ± 23.2 (2)
PMA		105.3 ± 2.5 (5)	109.5 ± 2.3 (2)	110.0 ± 0.5 (2)	107.1 ± 2.0 (5)	

[a] Data are means ± standard errors of the means. The number of experiments is shown in parenthesis. Some data for adenosine are from Hand et al. (submitted).

[b] Significant difference between control (no pentoxifylline or adenosine) and experimental groups ($P < 0.05$).

TABLE 4. Effects of pentoxifylline, adenosine, and NBTI on stimulated superoxide production by human PMN

Experimental group	Superoxide generation (% of control) at a drug concn of[a]:		
	10^{-5} M	10^{-4} M	10^{-3} M
FMLP			
Pentoxifylline		57.1 ± 3.1 (5), $P = 0.0001$	25.8 ± 1.5 (5), $P = 0.00001$
Adenosine		23.1 ± 3.3 (5), $P = 0.00002$	
Pentoxifylline + adenosine (10^{-4} M)		14.3 ± 3.2 (3), $P = 0.0007$	8.8 ± 3.3 (4), $P = 0.00006$
NBTI	65.6 ± 5.9 (5), $P = 0.003$		
Pentoxifylline + NBTI (10^{-5} M)		34.8 ± 7.8 (5), $P = 0.0007$	17.4 ± 4.5 (5), $P = 0.00004$
Zymosan			
Pentoxifylline		58.9 ± 4.4 (5), $P = 0.0005$	26.1 ± 3.6 (5), $P = 0.00002$
Adenosine		47.8 ± 3.4 (5), $P = 0.00006$	
Pentoxifylline + adenosine (10^{-4} M)		14.7 ± 4.0 (5), $P = 0.00002$	10.2 ± 5.0 (5), $P = 0.00004$

[a] Data are means ± standard errors of the means. The number of experiments is shown in parenthesis. P values reflect differences between control (no pentoxifylline or nucleoside) and experimental groups.

alter PMN production of superoxide. However, pentoxifylline had no effect on the reduction of cytochrome c by superoxide generated in a cell-free system (hypoxanthine-xanthine oxidase).

Antibiotic uptake. As we have previously shown (17, 31, 40), uptake of clindamycin by human PMN was striking (C/E = 10 to 11) (Table 5). Adenosine entry into neutrophils was modest, with a C/E value of ~2 at 60 min. Zymosan ingestion stimulated uptake of both clindamycin (C/E = 16.6) and adenosine (C/E = 3.2) by PMN. Exposure of PMN to FMLP produced a slight increase in adenosine (but not clindamycin) entry. Pentoxifylline at 10^{-3} to 10^{-6} M had little effect on entry of either clindamycin or adenosine into unstimulated PMN. However, pentoxifylline at a concentration of 10^{-3} M inhibited zymosan-stimulated uptake of adenosine at each time point and had a similar effect on FMLP-induced entry into PMN. The drug failed to inhibit and actually appeared to augment the zymosan-induced increase in clindamycin uptake by PMN.

Assays of cell viability and cytoplasmic LDH release. The possibility that the effects of pentoxifylline on PMN phagocytic and respiratory burst activity might be due to toxicity of the drug was considered. Cell viability (trypan blue exclusion) and release of the cytoplasmic enzyme LDH were evaluated as indicators of cellular injury after exposure to pentoxifylline. Approximately 95% of the cells were viable after exposure to pentoxifylline at of 10^{-3} to 10^{-6} M. Release of cytoplasmic LDH to the external environment by stimulated PMN (zymosan or FMLP) was not influenced by pentoxifylline (10^{-3} to 10^{-5} M).

DISCUSSION

Pentoxifylline, a methylxanthine derivative [1-(5-oxohexyl)-3,7-dimethylxanthine], is now known to produce major alterations of cellular membrane functions, especially in leukocytes (2, 14, 19, 24, 26, 34, 36, 42, 43). The drug was shown to increase chemotaxis, augment cell deformability, and decrease adherence of neutrophils. Recent data suggest that pentoxifylline has the ability to reverse or antagonize many of the changes which occur in stimulated leukocytes (PMN and macrophages) (2, 14, 42, 46). In addition to blocking the action of inflammatory cytokines (including tumor necrosis factor alpha) on neutrophils (14, 42), pentoxifylline also inhibits production of tumor necrosis factor (a block in message translation) by endotoxin-treated mouse

TABLE 5. Influence of pentoxifylline on uptake of clindamycin and adenosine by human PMN

