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The Effect of Pentoxifylline on Human Neutrophil Migration: A Possible Role for Cyclic Nucleotides

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ABSTRACT. Relatively low concentrations of pentoxifylline caused a stimulation of random migration, while high concentrations inhibited chemotactic migration activated by formyl-methionyl-leucyl-phenylalanine (fMLP). The stimulating effect of pentoxyfylline was partly chemokinetic and partly chemotactic, and was dependent on extracellular calcium. Activation of migration by pentoxifylline was not dependent on the pore size of the micropore filter, indicating that the effect was not mediated by the ability of the drug to induce membrane deformability. Inhibitors of guanylate cyclase and antagonists of cGMP-dependent protein kinase (G-kinase) inhibited stimulation of migration by pentoxifylline. Pentoxyfylline caused a transient increase in cGMP level, while only high concentrations of pentoxifylline caused an increase in cyclic adenosine monophosphate (cAMP) level. It is suggested that the increase of migration is caused by cGMP and is mediated by a G-kinase, while the inhibition of migration at high concentrations of pentoxifylline is mediated by cAMP. BIOCHEM PHARMACOL **54**;4:475–480, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. neutrophil; pentoxifylline; migration; cGMP; cAMP; G-kinase

Pentoxyfylline is a drug used to improve disturbed microcirculatory flow and to inhibit the inflammatory effects of tumor necrosis factor (αTNFα). Its effect on vaso-occlusive disease was thought to be a consequence of an effect on the fluidity of the plasma membrane, resulting in increased red blood cell deformability and decreased blood viscosity [1]. The inhibition of the effects of TNFα is the basis of a number of applications, such as in bone marrow transplantations and rheumatoid arthritis [2].

Several neutrophil functions are affected by pentoxifylline. The drug inhibits phagocytosis of several types of particles [3, 4]. Superoxide production induced by formyl-methionyl-leucyl-phenylalanine (fMLP)§ or zymosan was decreased by pentoxifylline at clinically achievable concentrations [3–5]. Degranulation induced by fMLP was equally inhibited by pentoxifylline [6].

Pentoxifylline affects neutrophil migration. The results obtained by different groups suggest that the effect is complex, because seemingly contradictory results were obtained. Most investigators reported a stimulatory effect of pentoxifylline on neutrophil migration [7–9]. *In vitro*, it reversed the chemotactic deficiency induced by interleukin-2 (IL-2) treatment [10]. Pentoxifylline corrected the developmental defects in activation and movement of neutrophils of neonates [11]. The drug restored the normal chemotactic capacity of patients with known functional defects, such as liver cirrhosis, myelodysplastic syndrome, or juvenile parodontitis [7]. It counteracted amphotericin B-induced inhibition of nondirected or directed migration [12]. In contrast, some investigators found an inhibitory effect of pentoxifylline on neutrophil migration [5], while others found no effect at all with pentoxifylline alone, and an inhibition of fMLP-activated chemotaxis by pentoxifylline [13].

Little is known about the mechanism of the inhibitory or stimulatory effect of pentoxifylline. Pentoxifylline is a methyxanthine derivative, and like the other methylxanthines, an inhibitor of phosphodiesterase. The rise in cyclic adenosine monophosphate (cAMP) level has been suggested as a cause of the inhibitory effect of pentoxifylline on the respiratory burst of neutrophils [3]. It seems unlikely that the change in cAMP is the only cause of the effects of pentoxifylline, because theophylline is as effective an inhibitor of phosphodiesterase as pentoxifylline, but lacks the effects of pentoxifylline on the respiratory burst [5]. Furthermore, with regard to migration, it is known that most agents that enhance cAMP level inhibit migration rather than stimulate migration (for references, see [14]).

