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DICLOFENAC SODIUM, A NEGATIVE CHEMOKINETIC FACTOR FOR NEUTROPHIL LOCOMOTION

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Abstract—Diclofenac sodium, a non steroidal anti-inflammatory agent, was studied for its influence on the locomotion of human polymorphonuclear neutrophils (PMN), in an attempt to define the mechanism governing the drug's anti-inflammatory properties. PMN locomotion was measured by the agarose technique under two conditions of stimulation of cell migration: in the presence of a gradient of stimuli (chemotaxis) and in the presence of various amounts of stimuli incorporated in the gel (chemokinesis).

At concentrations below 100 μg/ml, diclofenac in the gel reduced, in a dose-dependent manner, the directed locomotion of PMN induced by a gradient of C_{5a}-activated serum, peptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) or *Klebsiella pneumoniae* culture supernatant (KPCS). Diclofenac also inhibited the random locomotion of unstimulated PMN, as well as the PMN chemokinetic activity induced by various amounts of FMLP or activated serum. Inhibition of PMN locomotion by diclofenac decreased when the concentration of the stimulant was raised; this inhibition was inversely related to the concentration of heat-inactivated fetal calf serum in the medium. The directed locomotion and chemokinesis of PMN, induced by FMLP were also reduced in PMN preincubated with diclofenac before migration, suggesting a direct cellular effect of diclofenac. On the other hand, diclofenac did not affect the changes in shape induced in floating PMN by FMLP or activated serum. The observation that diclofenac did not alter the ingestion rate of bacteria by PMN indicates that this drug is not cytotoxic for PMN. Consequently, diclofenac reduces PMN locomotion by interfering with the PMN chemokinetic activity. Diclofenac is an anti-inflammatory drug possessing the original property of acting as a negative chemokinetic agent, for migration of both stimulated and unstimulated PMN. It should therefore be a useful tool for analyzing the elements controlling PMN locomotion speed.

Polymorphonuclear neutrophils (PMN) are involved in all types of inflammation and in host defence against microorganisms [1, 2]. In response to chemoattractants, PMN migrate towards damaged or infected tissues [3] where, under a variety of stimuli, they release oxygen by-products as part of the metabolic burst [4], and discharge the content of their cytoplasmic granules into phagosomes and extracellular spaces [5]. PMN may be detrimental to the host if a misdirected or excessive inflammatory response leads to self damage [6]. Anti-inflammatory agents (AIA) counter such damage and act in part by interfering with PMN locomotion. The latter may be impaired by a drug effect on the speed (chemokinesis) and/or the direction (chemotaxis) of PMN locomotion [7]. Diclofenac, an aryl alkanoid, is non-steroidal AIA which is widely employed in the treatment of chronic inflammation and whose mechanism of action is poorly understood. This study was conducted to analyse the *in vitro* mechanism of action of diclofenac-induced alterations in PMN locomotion. Special attention was paid to determining the nature of these alterations and in particular, whether they act on chemokinesis and/or chemotaxis.

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MATERIALS AND METHODS

Drugs and chemoattractants. Diclofenac sodium (Ciba-Geigy, Basel, Switzerland) was diluted immediately prior to use in dimethylsulfoxide (DMSO from Fischer) and then in 0.1 M Krebs Ringer Phosphate buffer (KRP), pH 7.4 to the final desired concentration. The final concentration of DMSO in the assays was less than 0.5% and had no effect on PMN locomotion. Stock solutions of formyl-methionyl-leucyl-phenylalanine (FMLP from Sigma Chemical Co., St. Louis, MO) were prepared in aliquots in 0.15 M NaOH and stored at −80° until use. *Klebsiella pneumoniae* culture supernatant (KPCS) and zymosan-activated serum were prepared as previously described [10, 11]. Sera obtained from 10 healthy volunteers were pooled and stored in aliquots at −80°. Whole blood was obtained from peripheral veins of healthy adults, in preservative-free lithium heparin (10 IU/ml of blood). Leukocytes were isolated as previously described [10]. The final cellular suspension contained 10^8 PMN/ml. All experiments were completed within 3 hr of blood collection (i.e. 2 hr after PMN preparation).