Experimental group	Antibiotic uptake (C/E)[a]:			
	5 min	15 min	30 min	60 min
Clindamycin				
Control	9.5 ± 1.7 (4)	11.1 ± 1.8 (7)	10.7 ± 1.5 (7)	10.0 ± 1.9 (5)
Pentoxifylline	8.6 ± 0.9 (2)	11.7 ± 2.2 (5), $P = 0.18$	11.2 ± 1.9 (5), $P = 0.28$	11.5 ± 3.3 (3), $P = 0.08$
Zymosan	9.6 ± 1.8 (4), $P = 0.46$[b]	13.6 ± 1.6 (7), $P = 0.003$	15.1 ± 1.7 (7), $P = 0.002$	16.6 ± 2.8 (5), $P = 0.012$
Zymosan + pentoxifylline	11.3 ± 1.2 (4), $P = 0.06$[c]	16.3 ± 1.8 (7), $P = 0.014$	18.4 ± 2.1 (7), $P = 0.07$	20.1 ± 3.6 (5), $P = 0.057$
Adenosine				
Control	0.2 ± 0.1 (9)	0.7 ± 0.1 (11)	1.2 ± 0.1 (16)	1.9 ± 0.2 (13)
Pentoxifylline	0.5 ± 0.2 (9), $P = 0.09$[b]	0.9 ± 0.2 (11), $P = 0.26$	1.3 ± 0.2 (16), $P = 0.32$	2.0 ± 0.4 (13), $P = 0.50$
Zymosan	0.9 ± 0.2 (9), $P = 0.0002$[b]	1.7 ± 0.1 (11), $P = 0.00002$	2.4 ± 0.2 (16), $P = 0.00001$	3.2 ± 0.3 (13), $P = 0.00001$
Zymosan + pentoxifylline	0.7 ± 0.2 (9), $P = 0.04$[c]	1.3 ± 0.1 (11), $P = 0.007$	1.9 ± 0.2 (16), $P = 0.0007$	2.8 ± 0.4 (13), $P = 0.13$
FMLP	0.45 ± 0.1 (16), $P = 0.61$[b]	1.0 ± 0.2 (27), $P = 0.12$	1.7 ± 0.2 (29), $P = 0.09$	2.7 ± 0.3 (25), $P = 0.004$
FMLP + pentoxifylline	0.5 ± 0.1 (16), $P = 0.79$[d]	0.9 ± 0.2 (27), $P = 0.32$	1.5 ± 0.2 (29), $P = 0.03$	1.9 ± 0.2 (25), $P = 0.0004$

[a] Data are means ± standard errors of the means. The number of experiments is shown in parenthesis.
[b] P values reflect differences between control and experimental (pentoxifylline, zymosan, or FMLP) groups.
[c] P values reflect differences between zymosan and zymosan + pentoxifylline groups.
[d] P values reflect differences between FMLP and FMLP + pentoxifylline groups.

macrophages (41). Perhaps as a consequence of this role in modulating leukocyte function, pentoxifylline proved to be protective in animal models of bacterial infection and inflammation (3, 7, 21, 24, 33, 43, 45–47). The mechanism(s) for the effects of pentoxifylline on leukocyte function has not been established, but associated cellular changes have included an increase in cyclic AMP levels, altered phosphatidyl nucleotide metabolism, and decreased intracellular calcium.

In this study, we examined the effects of pentoxifylline on specific membrane-associated activities in human PMN. The drug suppressed several stimulus-induced cell membrane functions in neutrophils. Pentoxifylline inhibited ingestion of microbial particles (*S. aureus* and zymosan) by human PMN. Furthermore, pentoxifylline exhibited a marked inhibitory effect on superoxide generation in PMN activated by soluble substances (FMLP and ConA) or microbial particles (zymosan). Pentoxifylline had little influence on superoxide production stimulated by PMA, which bypasses membrane receptor-related mechanisms of activation and directly stimulates protein kinase C (6). Pentoxifylline also decreased uptake (transport) of adenosine in zymosan- and FMLP-stimulated (but not control) PMN. Thus, pentoxifylline inhibited three cell membrane-associated functions (phagocytosis, respiratory burst activity, and nucleoside transport) in stimulated human PMN.

The evidence clearly indicates that pentoxifylline has a profound effect on neutrophil cell membrane function and structure. Although the exact means by which this drug interacts with the cell membrane is not known, other methylxanthines produce recognized alterations in cellular function (11, 12, 20). Methylxanthines inhibit phosphodiesterase activity, which could account for the observed elevation in cellular cyclic AMP levels. This sequence might lead to the biological changes observed after administration of these drugs. However, other findings have suggested that phosphodiesterase inhibition occurs only at high concentrations for many methylxanthine drugs (11, 12, 20).

