Vol. 109, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 16, 1982 Pages 14-20

PIROXICAM ENHANCES PHAGOCYTOSIS OF ESCHERICHIA COLI BY HUMAN POLYMORPHONUCLEAR NEUTROPHILS

Egil Lingaas and Tore Midtvedt

Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt

University of Oslo, Rikshospitalet, Oslo, Norway.

Received September 15, 1982

SUMMARY: Human polymorphonuclear neutrophils were exposed to piroxicam, 1.5 uM, 15 uM and 150 uM during phagocytosis of radiolabelled Escherichia coli in vitro. Preincubation of the cells for 30 minutes before phagocytosis stimulated the uptake of E. coli at all concentrations. The elimination of substances of bacterial origin from the neutrophils in the postingestion phase was, however, not influenced by piroxicam.

The polymorphonuclear neutrophil (PMN) is a cornerstone of the host defence against infection. On the other hand it also plays an important role in many forms of immunological tissue injury (1-3). Any disease or drug impairing the function of PMN may therefore render the host more susceptible to infection or modify the tissue damage. Neutrophil functions which can be influenced by chemical agents include chemotaxis, adherence, ingestion, the metabolic burst following surface stimulation, intracellular breakdown and elimination of ingested particles as well as external secretion of metabolic products and granule components. Antiinflammatory agents are designed to alleviate various inflammatory conditions, and many of these drugs have been shown to inhibit one or more of these PMN functions (4-6). Piroxicam is a new antiinflammatory agent shown to work through

Abbrevations: PMN, polymorphonuclear leukocytes or neutrophils; KRG, Krebs-Ringer phosphate buffer, pH 7.4, enriched with 10 mM glucose; PMA, phorbol myristate acetate; CTC, chlortetracycline; FMLP, N-formyl-methionyl-leucyl-phenylalanine.

0006-291X/82/210014-07$1.00/0

inhibition of prostaglandin biosynthesis (7), which also takes place in PMN (8). This study was undertaken to investigate the influence of piroxicam on phagocytosis by human PMN.

MATERIALS AND METHODS

Preparation of PMN: Heparinized blood was collected from healthy donors and separated according to the technique of Bøyum (9). The PMN were washed once in Krebs-Ringer phosphate buffer (NaCl 120 mM, KCl 4.9 mM, $CaCl_2$ 1.02 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 2.7 mM, Na_2HPO_4 13.3 mM) enriched with 10 mM glucose (KRG), pH 7.4, and resuspended to a final concentration of 2.5×10^6 cells per ml in KRG. At least 95% of the cells were neutrophils..

Preparation of bacteria: Escherichia coli, strain X-7, serogroup 0:86, B:7, was grown to a density of 10^9 bacteria per ml in 200 ml of a medium prepared according to Benacerraf et al (10) with 1 mC of [^{32}P]-labelled orthophosphate (Institutt for Atomenergi, Norway). The bacteria were washed two times in ice-cold KRG, and a suspension of 10^{10} bacteria per ml in KRG was prepared. This suspension was kept on ice until further use within 30 minutes. Immediately preceding the phagocytosis the suspension was diluted to 10^9 bacteria per ml with prewarmed (37°C) KRG. For opsonization, serum from the same donor as the neutophils was added to a final concentration of 10%.

Phagocytosis: Aliquots of 1 ml of the PMN suspension were preincubated for 60 minutes in Leighton tubes (16x125 mm, Bellco, USA). By this time a monolayer had been formed. In the uptake experiments piroxicam was added to a final concentration of 1.5 mM, 15 mM and 150 mM respectively for the last 30 minutes of the preincubation period. After washing the cells once with KRG at 37°C, the uptake was started by adding 1 ml of the suspension of non-opsonized or opsonized E. coli with or without piroxicam as indicated above. The uptake was stopped after 15 minutes by washing the cells 4 times with ice-cold KRG. For the study of elimination, however, the cells were washed with prewarmed KRG and further incubated for 60 minutes in KRG with or without piroxicam and supplied with 10% donor serum. The elimination was stopped by washing with ice-cold KRG, and the medium covering the cells during the elimination period was assayed for radioactivity before and after centrifugation at 10,000 x g for 10 minutes.

Quantitation of cell protein and radioactivity: After washing, the tubes were dried, and 1.5 ml of Lowry's alkaline copper solution was added to dissolve the cells at room temperature overnight. Aliquots of 1 ml were taken for protein determination according to the Oyama/Eagle modification of the method of Lowry et al (11). The reference was lyophilized human albumin, 96% purity (Sigma Chemical Company, USA). For quantitation of radioactivity, aliquots of 0.2 ml were counted in a Hewlett Packard liquid scintillation counter at 4°C.