PMN function tests. Two types of PMN migration assays were used. One served to measure PMN directed locomotion (i.e. speed + direction) in the presence of one of three chemoattractants-zymosan-activated human serum, KPCS or FMLP. The other

was used for measurement of undirected PMN locomotion (i.e. speed only) in the presence or absence of chemokinetic factors.

Directed PMN locomotion was assessed as previously described [10], by a modified version of the agarose technique [12, 13] except that the agarose contained various amounts of diclofenac and 1–10% of heat-inactivated fetal calf serum. Undirected PMN locomotion proceeded under similar conditions of agarose preparation, except that four sets of one well only instead of three were cut in the agarose with a template. The agarose contained various amounts of FMLP or zymosan-activated human serum, diclofenac and heat-inactivated fetal calf serum (FCS) whose concentrations are given in the results. Five microlitres of leukocyte suspension (5×10^5 PMN) was placed in each well and incubated at 37° for 90 min. At the end of incubation, the surface occupied by the PMN was a circle with the well in its centre. The radius of the circle minus that of the well was used to estimate undirected locomotion. The variation in the results obtained with this technique was less than 5% for duplicate experiments (two different petri dishes) of four measurements each.

Changes in the shape of floating PMN induced by FMLP or human activated serum were assessed by a modified version of the method of Smith *et al.* [14]. Suspensions of 5×10^5 PMN/ml in KRP containing 10% FCS were exposed for 10 min at 37° to various concentrations of diclofenac or buffer. FMLP final concentration: 5×10^{-8} M was then added. For changes of shape induced by human activated serum, the FCS concentration was 5% during the 10 min of PMN exposure to diclofenac; human activated serum (or human heat-inactivated serum) was then added to a total serum concentration of 10%. After 4 min the PMN were fixed with 1% (final concentration) of ice cold glutaraldehyde (v/v). PMN were examined by phase contrast microscopy (magnification, ×400) and 200 cells per field were classified into two groups, one consisting of round cells, and the other of cells with at least one lamellipod (front and/or tail). In some experiments an aliquot of fixed cells was cytocentrifuged and stained with hematoxylin-eosin. Results were similar to those obtained with unstained cells. The ingestion rate of heat-killed ^{14}C-labelled *Klebsiella pneumoniae* by PMN was measured exactly as previously described [15] in the presence of 100 microorganisms per PMN, except that the PMN were incubated with various concentrations of diclofenac prior to the addition of the microorganisms. Diclofenac was not removed during the assays.

Results used for statistical analysis are the means of assays performed in duplicate, after subtraction of appropriate blanks. Values were discarded when a variation of more than 10% was observed between the duplicate results.

Statistics were calculated on a Compucorp 445 Statistician. The significance of differences between control and assay experiments was assessed by the Student *t*-test (paired or unpaired). Linear regression parameters were calculated by the least square method.

RESULTS

PMN-directed locomotion, induced by human serum, 10^{-7} M FMLP (the concentration at which the maximal effect was obtained under our experimental conditions), or KPCS was reduced by the presence of diclofenac in the agarose (Fig. 1). The effect of this agent was dose-dependent. Observation of single cell locomotion under the microscope [10] during the migration induced by human serum, KPCS or FMLP suggested that diclofenac acted primarily on the speed of locomotion and not on the direction taken by the cells. Diclofenac also decreased FMLP-induced directed locomotion from 10^{-8} to 10^{-6} M