In this study we considered the effect of pentoxyfylline on neutrophil migration to characterize the nature of the migration-modulating effect and to determine the mecha-

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§ *Abbreviations:* fMLP, formyl-methionyl-leucyl-phenylalanine; G-kinase, cyclic GMP-dependent protein kinase; LY-83583, 6-anilino-5,8-quinolinedione; R_p-pCPT-cGMPS, R_p-8-(4-chlorophenylthio-guanosine-3′,5′-cyclic monophosphorothioate; R_p-Br-cGMPS, R_p-8-bromoguanosine-3′,5′-cyclic monophosphorothioate; R_p-Br-PET-cGMPS, R_p-8-bromo-β-phenyl-1,N^2-ethenoguanosine-3′,5′-cyclic monophosphorothioate; IBMX, isobutyl-methyl-xanthine; IL-2, interleukin-2; TNFα, tumor necrosis factor α; cAMP, cyclic adenosine monophosphate.

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nism of activation. The role of cyclic nucleotides, especially cGMP, was assessed and considered as a possible cause of modulation of migration.

MATERIALS AND METHODS

Isolation of Neutrophils

Neutrophils were isolated from the buffy coat of blood of healthy donors. The buffy coat was diluted with a four-fold volume of heparinized medium and layered on top of Ficoll-amidotrizoate (d = 1.077). After centrifugation the pellet was resuspended, and starch was added to sediment erythrocytes. After sedimentation the neutrophil-containing supernatant was collected and centrifuged. The remaining erythrocytes were removed by hypotonic hemolysis, and the neutrophils suspended in medium. The cells consisted of more than 95% neutrophils, and were more than 99% viable as determined by Trypan blue exclusion. The medium used consisted of 140 mM NaCl, 5 mM KCl, 10 mM glucose, 0.5% bovine serum albumin, and 20 mM Hepes pH 7.3. Unless otherwise stated, the medium was supplemented with 1 mM Ca^{2+} and 1 mM Mg^{2+} during the experiments. The final cell suspension during the experiments contained 3×10^6 neutrophils per mL.

Migration Measurements

Cell migration was measured with the Boyden chamber technique [15], as described by Boyden [16], and modified by Zigmond and Hirsch [17]. The two compartments of the chamber were separated by a cellulose acetate Millipore filter (Millipore Corporation, Bedford, MA) with a pore size of 3 μm, unless otherwise indicated. Chemotaxis was activated by the chemotactic peptide fMLP, which was placed in the lower compartment at a concentration of 10^{-9} M (which under the conditions of our experiments gave the strongest increase of migration). Neutrophils were placed in the upper compartment of the chamber and incubated for 35 min at 37°C. After migration, the filters were fixed and stained and the distance travelled in micrometers into the filter determined according to the leading front technique [17]. The assays were carried out in duplicate and the migration distance of the neutrophils was determined at five different filter sites. In some cases, Millipore filters with a pore size of 8 μm were used; in that event the same experimental procedure was followed as for filters with a pore size of 3 μm.

Electroporation of Neutrophils

Neutrophils were electroporated according to the method of Grinstein and Furuya [18], with minor modifications. The electropermeabilization procedure was carried out at room temperature. When permeabilization was carried out at 0°C, the cells were not able to migrate. Neutrophils (3×10^6 per mL) in permeabilization medium (135 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 20 mM Hepes pH 7.0, 10 mM glucose, and 0.5% BSA), were placed in the cuvette of a BioRad Gene Pulser (BioRad Laboratories, Veenendaal, The Netherlands). The cells were exposed to two discharges of 14.75 kV/cm from a 25 μF capacitor. Between the two discharges, the cell suspension was stirred with a plastic pipette. After permeabilization and mixing, 0.2 mL of the suspension were placed in the upper compartment of the Boyden chamber. When electroporated neutrophils were compared with control neutrophils, the latter cells were also suspended in permeabilization buffer.