Calculations: The uptake of E. coli by the PMN was expressed as counts of [^{32}P] radioactivity per mg cell protein and per minute. The elimination was expressed in the same way by subtracting the counts found after the elimination period from those found at the end of the uptake period. Each experiment was

TABLE 1

The effect of piroxicam on the uptake of radiolabelled non-opsonized and opsonized E. coli by human PMN. All data are expressed as % of control ± S.E.M.

PIROXICAM	UPTAKE OF E. COLI	
	NON-OPSONIZED	OPSONIZED
1.5 uM	138.0 ± 8.5	118.3 ± 4.4
15 uM	108.0 ± 6.2	110.4 ± 2.6
150 uM	172.6 ± 20.1	134.9 ± 9.2

performed with 5 replicates, and 4-6 experiments were done with each concentration of piroxicam. The results were expressed as a percentage of control tubes without drug, and the student-t test was used for statistical analysis.

RESULTS

The uptake of E. coli by the PMN is shown in table 1. With non-opsonized as well as opsonized bacteria piroxicam stimulated the uptake at all concentrations. However, the effect did not increase linearly with increasing concentrations of piroxicam. Even though the largest uptake was seen with 150 uM, it differed only significantly from the uptake with 15 uM ($P<0.01$). The uptake with 15 uM appeared smaller than with 1.5 uM, but the difference was not statistically significant ($P>0.05$). The uptake of non-opsonized bacteria was approximately 5% of the uptake of opsonized bacteria in all experiments (data not shown).

The elimination of radioactive substances from the PMN was not influenced by piroxicam as shown in table 2. In all experiments at least 90% of the radioactivity remained in the supernatant when the medium from the elimination phase was centrifuged at 10,000 x g for 10 minutes (data not shown).

DISCUSSION

Phagocytosis of microorganisms, immune complexes or other particles by PMN results in a burst of morphological and bio-

TABLE 2

The effect of piroxicam on the elimination of radiolabelled substances of bacterial origin from human PMN during a 1 hour postingestion period. All data are expressed as % of the cell-associated radioactivity at the end of the uptake period with untreated PMN.

PIROXICAM	ELIMINATION	
	NON-OPSONIZED	OPSONIZED
0	41.2 ± 11.9	53.5 ± 2.4
1.5 uM	39.5 ± 12.0	50.0 ± 3.2
15 uM	39.9 ± 9.0	45.6 ± 3.2
150 uM	50.9 ± 10.2	50.9 ± 3.7

chemical responses. Similar reactions can be induced by numerous soluble stimuli such as formylpeptides, phorbol myristate acetate (PMA), lectins such as concavalin A, B and C, the calcium ionophore A 23187, surface active agents as digitonin, and several substances having chemoattractant properties such as various complement factors, bacterial breakdown products and others.

Recently, Edelson et al (12) found that piroxicam, 50 uM, inhibited the aggregation, O_2^--generation, lysozyme secretion and chlortetracycline (CTC) fluorescence shown by PMN stimulated by f-met-leu-phe (FMLP). When concavalin A or PMA were used as stimuli, however, aggregation and lysozyme secretion was normal in the presence of piroxicam, whereas O_2^--generation and CTC fluorescence decrements were significantly inhibited. The binding of [^{3}H]-FMLP to the PMN, but not of [^{3}H]-concavalin A, was also inhibited in the presence of piroxicam.

The results of the present study show that piroxicam clearly stimulates the uptake of E. coli by human PMN, whereas the elimination of substances of bacterial origin from the cells is not affected. The mechanism by which piroxicam exerts this

effect, however, remains conjectural. Piroxicam is a selective inhibitor of the cyclooxygenase step of arachidonic acid metabolism, and an inhibitor of cellular prostaglandin synthesis (13). Even though the role of prostaglandins in the inflammatory process is not fully clarified, data from several studies indicate an inhibitory effect on phagocytosis (14,15). The stimulatory effect of piroxicam on the uptake of E. coli may thus be due to the inhibitory effect on prostaglandin synthesis.

Arachidonic acid, when given to rabbit PMN in the presence of cytochalacin B, serves as an excellent secretagogue. The degranulation response is dependent on arachidonic acid metabolism (16). However, quantitatively, at least rabbit PMN produce considerably more lipooxygenase products than cyclooxygenase products of arachidonic acid (17). Several of these lipooxygenase products have been shown to induce degranulation in human PMN (18). The present study shows that the breakdown of E. coli in the PMN proceeds normally in the presence of piroxicam. The degranulation into the phagocytic vacuole thus seems to be unaffected by piroxicam. This is in accordance with the fact that piroxicam has little blocking activity at the lipooxygenase step of arachidonic acid metabolism.

