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Effects of indomethacin and diclofenac on some functions of polymorphonuclear neutrophils

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Abstract—The effects in-vitro of two non-steroidal anti-inflammatory drugs, diclofenac and indomethacin, have been studied on human polymorphonuclear functions. Adhesivity and chemotaxis were decreased by the two drugs, but in a manner that varied with the attractant used. As shown by the nitro blue tetrazolium slide test, all cells remained active but the production of oxygen metabolites by opsonized zymosan was decreased in the presence of diclofenac.

The effects of non-steroidal anti-inflammatory drugs on the functions of polymorphonuclear neutrophils (PMNs) has received attention because of the role of these cells in the inflammatory process. Bird & Giroud (1985) suggested measuring the chemiluminescence of PMNs to detect the anti-inflammatory activity of a drug. Chemotaxis in PMNs is inhibited for example by phenacetin, indomethacin and piroxicam (Austin & Truant 1978; Bandmann et al 1975; Pecoud et al 1978; Rivkin et al 1976; Tanaka et al 1984). Maderazo et al (1984) and Tanaka et al (1984) reported that the release of lysosomal enzymes is inhibited by piroxicam, ibuprofen, and indomethacin. Similarly, the oxidative metabolism of PMNs is inhibited by most anti-inflammatory drugs, except ibuprofen (Biemond et al 1986; Maderazo et al 1984; Tanaka et al 1984).

We report the results of a study in-vitro of the effects of two commonly used anti-inflammatory drugs, diclofenac and indomethacin, on the functions of PMNs.

Materials and methods

Reagents. Dextran T500, 0·87% NH_4Cl in distilled water, phosphate-buffered saline (PBS), sterile distilled water, Minimum Essential Medium (MEM) (Gibco), 7·5% sodium bicarbonate (Gibco), heat-inactivated human serum stored at −80°C, agarose (indubiose A37 from IBF), fresh AB serum, Zymosan A (Sigma), methanol, formalin, a 1% solution of fuchsin in alcohol, 10 mg mL^{-1} zymosan-activated serum (ZAS), 100 nM formyl-methionyl-leucyl-phenylalanine (FMLP) (Sigma), a freshly prepared 1 g L^{-1} suspension of nitro blue tetrazolium (NBT) (Sigma), latex (Bactolatex, from Difco), cytochrome C (Boehringer), diclofenac (Ciba-Geigy), indomethacin (Sigma).

Blood was taken from 10 healthy volunteer blood donors and leucocytes were obtained by dextran accelerated sedimentation. Contaminating red blood cells were haemolysed with ammonium chloride and the leucocytes were rinsed and then resuspended in PBS at 10 000 cells μL^{-1}. These cells were incubated for 30 min with various concentrations of the drug being tested and were then rinsed with PBS.

Concentrations of drugs were $0{\cdot}8 \times 10^{-3}$ to 8×10^{-3} mg mL^{-1} for diclofenac and 10^{-3} to 10×10^{-3} mg mL^{-1} for indomethacin. These concentrations represent the level reached in therapy.

Adhesivity. This was studied on glass beads, according to Lorente et al (1978), each experiment being in duplicate. Blood (1 mL) was loaded onto glass beads in disposable syringes, which were left in an incubator at 37°C for 30 min (the syringes were turned once, after the first 15 min). Next, PBS (1 mL) was loaded into each syringe and the effluent then collected in a plastic tube. A differential blood count was then made in a Coulter Counter on the original sample and on the recovered effluents. Adhesivity was expressed as the percentage of adherent PMNs. Drug concentrations in the incubation medium were typical of those reached in therapy: 0·8, 2 and 8 $\mu g\ mL^{-1}$ for diclofenac and 1, 5 and 10 $\mu g\ mL^{-1}$ for indomethacin.

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Chemotaxis. Migration in an agarose gel was studied using a technique which we have described elsewhere Marchand-Arvier & Vigneron (1982). Two chemotactic substances were used: ZAS (zymosan-activated serum) and FMLP (the synthetic pepide formyl-methionyl-leucyl-phenylalanine). The chemotactic mobility (Mc) and the spontaneous mobility (Ms) were determined under a microscope as distances across a calibrated grid.

From these, the chemotactic differential (Mc–Ms) and the chemotactic index (Mc/Ms) were calculated.

Cytochemical test of NBT reduction (Holmes test). PMNs were incubated in advance with the drugs being tested and then with NBT and latex particles for phagocytosis. They were then smeared, for observation of the blue-black grains of reduced NBT. The results were expressed as a percentage: the number of cells that had reduced the NBT per 100 cells which had phagocytosed more than 10 latex beads.

Superoxide ion production through the reduction of cytochrome C, using the technique of De Chatelet et al (1975). The reduction of cytochrome C was measured from the superoxide anion O_2^- released by zymosan-activated PMNs previously incubated with one of the two drugs being tested. In a parallel experiment, this reduction was inhibited by adding superoxide dismutase (SOD) to the reaction medium.