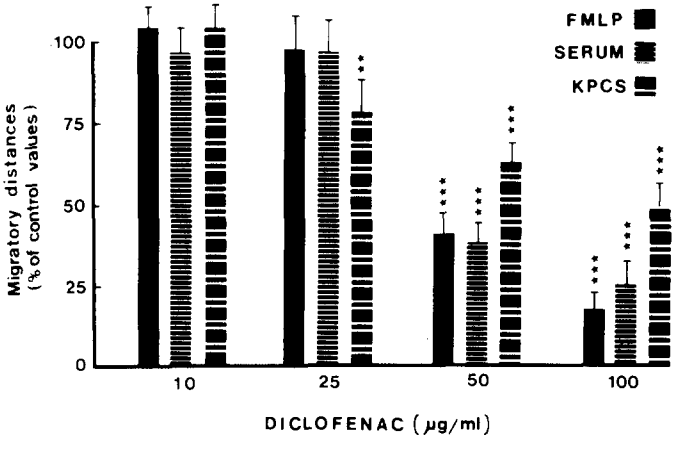

Fig. 1. Effect of diclofenac on FMLP, KPCS and serum-induced directed migration of PMN. Diclofenac was present at the indicated final concentration in the agarose gel containing 10% heat-inactivated FCS. Directed PMN locomotion was induced by undiluted serum, KPCS or FMLP at the concentration of 10^{-7} M. Control values (obtained in the absence of diclofenac) were, 1.90 ± 0.20, 1.30 ± 0.13 and 1.54 ± 0.15 mm in the presence of FMLP, KPCS and serum respectively. Results are expressed as percentages of control values and represent the means ± 1 S.D. of 5–7 different experiments. Significant differences between control and assay values (paired data) are indicated by ** ($P < 0.01$) and by *** ($P < 0.001$).

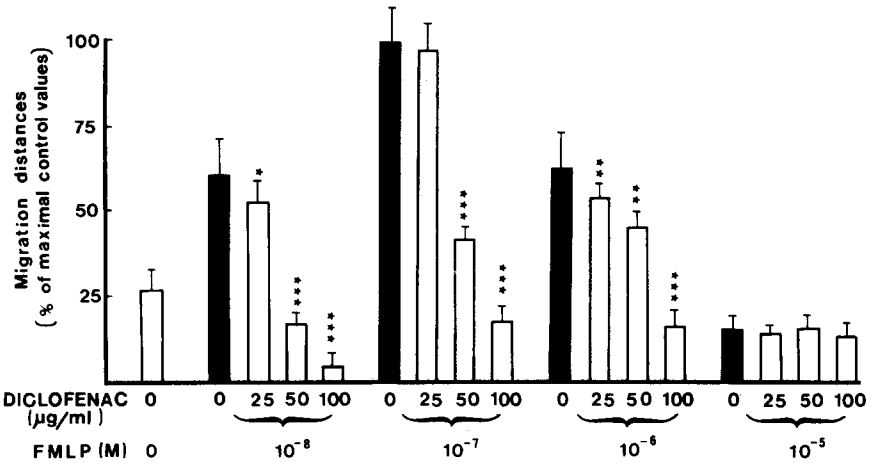

Fig. 2. Effect of diclofenac on the directed locomotion of PMN induced by various FMLP concentrations. Diclofenac was present in the agarose gel which contained 10% FCS. Increasing concentrations of FMLP were added to the chemoattractant wells to induce oriented PMN locomotion. Results are expressed in percentages of the maximal control values obtained in the presence of 10^{-7} M of FMLP. The maximal control value represented a migration distance of 1.90 ± 0.20 mm after incubation for 90 min. Each value is the mean ± 1 S.D. of 5–7 different experiments. For each series of experiments, a statistically significant difference between corresponding control and assay values (paired data) is indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

(Fig. 2). At 10^{-5} M FMLP, a concentration at which directed locomotion of control PMN was much lower than PMN random locomotion, no inhibitory effect by diclofenac could be observed. Moreover, more than 95% of PMN incubated for 90 min with the highest concentration of diclofenac used in these assays, i.e. 100 μg/ml, excluded Trypan blue. This suggested that diclofenac was not cytotoxic for the PMN at the concentrations used. In addition, 25–100 μg/ml diclofenac did not alter the ingestion rate of heat-killed opsonized *Klebsiella pneumoniae* by PMN (results not shown). This confirmed that diclofenac was not cytotoxic.

Because diclofenac appeared to act on PMN chemokinesis rather than on human serum, KPCS or FMLP-induced chemotaxis, we checked that diclofenac-treated PMN were still able to respond to stimulation by FMLP or human activated serum by changing their shape. This was indeed the case, as shown in Table 1.