On the other hand, piroxicam and other anti-inflammatory agents have been shown to inhibit the exocytotic release of granule associated enzymes from PMN (12,19,20). During phagocytosis, however, degranulation is normally an intracellular event. These data, together with the results of the present study, might therefore suggest that piroxicam acts selectively at exocytotic release as opposed to phagocytotic release of granule associated enzymes in PMN.

It is well known that enzymes released from the lysozomes of PMN are important factors in the development of the inflamma-

tory process, as in rheumatoid arthritis (21,22,23). Piroxicam, increasing the uptake of particles to be ingested as well as decreasing the extracellular release of lysozomal enzymes, seems to be well suited to serve as an anti-inflammatory agent. However, even though a concentration of 15 uM is commonly achieved in the serum of persons treated by piroxicam (24), these in vitro data do not necessarily imply any in vivo influence on PMN function. Studies of PMN from patients treated with piroxicam would help to clarify this. So far, no other anti-inflammatory agent have been shown to stimulate phagocytosis. On the contrary, many have been shown to be inhibitors of phagocytosis (15,25).

Acknowledgements:This study was supported by grants from The Norwegian Research Council for Science and Humanities and from Statssekretær Jacob Aall ag hustrus legat.
The technical assistance of David Bradfield, Patricia Engen and Ingrid Fagerheim was greatly appreciated.

REFERENCES

1. Weissmann, g., Zurier, R.B., and Hoffstein, S. (1973) Agents Actions 3, 370-379.

2. Baggiolini, M., Bretz, U., Dewald, B., and Feigenson, M. (1978) Agents Actions 8, 3-10.

3. Janoff, A. (1972) Annu. Rev. Med. 23, 177-190.

4. Rivkin. I., Foschi, G.V., and Rosen, C.H. (1976) Proc. Soc. exp. Biol. Med. 153, 236-240.

5. Simchowitz, L., Mehta, J., and Spilberg, I. (1979) Arthritis Rheum. 22, 755-763.

6. Mikulikova, D., and Trnavsky, K. (1982) Biochem. Pharmacol. 31, 460-463.

7. Wiseman, E.H., and Lombardino, J.G. (1981) Eur. J. Rheumatol. Inflam. 4, 280-297.

8. Zurier, R.B., and Sayadoff, D.M. (1975) Inflammation 1, 93-101.

9. Bøyum, A. (1964) Scand. J. clin. Lab. Invest. 21, Suppl 9, 31-50.

10. Benacerraf, B., Sebestyen, M.M., and Schlossman, S. (1959) J. exp. Med. 110, 27-48.

11. Oyama, V.I., and Eagle, H. (1956) Proc. Soc. exp. Biol. 91, 305-307.

12. Edelson, H.S., Kaplan, H.B., Korchak, H.M., Smolen, J.E., and Weissmann, G. (1982) Biochem. Biophys. Res. Commun. 104, 247-253.

13. Carty, T.J., Stevens, J.S., Lombardino, J.G., Parry, M.J., and Randall, M.J. (1980) Prostaglandins 19, 671-682.

14. Smith, R.J. (1977) J. Pharmacol. exp. Ther. 200, 647-657.

15. Cox, J.P., and Karnovsky, M.L. (1973) J. Cell Biol. 59, 480-490.

16. Naccache, P.H., Showell, H.J., Becker, E.L., and Sha'afi, R.I. (1979) Biochem. Biophys. Res. Commun. 87, 292-299.

17. Walenga, R.W., Showell, H.J., Feinstein, M.B., and Becker, E.L. (1980) Life Sci. 27, 1047-1053.

18. Becker, E.L., Naccache, P.H., Showell, H.J., and Walenga, R.W. (1981) Lymphokines 4, 297-334.

19. Smith, R.J., and Iden, S.S. (1980) Biochem. Pharmacol. 29, 2389-2395.

20. Nakanishi, M., and Goro, K. (1975) Biochem. Pharmacol. 24, 421-424.

21. Weissmann, G. (1972) New Engl. J. Med. 286, 141-147.

22. Barret, A.J. (1978) Agents Actions 8, 11-18.

23. Sandy, J.D., Spiratana, A., Brown, H.L.G., and Lowther, D.A. (1981) Biochem J. 193, 193-202.

24. Schoog, M., Laufen, H., and Dessain, P. (1981) Eur. J. Rheumatol. Inflam. 4, 298-302.

25. Smith, R.J. (1978) J. Pharmacol. exp. Ther. 207, 618-629.