The inhibition of cytochrome C reduction by SOD was determined in a spectrophotometer a 550 nm. The difference in optical density (OD) between the medium containing SOD and that without SOD corresponds to the amount of cytochrome C reduced by the actively phagocytosing PMNs and thereby to the quantity of superoxide anion produced. The results were expressed as the difference in optical density between the tube without SOD and the tube with SOD per 10^6 PMNs.

Results

Adhesivity. Fig. 1 shows a significant inhibition of adhesivity in the presence of each of the two drugs tested. This inhibition was concentration-dependent, but was greater with diclofenac than with indomethacin ($P < 0{\cdot}001$).

Chemotaxis. Spontaneous mobility (Ms) of PMNs was measured in the presence of diclofenac or indomethacin. Diclofenac inhibited Ms by 12 to 22%, depending on the concentration (Table 1). The inhibition was greater than that of indomethacin at all the concentrations tested (Table 2), and was maximal at moderate concentrations.

The chemotactic mobility (Mc) of PMNs in response to ZAS and in the presence of diclofenac and of indomethacin showed

Table 1. Influence of diclofenac on human polymorphonuclear migration.

Random migration				
Diclofenac mg mL^{-1}	0	$0{\cdot}8\times10^{-3}$	2×10^{-3}	8×10^{-3}
Spontaneous migration (mm)	$0{\cdot}82\pm0{\cdot}024$	$0{\cdot}72\pm0{\cdot}025$	$0{\cdot}64\pm0{\cdot}026$	$0{\cdot}68\pm0{\cdot}024$
Ms: mean ± 2 s.e.m.				
Significance		NS	$P<0{\cdot}02$	$P<0{\cdot}05$

	Attractants							
Activated migration	ZAS				FMLP			
Diclofenac mg mL^{-1}	0	$0{\cdot}8\times10^{-3}$	2×10^{-3}	8×10^{-3}	0	$0{\cdot}8\times10^{-3}$	2×10^{-3}	8×10^{-3}
Chemotactic migration (mm)	1·69	1·53	1·28	1·30	1·72	1·58	1·71	1·22
Mc: mean ± 2 sem	±0·14	±0·14	±0·14	±0·15	±0·18	±0·14	±0·24	±0·11
Significance		NS	NS	NS		NS	NS	NS
Chemotactic index	1·98	2·39	1·96	2·09	2·13	2·12	2·05	2·01
Mc/Ms: mean ± 2 s.e.m.	±0·11	±0·20	±0·20	±0·12	±0·14	±0·14	±0·17	±0·13
Significance		NS	NS	NS		NS	NS	NS

Table 2. Influence of indomethacin on human polymorphonuclear migration.

Random migration					
Indomethacin mg mL^{-1}	0	10^{-3}	5×10^{-3}	10×10^{-3}	
Spontaneous migration (mm)	$0{\cdot}88\pm0{\cdot}03$	$0{\cdot}79\pm0{\cdot}03$	$0{\cdot}79\pm0{\cdot}03$	$0{\cdot}79\pm0{\cdot}04$	
Ms: mean ± 2 s.e.m.					
Significance	NS	NS	NS	NS	NS

	Attractants							
Activated migration	ZAS				FMLP			
Indomethacin mg mL^{-1}	0	10^{-3}	5×10^{-3}	10×10^{-3}	0	10^{-3}	5×10^{-3}	10×10^{-3}
Chemotactic migration (mm)	2·09	1·64	1·75	1·63	1·83	1·83	1·75	1·72
Mc: mean ± 2 s.e.m.	±0·11	±0·14	±0·10	±0·13	±0·16	±0·13	±0·15	±0·13
Significance		NS	NS	NS		NS	NS	NS
Chemotactic index	2·08	2·28	2·26	2·02	2·21	2·06	2·07	2·32
Mc/Ms: mean ± 2 s.e.m.	±0·09	±0·15	±0·11	±0·13	±0·16	±0·21	±0·16	±0·15
Significance		NS	NS	NS		NS	NS	NS

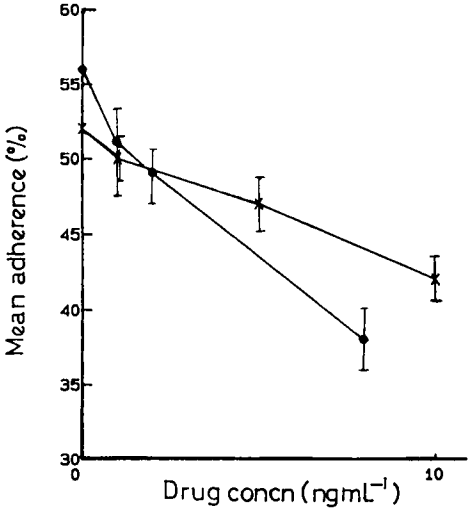

FIG. 1. Effect of diclofenac (●) and indomethacin (×) on polymorphonuclear adherence.

that Mc was inhibited by both drugs, the degree of inhibition varying with the concentration. At low concentrations indomethacin had the stronger inhibiting effect, at high concentrations both two drugs had about the same effect.