PMN undirected locomotion, i.e. in the absence of chemoattractant gradient, was estimated, as stated

Table 1. Effect of diclofenac on changes in the shape of floating neutrophils induced by FMLP or activated serum

Stimuli	Diclofenac (μg/ml)	% of cell shape ± 1 S.D. Round PMN	Morphologically modified PMN
FMLP	0	14.2 ± 7.8	85.8 ± 7.8
	25	14.0 ± 5.4	86.0 ± 5.4
	50	11.5 ± 6.4	88.0 ± 6.4
	100	12.8 ± 8.7	87.2 ± 8.7
Activated serum	0	8.3 ± 3.3	91.7 ± 3.3
	25	5.4 ± 3.5	94.6 ± 3.5
	50	6.9 ± 4.3	93.1 ± 4.3
	100	7.3 ± 3.4	92.3 ± 3.4

A suspension of 5×10^5 PMN (i.e. 96% of leukocytes) was preincubated for 10 min at 37° in the presence or absence (control) of various amounts of diclofenac and 10% heat-inactivated FCS. The cells were then stimulated by FMLP (5×10^{-8} M) and incubated for 4 min and fixed by adding 500 μl of 2% glutaraldehyde to the incubation medium. When the PMN were stimulated with activated serum (5%), the FCS concentration in the medium was 5%. PMN which were round or showed morphological changes were counted under the microscope. At least 200 cells per field were counted. Results are expressed as percentages of cell shapes and represent the means ± 1 S.D. of 6 experiments. In the absence of chemotactic stimuli, more than 77% of the PMN were round.

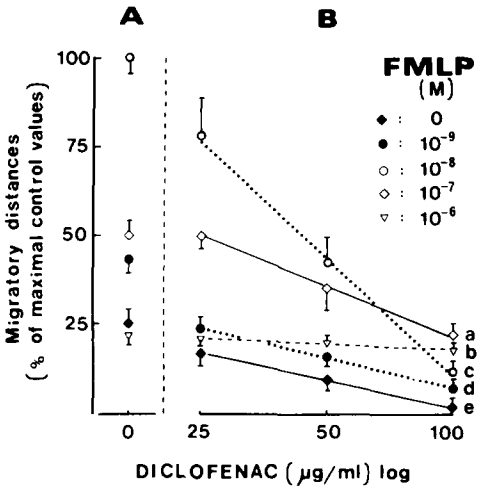

Fig. 3. Effect of diclofenac on PMN chemokinesis induced by various FMLP concentrations. Various concentrations of FMLP and/or diclofenac were incorporated into the agarose containing 10% FCS. Results are expressed as the percentages of maximal control values obtained in the presence of 10^{-8} M FMLP. The corresponding maximal control values represented a migration distance of approximately 2.22 ± 0.07 mm (mean ± 1 S.E.M. of 6 experiments) after incubation for 90 min. Regression parameters calculated for each correlation coefficient (r) and slope (s) were $r = 0.950$, $s = -28.1$ for curve e (absence of FMLP); $r = 0.990$, $s = -27.5$ for curve d (10^{-9} M FMLP); $r = 0.998$, $s = -110$ for curve c (10^{-8} M FMLP); $r = 0.999$, $s = -48.7$ for curve a (10^{-7} M FMLP) and $r = 0.900$, $s = -4.9$ for curve b (10^{-6} M FMLP).

in Materials and Methods, in the presence of various doses of chemotactic factors included in the gel. Results in Fig. 3B (curve e) show that undirected and unstimulated locomotion (i.e. in the absence of a chemokinetic factor) decreased linearly as a function of the log of the diclofenac concentration in the agarose. At 100 µg/ml of diclofenac, undirected and unstimulated locomotion was inhibited by more than 95%.

Both FMLP and serum are known to be not only chemotactic, but also to possess chemokinetic activity [11, 16]. Results in Fig. 3A confirm that FMLP inclusion in the agarose has a positive chemokinetic action on the PMN, except at high concentrations (10^{-6} M). The maximal positive chemokinetic activity of FMLP was recorded for 10^{-8} M of FMLP in the agarose; above and below this concentration, FMLP was less effective (Fig. 3A). As shown in Fig. 3B (curves a, c, d), diclofenac inhibited the positive chemokinetic effect of FMLP on PMN. Moreover, this inhibition was linearly correlated to the log of the diclofenac concentration (Fig. 3B). The inhibitory effect of diclofenac was weak in the presence of high concentrations of FMLP (10^{-6} M), which exhibited no detectable positive chemokinetic activity (Figs 3A and B, curve b), but marked in the absence of FMLP (Fig. 3B, curve e). Figure 4 confirms the chemokinetic activity of human serum. It also shows that diclofenac reduced the PMN chemokinetic activity at all the serum concentrations used (from 1 to 10%). These results indicate that diclofenac possesses a negative chemokinetic effect which is obvious in unstimulated cells, and in cells stimulated by serum or 10^{-7} to 10^{-9} M FMLP. In order to determine whether or not the diclofenac inhibition effect on PMN locomotion was due to cellular alterations, we measured the migration of PMN preincubated with diclofenac. Results given in Table 2 indicate that both chemokinesis and directed migration induced by FMLP were reduced in diclofenac-treated PMN. This argues for a cellular effect of diclofenac.