Cyclic GMP Assay

Neutrophils (final concentration 2×10^7 cells per mL) were exposed to reagents at 37°C for the indicated time. Subsequently, 1 mL of 3.5% (v/v) perchloric acid was added, and the resulting mixture was stored overnight in the freezer. The solution was neutralized by adding 0.5 mL saturated (22°C) $NaHCO_3$. After 10 min, the mixture was centrifuged for 3 min at 2000 rpm. To 100 μL of the supernatant 50 μL of radioactive cGMP and 50 μL antibody from the radioimmunoassay kit (Amersham, Little Chalfont, UK) were added. After mixing the solution was kept on ice for 90 min, after which 1 mL icecold 60% $(NH_4)_2SO_4$ was added. The solution was mixed, and kept on ice for a further 10 min, and centrifuged. The supernatant was carefully removed, and the residue taken up in 1.1 mL water. The solution (1 mL) was mixed with 4 mL scintillation fluid (299, Packard), and counted in the scintillation counter. Known amounts of cGMP were treated in the same way as the cells, and were used for the calibration curve.

Cyclic AMP Assay

Neutrophils (final concentration 2×10^7 cells per mL) were exposed to reagents at 37°C for the indicated time. Subsequently, 1 mL of 3.5% (v/v) perchloric acid was added, and the resulting mixture was stored overnight in the freezer. The solution was neutralized by adding 0.5 mL saturated (22°C) $NaHCO_3$. After 10 min the mixture was centrifuged for 3 min at 2000 rpm. To 50 μL of the supernatant 50 μL of radioactive cAMP and 100 μL antibody from the radioimmunoassay kit (Amersham) were added. After mixing, the solution was kept on ice for 120 min, after which 100 μL ice-cold charcoal suspension was added. The solution was mixed, kept on ice for a further 2 min, and centrifuged. To 200 μL of the supernatant 10 mL scintillation fluid (Emulsifier Scintillator Plus, Packard, Meriden, CT) was added, and the mixture was counted in the scintillation counter. Known amounts of cAMP were treated in the same way as the cells, and were used for the calibration curve.

Statistical Analysis

All mean values for the chemotactic assays are arithmetical means ± SEM of at least four different experiments. Significances were calculated with Student's *t*-test; a value of $P < 0.01$ was considered as statistically significant.

Materials

Pentoxifylline, fMLP, and methylene blue were purchased from Sigma Chemical Co (St. Louis, MO). The compound LY-83583 (6-anilino-5,8-quinolinedione) was obtained from Calbiochem, Bierges, Belgium. The cGMP-dependent protein kinase (G-kinase) antagonists R_p-pCPT-cGMPS (R_p-8-(4-chlorophenylthio-guanosine-3',5'-cyclic monophosphorothioate), R_p-Br-PET-cGMPS (R_p-8-bromo-β-phenyl-1,N^2-ethenoguanosine-3',5'-cyclic monophosphorothioate), and R_p-Br-cGMPS (R_p-8-bromoguanosine-3',5'-cyclic monophosphorothioate) were from Biolog, Bremen, Germany. The other chemicals were obtained from Sigma, and were of the highest purity available.

RESULTS

Migration

Pentoxifylline caused a concentration-dependent increase in migration (in the absence of other chemoattractants). Stimulation occurred both when pentoxifylline was present in both compartments of the Boyden chamber or in the lower compartment only, but was most pronounced when pentoxifylline was in the latter case (Fig. 1). Stimulation of migration was strongest at a concentration of 4×10^{-5} M pentoxifylline, whereas higher concentrations were less effective, or ineffective.

Because the decrease in activation at high pentoxifylline concentrations could suggest that pentoxifylline was inhibitory at high concentrations, we tested the effect of pentoxifylline on fMLP-activated chemotaxis. When cells were preincubated with pentoxifylline, migration towards the chemotactic peptide fMLP was moderately inhibited at concentrations higher than 10^{-4} M (Fig. 1).

Migration of neutrophils was slightly dependent on the pore size of the filters used. Migration was higher in filters with a pore size of 8 μm than in those with a pore size of 3 μm. However, the change in migration was not different for pentoxifylline-stimulated cells as compared with fMLP-stimulated cells (Table 1).