The modulation of cellular functions by pentoxifylline is similar to the changes caused by nucleosides. Adenosine plays a role in regulation of activation for many types of cells (12, 25, 35). For example, this nucleoside inhibits respiratory burst activity (generation of superoxide and hydrogen peroxide) in stimulated human PMN (9, 10; Hand et al., submitted). This regulatory activity of nucleosides appears to be mediated through external cell membrane receptors which are either stimulatory (A_2) or inhibitory (A_1) (12, 25) for guanine nucleotide regulatory (G or N) protein-adenylate cyclase activity (35, 38). Furthermore, specific receptor-G protein complexes may also regulate phospholipase C activity, with an effect on polyphosphoinositide breakdown and intracellular Ca^{2+} mobilization, thereby altering the functions of the respiratory burst enzyme and other cellular activities (37). Adenosine inhibition of FMLP-stimulated superoxide production in PMN is probably mediated by binding to A_2 receptors (9, 10).

Methylxanthines are inhibitors of nucleoside receptor binding (5, 11, 12, 20) and nucleoside transport (29, 48), and it has been shown that these agents block adenosine activity mediated by regulatory receptors. Therefore, the possibility that pentoxifylline alters PMN function by binding to nucleoside-regulatory membrane receptors has been considered. Indeed, certain findings in our study are compatible with the concept that the effects of pentoxifylline on neutrophil function are mediated through nucleoside cell membrane receptor pathways. Both adenosine (9, 10, 32; Hand et al., submitted) and pentoxifylline inhibit stimulated respiratory burst activity and promote chemotaxis in neutrophils. We also found that pentoxifylline is an effective inhibitor of stimulated adenosine uptake in PMN. NBTI, which binds to cell membrane nucleoside transport receptors and competitively inhibits adenosine uptake (29, 48; Hand et al., submitted), produced an effect on PMN superoxide production which is similar to and additive with that of pentoxifylline.

However, there are also major differences between pentoxifylline and adenosine in their effects on PMN function. Pentoxifylline (at concentrations greater than 10^{-5} M) had a progressive, concentration-dependent inhibitory effect on stimulated superoxide production in PMN. In contrast, the activity of adenosine was essentially maximal at 1×10^{-5} to 2.5×10^{-5} M (presumably because of saturation of external membrane receptors). Adenosine was a potent inhibitor of PMN superoxide production induced by soluble mediators (FMLP and ConA) but had less effect on microbial particle-induced prduction of superoxide. This may be related to the recent finding that microbial particle (opsonized zymosan) activation of the neutrophil respiratory burst differs from activation by certain other stimulating agents (28, 44). In contrast to adenosine, pentoxifylline had concentration-dependent inhibitory activity which was similar for cells exposed to FMLP, ConA, or zymosan.

Although pentoxifylline is a methylxanthine derivative, the drug failed to block the inhibitory activity of adenosine on PMN superoxide production. Indeed, pentoxifylline and adenosine were additive in their modulation of superoxide production. This suggests that these two agents have different pathways of action. Of considerable interest is the fact that NBTI and dipyridamole, but not pentoxifylline, decrease adenosine uptake (transport) in unstimulated neutrophils, implying that pentoxifylline does not bind to nucleoside transport receptors. We have shown that clindamycin enters PMN and macrophages by active membrane transport, at least in part via the nucleoside transport system (15, 40). Pentoxifylline, but not the nucleoside derivative NBTI, actually increased clindamycin uptake in zymosan-stimulated PMN. This also indicates that pentoxifylline does not bind to nucleoside transport receptors.

These findings make it highly unlikely that the modulatory effects of pentoxifylline on neutrophil function are mediated through external membrane nucleoside regulatory or transport receptors, either directly by receptor binding or indirectly by increased extracellular adenosine (due to inhibition of adenosine transport by pentoxifylline). This latter possibility is excluded by our finding that adenosine deaminase, which blocks the effect of adenosine on superoxide production, failed to alter suppression of superoxide generation due to pentoxifylline.

Thus, the findings suggest that the modulatory effects of pentoxifylline on neutrophil function are not caused by binding of external cell membrane receptors. The exact site of interaction in the activation signal chain is not known. As noted above, pentoxifylline has little effect on PMA-stimulated superoxide production mediated through protein kinase C (6). Therefore, pentoxifylline presumably affects another pathway of activation. Changes in cellular cyclic nucleotide levels or phospholipase C activity (which affects polyphosphoinositide metabolism and intracellular calcium mobilization) are potential sites of action. It is certainly possible that inhibition of phosphodiesterase, with an increase in cyclic AMP levels, plays a role in pentoxifylline activity. Whatever the mechanism of action, pentoxifylline has a profound effect on leukocyte function and may become

an important therapeutic agent for amelioration of certain infectious diseases and inflammatory states.

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