All the above experiments were performed with

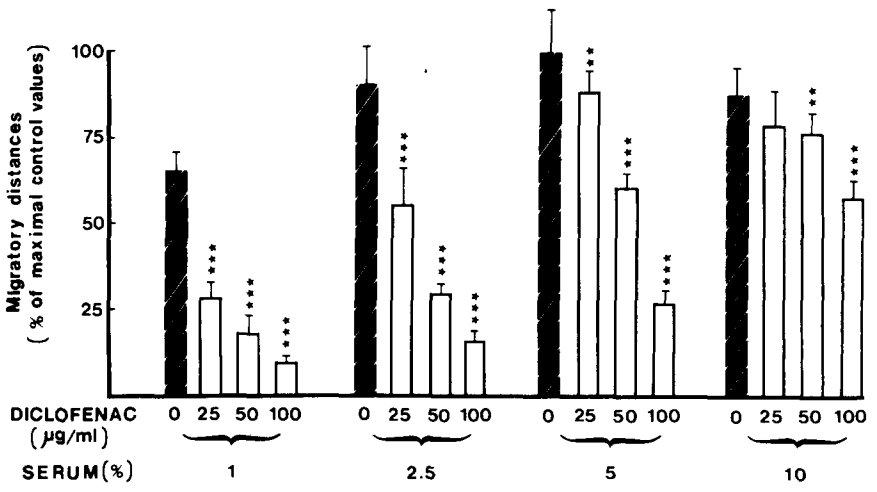

Fig. 4. Effect of diclofenac on the chemokinetic activity of PMN induced by activated serum. Diclofenac was present into the agarose gel which contained various indicated amounts of activated serum and inactivated FCS giving a total serum concentration of 10% (v/v). Results are expressed as the percentages of the maximal control values obtained in the presence of 5% activated serum. The maximal control value represented a migration distance of 1.75 ± 0.23 mm after 2 hr incubation. Each value is the mean ± 1 S.D. of 7 different experiments. A statistically significant difference between corresponding control and assay values (paired data) is indicated by ** ($P < 0.01$) and *** ($P < 0.001$).

Table 2. Effect of the preincubation of PMN with diclofenac on directed locomotion and chemokinesis induced by FMLP

Diclofenac (μg/ml)	Migratory distances ± 1 S.D. (in % of control values)	
	PMN directed locomotion	PMN chemokinesis
0	100 ± 9.8	100 ± 6.8
	(1.65 ± 0.16)	(1.84 ± 0.12)
25	94.9 ± 9.2	95.9 ± 5.2
50	88.8 ± 5.9**	82.3 ± 1.4***
100	84.6 ± 6.6**	77.9 ± 6.9**
200	55.2 ± 6.3***	56.8 ± 9.2***

PMN were preincubated for 15 min at 37° in the absence (control) or presence of diclofenac at various concentrations. Suspensions of PMN (5×10^5 cells) were added to the PMN wells. 10^{-7} M FMLP was placed in the attractant wells for measurement of PMN directed locomotion. PMN chemokinesis was assessed in the presence of 10^{-8} M FMLP incorporated in the gel. Results are expressed as the percentages of control values and represent the mean ± 1 S.D. of 5 different experiments. Each control value (100%) is given in parentheses and is expressed in mm. Significant differences between control and assay values are indicated by ** ($P < 0.01$) and *** ($P < 0.001$).

10% serum (FCS and/or human serum) in the agarose. To establish whether or not FCS altered the effect of diclofenac, we assessed this effect on PMN locomotion in relation to the FCS concentration in the agarose medium. When this concentration was reduced, inhibition of the PMN chemokinesis by diclofenac in the presence of 10^{-8} M FMLP greatly increased (Table 3). Similar results were observed with human activated serum. It is worth noting that in control experiments the decrease in the heat-inactivated serum concentration from 10 to 1% did not modify PMN locomotion.