Calcium

Stimulation of migration by pentoxifylline was strongly calcium dependent. Although there was some stimulation of migration in the absence of calcium, it was strongly reduced as compared with stimulation in the presence of extracellular Ca^{2+} (Fig. 2). In contrast, stimulation by fMLP was largely independent of calcium. Although there was some difference between migration in the presence and the absence of calcium, the fMLP-induced increase in migration was approximately the same in the presence of EGTA and the presence of Ca^{2+} (Fig. 2).

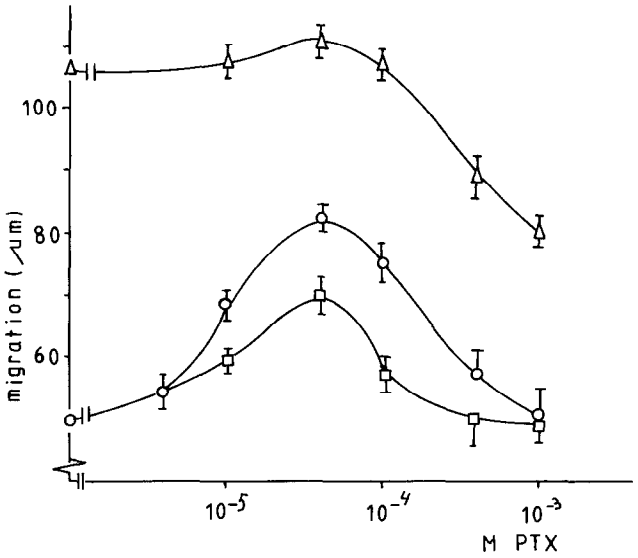

FIG. 1. The effect of increasing concentrations of pentoxifylline on neutrophil migration and on fMLP-activated chemotaxis. Pentoxifylline was either present in both compartments ([□]) or in the lower compartment only (○). For the effect of pentoxifylline on fMLP-activated chemotaxis (△), the cells were preincubated with the indicated concentration of pentoxifylline for 10 min, and then placed in the upper compartment of the Boyden chamber; in the lower compartment 10^{-9} M fMLP was present.

We could not observe a change in intracellular calcium upon application of pentoxifylline to neutrophils, as measured with the Fura technique. A series of concentrations, ranging from 0.01 to 1 mM pentoxifylline, was tested. Under the same conditions, fMLP (1 and 10 nM) as a positive control gave a strong enhancement of cytosolic free calcium.

Cyclic Nucleotides

Pentoxifylline caused an increase in cellular cGMP content. The effect was transient; after 2 min the cGMP level was even lower than the level of resting cells (Table 2). Stimulation occurred both at a pentoxifylline concentra-

TABLE 1. The effect of pore size on the activation by pentoxyfylline of neutrophil migration

	Migration (μm)	
	Pore size 3 μm	Pore size 8 μm
—	50.7 ± 1.9	64.6 ± 1.6
Pentoxifylline	88.4 ± 1.3*	96.3 ± 1.8*
fMLP	98.5 ± 1.8*	110.1 ± 1.6*

Either pentoxyfilline (40 μM) or fMLP (10^{-9} M) was present in the lower compartment of the Boyden chamber. For both pentoxifylline and fMLP, and for both filtertypes, the results in the presence of activator were significant different (*$P < 0.001$, compared with random migration).