The chemotactic mobility (Mc) of PMNs in response to FMLP and in the presence of diclofenac and of indomethacin was inhibited more by diclofenac than by indomethacin at low and at high concentrations, but at intermediate concentrations indomethacin was the stronger inhibitor. This suggests that the two drugs do not act on the same receptors, since they arouse different (even opposite) responses depending on the chemotactic attractant used to provoke chemotaxis of the PMNs.

The chemotactic differentials and indexes were not much affected. The chemotaxis results show: (i) decrease of spontaneous and chemotactic mobilities of PMNs under the influence of both drugs; (ii) inhibition that varied depending on the concentrations tested and on the attractant used; (iii) from chemotactic mobilities and chemotactic differentials that diclofenac does not appear to act on the same receptors as indomethacin.

Diclofenac may act on the receptor for the peptide (FMLP), whereas indomethacin acts specifically on a receptor for complement (ZAS).

Reduction of nitro blue tetrazolium (NBT). The NBT-reducing property of PMNs was practically unchanged in the presence of diclofenac and of indomethacin. Neither drug affected the number of PMNs that reduced NBT.

Production of superoxide anion. In the presence of diclofenac the production of superoxide anion was reduced at all three concentrations tested (between 6·6 and 19·4%, depending on the concentration), compared with production by non-incubated cells ($P<0{\cdot}02$).

In the presence of indomethacin the results were erratic, varying with the individual and the concentration (low concentrations seemed to inhibit the generation of superoxide anion while a high concentration seemed to activate it). Results are not significant.

Statistical analysis. Student's *t*-test was used for differences between groups. Probabilities below 0·05 were considered significant.

Discussion

Published reports have often used higher than therapeutic concentrations and hence higher than those used in our study, so there are difficulties in comparing our results with earlier ones.

Adhesivity was decreased in the presence of the drugs at their therapeutic concentrations. This decrease was greater for diclofenac than for indomethacin. We have not found any earlier reports with which to compare our results.

Diclofenac and indomethacin decreased the spontaneous and chemotactic mobility of PMNs towards the attractants used. Furthermore, our results suggest some specificity of action by indomethacin on a complement receptor (ZAS) and by diclofenac on the peptide receptor (FMLP). The study of Magous et al (1985) had already shown that FMLP is not recognized by the platelet receptors for indomethacin.

However, Tanaka et al (1984), using Boyden's chamber technique, showed that with 10 μmol L^{-1} indomethacin (about 4 $\mu g\ mL^{-1}$), chemotaxis in response to FMLP is inhibited by 19·6%. We found a 10·2% inhibition at this concentration, using the technique of migration under agarose gel.

The number of cells that reduced NBT did not change in the presence of either anti-inflammatory agent, which means that both act on all the cells rather than selecting one cell population rather than another.

There was a decrease in the production of superoxide ions by phagocytically active PMNs in the presence of diclofenac at the therapeutic concentrations tested. There do not appear to be any earlier studies on this subject for comparisons to be made. Indomethacin gave highly variable results; in the absence of any simpler explanation, we assume that susceptibility to this drug varies with the individual.

However, studies by Gay et al (1984) showed that indomethacin increased the release of O_2^- in the presence of opsonized zymosan, but the indomethacin concentrations they used exceeded those reached in therapy. Gay et al also used other stimuli. For example, with FMLP there was a concentration-dependent inhibition of the release of O_2^-. The concentration producing a 50% inhibition of O_2^- production was reported to be 44 $\mu g\ mL^{-1}$ by Gay et al (1984) and 35·7 $\mu g\ mL^{-1}$ by Tanaka et al (1984). These exceed the maximum serum concentrations reached in therapy.

Thus it appears that in an inflammatory process, diclofenac and indomethacin act at different stages of the involvement of PMNs: from diapedesis to the release of phlebogenic factors.

The adhesivity and chemotaxis of PMNs induced naturally by attractants during the inflammatory process are inhibited by these two drugs. This may induce a decrease in the influx of the cells to the focus of inflammation under the influence of the drugs. The number of PMNs that reduce NBT was not affected, signifying that the cells retain their activity. The production of superoxide ion by the cells was decreased by diclofenac but not by indomethacin.

Conclusion. The study of the adhesivity of PMNs in-vitro has shown a decrease of this activity by indomethacin and diclofenac, which supports their involvement at an early stage in the inflammatory process. Furthermore the chemotaxis of the cells is also inhibited by the two drugs, but in a manner that varies with the attractant used. Indeed, our study suggests that diclofenac may act specifically on the peptide receptor and that indomethacin may act more specifically on complement receptors.

Also, the number of PMNs that reduce nitro blue tetrazolium was not changed by the presence of the drugs: the cells all remained active. But we did observe a decrease in the production of superoxide anion by diclofenac in the presence of a particular stimulus (opsonized zymosan). This emphasizes the probable usefulness of the drug in preventing tissue damage by any oxygenated metabolites produced during the "respiratory burst" of PMNs. The results obtained with indomethacin showed no such effect.

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