DISCUSSION

The results reported here indicate that *in vitro*, diclofenac acts on PMN locomotion as a direct negative chemokinetic agent. This property of diclofenac will be discussed, bearing in mind that the drug is not toxic for the PMN.

The dose-related inhibition by diclofenac of both unstimulated (Fig. 3) and serum-stimulated locomotion (Figs 1 and 4) is open to question. It is conceivable that in actual fact, diclofenac exerts its effect on the chemokinetic activity of any bacterial products such as FMLP [17] which happen to be present in the agarose. Accordingly, if under our experimental conditions of measurement of undirected and "unstimulated" PMN locomotion the agarose is contaminated by minute amounts of bacterial products, they would act as positive chemokinetic factors. If this were the case, then our results for "unstimulated" and undirected locomotion would reflect a PMN locomotion stimulated by bacterial products, and diclofenac might act by inhibiting this stimulation. Several factors, however, argue against this hypothesis. Phenylbutazone has been shown by several authors to inhibit FMLP binding to its specific receptors in the PMN and to abolish the triggering effects of FMLP on PMN functions [10, 18, 19]. However, we and others have shown that phenylbutazone does not alter undirected PMN locomotion in the absence of any stimuli [10, 18], which was confirmed under our present experimental conditions (results not shown). Furthermore, phenylbutazone does not inhibit directed PMN locomotion induced by activated serum [10, 18] and this was also confirmed here (results not shown). In contrast, diclofenac decreased undirected and directed PMN locomotion induced by serum, and acted almost exclusively on PMN chemokinesis. It is well known that the stimulating effect of serum on PMN locomotion is largely due to the presence of C_{5a} fraction of activated complement [16]. C_{5a} acts through its binding to specific receptors on the PMN, which are different from the PMN receptors binding FMLP [16]. The binding of C_{5a} also induces morphological changes in the shape of PMN. Under all our experimental conditions, diclofenac inhibited the PMN locomotion in the absence or presence of FMLP, serum and KPCS, without altering the cell changes in PMN morphology induced by FMLP or activated serum. This suggests that diclofenac-treated PMN are still able to recognize chemotactic factors and

Table 3. Effect of diclofenac on the PMN chemokinetic activity induced by FMLP in relation to the serum concentration

Diclofenac (μg/ml)	Migratory distances (% of control values ± 1 S.D.)			
	Fetal calf serum concentration (%)			
	1	2.5	5	10
0	100.00 ± 8.6	100.00 ± 10.2	100.00 ± 9.2	100.00 ± 8.6
	(1.84 ± 0.16)	(1.70 ± 0.16)	(1.91 ± 0.17)	(1.79 ± 0.15)
10	84.6 ± 7.1*	90.5 ± 10.3	84.00 ± 6.3*	99.2 ± 7.9
25	39.1 ± 1.1***	55.3 ± 7.9***	60.8 ± 8.5***	78.00 ± 7.6**
40	28.8 ± 1.0***	31.2 ± 4.7***	36.5 ± 6.0***	67.00 ± 9.0***

Diclofenac and heat-inactivated FCS were present in the agarose gel at the indicated final concentrations. FMLP was also incorporated into the agarose at the optimal concentration of 10^{-8} M. Each bar represents the mean ± 1 S.D. of 5 different experiments. Results are expressed as the percentages of the corresponding control values (obtained in the absence of diclofenac). Each control value (100%) is given in parentheses and is expressed in mm. Significant differences between control and assay values are indicated by * ($P < 0.05$), ** ($P < 0.001$) and *** ($P < 0.001$).

possibly small differences in the concentrations of the stimuli controlling cell orientation during migration. The mechanism by which diclofenac alters PMN locomotion remains speculative. It is however probably due to cellular alterations as suggested by the decrease in the migration of diclofenac-pretreated cells (Table 2). Further analysis of diclofenac binding to the PMN will show whether the drug binds to specific sites on the PMN or binds non-specifically to its membrane. Many other anti-inflammatory drugs such as indomethacin [20], auranofin [21] and corticosteroids [22] have been shown to inhibit PMN locomotion, but none of them has been reported to possess negative chemokinetic activity on unstimulated PMN.