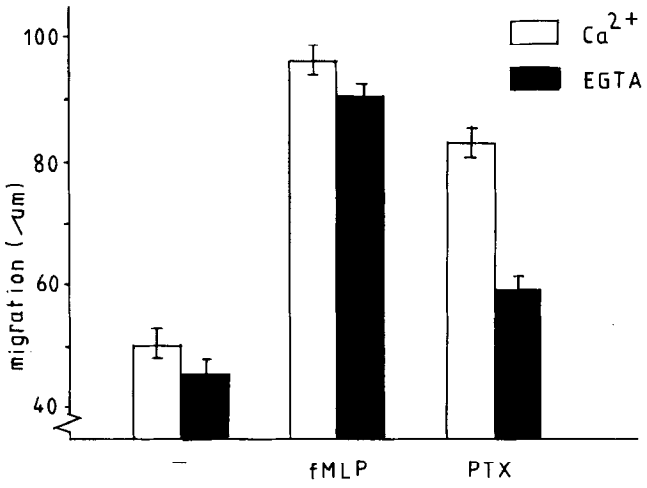

FIG. 2. The effect of extracellular calcium on random migration and on migration stimulated by pentoxyfylline (PTX) or fMLP. [□]: migration in the presence of 1 mM Ca^{2+}; [■]: migration in the presence of 1 mM EGTA. In both cases 1 mM Mg^{2+} was present. Pentoxyfylline (40 µM) or fMLP (10^{-9} M) was present in the lower compartment of the Boyden chamber.

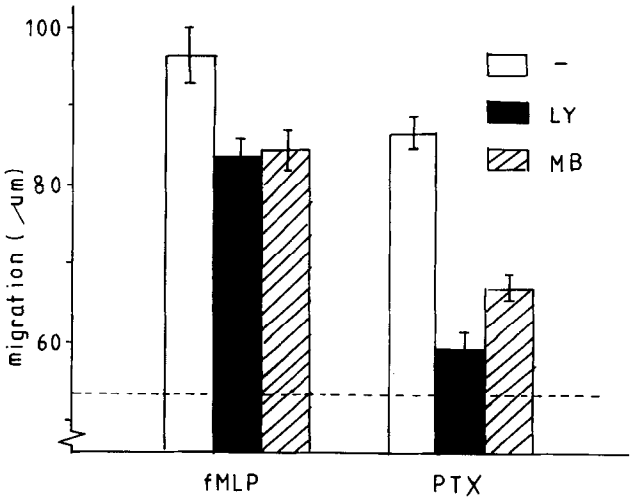

FIG. 3. The effect of LY83583 (LY) and methylene blue (MB) on migration stimulated by fMLP or pentoxyfylline (PTX). Cells were preincubated, without agent, with 5 µM LY83583 or 20 µM methylene blue for 30 min at 37°C, and subsequently placed in the upper compartment of the Boyden chamber. Pentoxyfylline (40 µM) or fMLP (10^{-9} M) was present in the lower compartment of the Boyden chamber.

tion, which stimulated migration (40 µM), and a concentration (1 mM), which inhibited fMLP-activated migration (Table 2). Cyclic AMP level was not affected by exposure to 40 µM pentoxifylline, but increased after exposure to 1000 µM pentoxifylline (Table 2); comparable results for cAMP were found previously [3].

Two inhibitors of guanyl cyclase, LY-83583 and methylene blue [19–21], strongly inhibited pentoxifylline-induced stimulation of migration (Fig. 3). Migration activated by fMLP was also inhibited by these agents, but to a lesser degree. To study the effect of antagonists of G-kinase, we used electroporated cells, because this allows a direct comparison with the effect on migration activated by cGMP. Three antagonists of G-kinase, R_p-pCPT-cGMPS, R_p-Br-PET-cGMPS, and R_p-Br-cGMPS [22, 23] inhibited pentoxifylline-stimulated migration by electroporated neutrophils. Under conditions where pentoxifylline-activated migration was 74.0 ± 1.9 µm, this migration in the presence of 4 nM R_p-pCPT-cGMPS was reduced to 59.4 ± 1.8 µm, in the presence of 10 nM R_p-Br-PET-cGMPS to 38.4 ± 2.0 µm, and in the presence of 100 nM R_p-Br-cGMPS to 59.5 ± 2.1 µm. Isobutylmethyl xanthine (IBMX), a nonselective inhibitor of phosphodiesterases, had no effect on fMLP-activated migration. Pentoxifylline-activated migration was strongly inhibited: fMLP-activated migration was 96.3 ± 2.1 µm in the absence of IBMX, and 95.6 ± 1.8 µm after pretreatment with 1 mM IBMX. Pentoxifylline-activated migration was 80.5 ± 2.0 µm in the absence of IBMX, and 69.8 ± 1.5 µm after pretreatment with 1 mM IBMX.

