Inhibition of human leucocytes locomotion by anti-inflammatory drugs[1]

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Summary. In this study we have demonstrated that acidic non-steroidal anti-inflammatory drugs in low concentrations inhibited human PMN locomotion in vitro. A speculative mechanism of action is proposed.

Polymorphonuclear leucocytes (PMN) play an important role in the inflammatory process, and their functions are likely to be regulated by pharmacological agents. In this paper we report the in vitro effect of acidic non-steroidal anti-inflammatory drugs (ANSAI) on random and chemotactic motility of human PMN, and demonstrate that the 2 forms of movement, spontaneous and directional, are differently inhibited by low concentrations of these agents.

Materials and methods. The ANSAI used were: aspirin from Carlo Erba, sodium salicylate: a gift of Ciba-Geigy, ibuprofen and indomethacin: a gift of Roche, ketoprofen and naproxen: a gift of Dr Pollini from Chemistry Institute, University of Ferrara. These drugs were dissolved in Gey's solution containing 1 mg/ml bovine serum albumin from Sigma (Gey's-BSA) at pH 7.2[2]. Each drug was assayed both in the chemotaxis chambers and in pre-incubating experiments performed before the chemotactic test.

Human neutrophil leucocytes were derived from fresh heparinized venous blood samples and used within a few hours of removal. Leucocyte fractions were prepared by dextran sedimentation[3], a method which yielded about 85% granulocytes and 15% mononuclear cells. The chemotaxis assay (positive control) was performed using modified Boyden chamber as previously described[4,5] which utilized 1×10^6 PMN/ml in the upper compartment and the leading front method of Zigmond and Hirsch was adopted[6] for evaluating the cell motility. Chambers were incubated at 37 °C for 60 min; after which filters were stained according to Wilkinson[5], and examined with a ×40 objective. This measure was taken for 5 fields across the filter. Duplicate chambers were always run and the data presented are the mean ±SE of separate experiments with blood samples from 6 different healthy donors. Casein (according to Hammersten, obtained from Merck, Darmstadt) at a concentration of 1 mg/ml was used as leuco-attractant solution in the lower compartment. The random migration (negative control) was measured in the absence of a leucotactic gradient. 1×10^6 cells/ml were suspended in Gey's-BSA and a similar medium was placed in the bottom compartment. None of the agent tested, when added to the attractant side alone, exhibited chemotactic activity for PMN.

Results and discussion. We demonstrated that the acidic non-steroidal anti-inflammatory agents commonly used as pharmacological drugs, inhibited the directional movement of PMN. In fact, as shown in figure 1, when the drugs were added in the chemotactic chamber, all substances exhibited the inhibitory effect on the directional locomotion, particularly ibuprofen that completely abolished the cell migration. Therefore, low concentration of all the anti-inflammatory drugs tested in vitro effected the locomotion of PMN. There is a considerable discrepancy between those results and those reported by Borel for rabbit PMN[7]; but Norris et al. observed that incubation of human PMN with high concentration of steroidal anti-inflammatory drugs inhibited the directional movement[8]. To elucidate the effect of anti-inflammatory agents on specific types of PMN motility, random and directional movements were tested, either when varying drug concentrations were used in both compartments of the chamber, or when PMN were pre-incubated with the same drug concentrations. The results indicated that aspirin, when added to the PMN in the chamber, showed a dose response curve in inhibiting the chemotactic activity of cells (figure 2A), while at a concentration that affected the directional movement, it failed to induce changes in the random motility. Pre-incubation of leucocytes with aspirin resulted in an inhibitory effect on both types of movements. Comparable experiments were per-

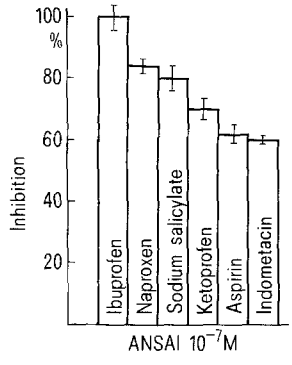

Fig. 1. Comparison of inhibitory effect of ANSAI on PMN chemotaxis. Drugs were added to both top and bottom compartments of chemotaxis chamber. Concentrations other than those here indicated has also been used and 10^{-7} M was chosen because expressive. Inhibition was evaluated as percentage of chemotaxis in the absence of ANSAI (positive control). The bars denote ±SE of the mean of separate experiments with blood samples from 6 different healthy donors.