The diclofenac concentrations giving half inhibition of the maximal chemokinetic activity of 10^{-8} M FMLP and of human activated serum were 45 and 65 μg/ml respectively, when the agarose contained 10% serum comprising FCS and/or human serum. These values are much higher than those obtained in the blood of treated patients. Since the anti-inflammatory agents act in extracellular fluid where the protein concentration is low, we analysed the effects of diclofenac at lower concentrations. Reducing the serum concentration in PMN locomotion studies clearly showed that inhibition by diclofenac increased. The most probable explanation for this finding is that diclofenac may be bound to serum factors, and is no longer active when bound to proteins. This has already been shown for the inhibition by indomethacin of several FMLP-induced PMN functions, which is obvious in protein-free buffer systems [23–25] but completely disappears on addition of albumin or heat-inactivated serum [24, 25].

In conclusion, diclofenac inhibits PMN chemokinetic activity in a dose-dependent fashion. This property was observed in the absence and presence of two positive chemokinetic factors (activated human serum and FMLP) which act on two different specific receptors on the PMN membrane. This negative chemokinetic effect of diclofenac which does not seem to interfere with the chemotactic activity of FMLP or human activated serum, might make the drug a very useful probe in PMN locomotion studies.

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REFERENCES

1. T. P. Stossel, *New Engl. J. Med.* **290**, 717, 774, 833 (1974).
2. R. L. Baehner, *Clin. Haematol.* **4**, 609 (1975).
3. J. I. Gallin, *Clin. Haematol.* **4**, 567 (1975).
4. S. J. Klebanoff, *Ann. Int. Med.* **93**, 480 (1980).
5. S. J. Klebanoff and R. A. Clark, in *The Neutrophil: Function and Clinical Disorders*, p. 283. Elsevier/North-Holland, Amsterdam.
6. K. F. Austen, *J. Immunol.* **121**, 793 (1978).
7. H. U. Keller, P. C. Wilkinson, M. Abercrombie, E. L. Becker, J. G. Hirsh, M. E. Miller, S. W. Ramsey and S. H. Zigmond, *Clin. exp. Immunol.* **27**, 377 (1977).
8. A. Bijlsma and J. G. ten Pas, *Scand. J. Rheumatol. Suppl.* **22**, 46 (1978).
9. A. Kajander and J. Martio, *Scand. J. Rheumatol. Suppl.* **22**, 57 (1978).
10. A. Perianin, M. T. Labro and J. Hakim, *Biochem. Pharmac.* **31**, 3071 (1982).
11. A. Ferrante and Y. H. Thong, *J. Immunol. Methods* **36**, 107 (1980).
12. J. E. Cutler, *Exp. biol. Med.* **147**, 471 (1974).
13. R. D. Nelson, P. G. Quie and R. L. Simmons, *J. Immunol.* **115**, 1560 (1975).
14. C. W. Smith, J. C. Hollers and R. A. Patrick, *J. clin. Invest.* **63**, 21 (1979).
15. M. Torres, D. de Prost, J. Hakim and M. A. Gougerot, *Eur. J. clin. Invest.* **9**, 209 (1979).
16. P. C. Wilkinson, in *Chemotaxis and Inflammation*, p. 67. Churchill Livingston, Edinburgh (1982).
17. E. Schiffmann, B. A. Corcoran and S. A. Wahl, *Proc. natn. Acad. Sci. U.S.A.* **72**, 1059 (1975).
18. C. Dahinden and J. Fehr, *J. clin. Invest.* **66**, 884 (1980).
19. A. Perianin, M. Torres, M. T. Labro and J. Hakim, *Biochem. Pharmac.* **32**, 2819 (1983).
20. M. J. M. Smith and J. R. Walker, *Br. J. Pharmac.* **69**, 473 (1980).
21. T. D. Coates, B. Wolach, D. Y. Tzeng, C. Higgins, R. L. Baehner and L. A. Boxer, *Blood* **62**, 1070 (1983).
22. K. M. Skubitz, P. R. Craddock, D. E. Hammerschmidt and J. T. August, *J. clin. Invest.* **68**, 13 (1981).
23. G. M. Bokoch and P. W. Reed, *Biochem. biophys. Res. Commun.* **90**, 481 (1979).
24. J. T. O'Flaherty, H. J. Showell, E. L. Becker and P. A. Ward, *Prostaglandins* **17**, 915 (1979).
25. J. E. Smolen and G. Weissman, *Biochem. Pharmac.* **29**, 533 (1980).