DISCUSSION

The results show that pentoxifylline may have different effects on migration, depending on its concentration. At low concentrations, it causes an increase of random migration, which comprises a chemokinetic and a chemotactic

TABLE 2. The effect of pentoxifylline on cyclic nucleotide level

		Cyclic nucleotide (% of control)	
	Tim	cGMP	cAMP
—		100 ± 12	100 ± 7
40 µM pentoxifylline	0.5 min	304 ± 43**	99 ± 4
40 µM pentoxifylline	1 min	199 ± 22**	103 ± 6
40 µM pentoxifylline	2 min	76 ± 19*	101 ± 3
1000 µM pentoxifylline	0.5 min	321 ± 51**	117 ± 9
1000 µM pentoxifylline	1 min	181 ± 24*	216 ± 34**
1000 µM pentoxifylline	2 min	150 ± 29*	206 ± 22**

The cyclic nucleotide level is expressed as a percentage of the level of control cells, because the levels of different cell batches were different. The average of these values was 1.97 ± 0.24 pmol cGMP/10^7 cells, and 3.0 ± 0.21 pmol cAMP/10^7 cells. After incubation of cells at 37°C for the time indicated, the reaction was terminated and the cyclic nucleotide level was determined. No significant change occurred within the control cells during this time. *$P < 0.01$; **$P < 0.001$.

component. At higher concentrations, it causes an inhibition of fMLP-activated chemotaxis. These observations reconcile the seemingly contradictory results in the literature, where both stimulation and inhibition of migration have been reported [5, 7–9, 13]. The differences are probably not real, but reflect more or less subtle differences in the experimental procedure used.

A number of the effects of pentoxifylline are related to its induction of a change in membrane deformability. If that effect played a role in activation of migration, it is likely that migration into 8 μm pores would be much different from that in pores of 3 μm, deformability being greater in the latter case than the former. As compared with fMLP-activated cells, the change in migration is not significant, so we may conclude that the ability of pentoxifylline to induce membrane deformability is not related to its effect on migration. The normal dose of pentoxifylline used in clinical medicine, i.e. 1200 mg a day, yields a plasma concentration of ca. 5 μM [24]. However, in several animal models in which pentoxifylline is found to have profound antiinflammatory effects, much higher doses of pentoxifylline have been used (20–80 mg/kg) [25]. Treatment protocols with high dose pentoxifylline are currently being evaluated [26] and may very well reveal that these doses have a significant effect on neutrophil migration.

Neutrophil migration can proceed without a change in cytosolic free calcium or an influx of extracellular calcium. This is apparent from the fMLP-activated migration of intact cells, which can proceed in the absence of extracellular calcium, and from the fMLP-activated migration of electroporated cells, which can migrate in a calcium-free medium [27]. The activating effect of pentoxifylline on migration is strongly calcium dependent. As we could not find an effect of pentoxifylline on the level of intracellular calcium, it is possible that the effect of pentoxifylline is related to an effect of calcium on constituents of the outer plasma membrane. On the other hand, the observation that there is no measurable increase of cytosolic free calcium at the maximal stimulatory concentration of pentoxifylline does not mean that there is no influx of Ca^{2+} at all. The fura-fura/Ca^{2+} system, used to determine changes in intracellular Ca^{2+}, functions as a calcium buffer, which makes it impossible to see small changes in cytosolic free calcium. The situation is comparable to what we found previously with endothelin-2 [28]. Endothelin-2 does not cause an increase in cytoplasmic free calcium in neutrophils at a concentration where migration is enhanced. However, we were able to demonstrate that intracellular calcium, derived from influx of extracellular calcium, was required [28]. The nature of the Ca^{2+}-dependent target remains to be elucidated. Apparently, there is a Ca^{2+}-dependent process that is required for the effect of pentoxifylline, but that is not required for fMLP-activated migration. This implies that for the effect of pentoxifylline more steps are required than for the classic chemoattractants.

A large number of studies show that an increase in cGMP level is associated with an increase of migration (see [14, 29] for a review). We have shown that inclusion of cGMP into the cell interior migration of electroporated neutrophils causes a calcium-dependent increase of migration; high concentrations of cGMP, on the other hand, cause an inhibition of chemotaxis [29]. Agents that cause an increase in cAMP, in most cases provoke an inhibition of migration [14]. The results obtained can be explained via an effect on the level of cyclic nucleotides. Pentoxifylline in low concentrations causes an increase in cGMP level and has little effect on cAMP, resulting in an increase of migration. The results with inhibitors of guanylate cyclase and with antagonists of G-kinase support this view. At higher concentrations of pentoxifylline, the effect on cAMP level becomes prominent and overrules the enhanced cGMP level, resulting in an inhibition of migration. Given our limited knowledge concerning the mechanism of cGMP action, the hypothesis is speculative. This is partly due to the possible compartmentalization of the action of cyclic nucleotides and (in the neutrophil) the rapid regulation of the cGMP level by phosphodiesterase inhibitors. Inhibition of pentoxifylline-activated migration by inhibitors of guanylate cyclase and antagonists of G-kinase is, therefore, more indicative of a role for cGMP than changes in the overall cGMP level. The finding that antagonists of G-kinase inhibit pentoxifylline-activated migration further indicates that the effect of cGMP is mediated by G-kinase.

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References

1. Ward A and Clissold SP, Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* **34:** 50–97, 1987.
2. Anaya J-M and Espinoza LR, Phosphodiesterase inhibitor pentoxifylline: An antiinflammatory/immunomodulatory drug potentially useful in some rheumatic diseases. *J Rheumatol* **22:** 595–599, 1995.
3. Bessler H, Gilgal R, Djaldetti M and Zahavi I, Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. *J Leukoc Biol* **40:** 747–754, 1986.
4. Hand WL, Butera ML, King-Thompson NL and Hand DL, Pentoxifylline modulation of plasma membrane functions in human polymorphonuclear leukocytes. *Infect Immun* **57:** 3520–3526, 1989.
5. Hammerschmidt DE, Kotasek D, McCarthy T, Huh P-W, Freyburger G and Vercelotti GM, Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. *J Lab Clin Med* **112:** 254–263, 1988.
6. Currie MS, Murali K, Padmanabhan J, Jones A, Crawford J and Cohen HJ, Stimulus-specific effects of pentoxifylline on neutrophil CR3 expression, degranulation, and superoxide production. *J Leukoc Biol* **47:** 244–250, 1990.
7. Boogaerts MA, Mabrain S, Meeus P, Van Hove L and Verhoef GEG, *In vitro* modulation of normal and diseased human neutrophil function by pentoxifyline. *Blut* **61:** 60–65, 1990.
8. Crocket KV, Lackie JM and Rogers AA, Effect of pentoxifyl-

line on neutrophil behaviour: Stimulation of movement without adhesion changes. *Biomed Pharmacother* **42:** 117–120, 1988.
9. Freyburger G, Bellock F and Boisseau MR, Pentoxifylline inhibits actin polymerization in human neutrophils after stimulation by chemoattractant factor. *Agents Actions* **31:** 72–78, 1990.
10. Fossat C, Sainty D, Stoppa AM, Maraninchi D and Juhan-Vague I, Pentoxifylline *in vitro* reverses neutrophil chemotactic deficiency induced by interleukin-2 treatment. *Nouv Rev Fr Hematol* **34:** S61–S64, 1994.
11. Hill HR, Augustine NH, Newton JA, Shigeoka O, Morris E and Sacchi F, Correction of a developmental defect in neutrophil activation and movement. *Am J Pathol* **128:** 307–314, 1987.
12. Sullivan GW, Carper HT and Mandell GL, Pentoxifylline modulates activation of human neutrophils by amphotericin B *in vitro*. *Antimicrobiol Agents Chemother* **36:** 408–416, 1992.
13. Roberts PJ, Yong KL, Khwaja A, Johnson BV, Pizzey AR, Carver JE, Addison IE and Linch DC, Pentoxifylline at clinically achievable levels inhibits FMLP-induced neutrophil responses, but not priming, upregulation of cell-adhesion molecules, or migration induced by GM-CSF. *Eur J Haematol* **50:** 1–10, 1993.
14. Elferink JGR and VanUffelen BE, The role of cyclic nucleotides in neutrophil migration. *Gen Pharmacol* **27:** 387–393, 1996.
15. Elferink JGR and Deierkauf M, The effect of quin2 on chemotaxis by polymorphonuclear leukocytes. *Biochim Biophys Acta* **846:** 364–369, 1985.
16. Boyden SV, The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. *J Exp Med* **115:** 453–466, 1962.
17. Zigmond SH and Hirsch JG, Leukocyte locomotion and chemotaxis: New methods for evaluation and demonstration of a cell-derived chemotactic factor. *J Exp Med* **137:** 387–410, 1973.
18. Grinstein S and Furuya W, Receptor-mediated activation of electropermeabilized neutrophils. Evidence for a Ca^{2+}-and protein kinase C-independent signalling pathway. *J Biol Chem* **263:** 1779–1783, 1988.
19. Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ and Ignarro LJ, Relationship between cyclic guanosine 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. *J Pharmacol Exp Ther* **219:** 181–186, 1981.
20. Mülsch A, Busse R, Liebau S and Föstermann U, LY 83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase. *J Pharmacol Exp Ther* **247:** 283–288, 1988.
21. Schmidt MJ, Sawyer BD, Truex LL, Marshall WS and Fleisch JH, LY83583: An agent that lowers intracellular levels of cyclic guanosine 3′,5′-monophosphate. *J Pharmacol Exp Ther* **232:** 764–769, 1985.
22. Butt E, Van Bemmelen M, Fischer L, Walter U and Jastorff B, Inhibition of cGMP-dependent protein kinase by (Rp)-guanosine 3′,5′-monophosphorothioates. *FEBS Lett* **263:** 47–50, 1990.
23. Butt E, Eigenthaler M and Genieser H-G, (Rp)-8-pCPT-cGMPS, a novel cGMP-dependent protein kinase inhibitor. *Eur J Pharmacol* **269:** 265–268, 1994.
24. Maksymowych PM, Avinasubieta A, Luong MH and Rusell AS, An open study of pentoxifylline in the treatment of severe refractory rheumatoid arthritis. *J Rheumatol* **22:** 625–629, 1995.
25. Lundblad R, Ekstrom P and Giercsky KE, PTX improves survival and reduces TNF, IL-6 and endothelin-1 in fulminant intra-abdominal sepsis in rats. *Shock* **3:** 210–215, 1995.
26. Dezube BJ, Lederman MM, Sprizler JG, Chapman B, Korvich JA and Flexner C, High dose pentoxifylline in patients with AIDS: Inhibition of TNF production. *J Infect Dis* **171:** 1628–1632, 1995.
27. Elferink JGR, Boonen GJJC and de Koster BM, The role of calcium in neutrophil migration: The effect of calcium and calcium antagonists in electroporated neutrophils. *Biochem Biophys Res Commun* **182:** 864–869, 1992.
28. Elferink JGR and de Koster BM, The effect of endothelin-2 (ET-2) on migration and changes in cytosolic free calcium of neutrophils. *Naunyn Schmiedebergs Arch Pharmacol* **252:** 130–135, 1996.
29. Elferink JGR and de Koster BM, The effect of cyclic GMP and cyclic AMP on migration by electroporated human neutrophils. *Eur J Pharmacol* **246:** 157–161, 1993.