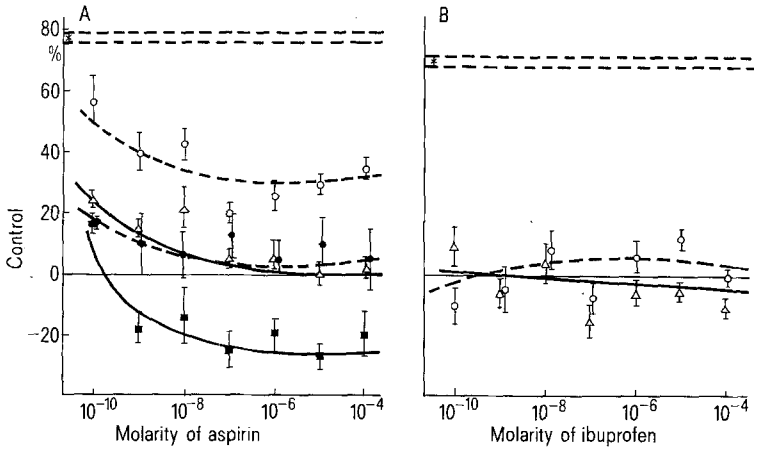

Fig. 2. Effect of aspirin (A) and ibuprofen (B) on random motility and chemotaxis of PMN. Random motility (—△—) and chemotaxis (——○——) were evaluated in the presence of increasing concentration of drugs in the range of 10^{-10}-10^{-4} M. Pre-incubating experiments were performed in which, before the chemotactic test, PMN were treated with different concentrations of drugs for 40 min at 20 °C. Thereafter cells were washed twice and placed in the upper compartment of the chamber and random motility (—■—) and chemotaxis (——●——) were tested. Chemotaxis (positive control) (= = =) and random motility (negative control) (---) were evaluated in the absence of drugs. Results are expressed as percentage of negative control. The bars denote the ±SE of the mean of separate experiments with blood samples from 6 different healthy donors.

formed with the other ANSAI, and ibuprofen was the most effective drug in blocking directional movement when added in the chamber (figure 2B). The chemotactic activity was completely abolished starting from the lowest concentrations of ibuprofen, whereas the other drugs exhibited their effect in the range of 10^{-8}–10^{-4} M. This cell behaviour was confirmed by retesting PMN from the same individuals in separate blood samples. On the other hand, pre-incubation of PMN with this anti-inflammatory drug, which selectively inhibited the directional movement, did not interfere with random movement. The mechanism by which acidic non-steroidal anti-inflammatory agents inhibited PMN cellular functions are speculative. Since ANSAI have been shown to interfere specifically with PG synthetase system, which transforms the suitable fatty in prostaglandins[9-11], it is likely, therefore, that these agents may carry on their pharmacological effect on human PMN throughout such a mechanism. Most recently it has been reported that low concentration of indomethacin, as 10^{-8}–10^{-5} M, inhibited PG production in PMN from rat[12] and human eosinophil[13], respectively. The data obtained, added to those with endogen mediators of inflammation[14], suggested that it is possible to hypothesize that the 2 forms of locomotions, random motility and chemotaxis, are 2 dissociable processes.

1 This work was supported by a grant from C.N.R.
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Protective effect of native levan on endotoxin toxicity in mice and rats[1]

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Summary. High polymer levan administered to mice and rats before the injection of endotoxin partly protects the animals from the lethal effects of the LPS.

Endotoxic shock is a common cause of morbidity and mortality in patients suffering from a variety of disease processes[3]. It probably represents an important pathogenetic mechanism underlying tissue damage in sepsis, burns and hypovolemic shock. The mortality rate in endotoxic shock has been reported to amount to 50–70% of patients[4,5]. Native levan, a polyfructoside of an average mol. wt of 2×10^7, is known to inhibit the passage of proteins and cells across the endothelial barrier[6-8]. Coating of endothelial surfaces by levan might be the main reason for its inhibitory effect on processes such as acute inflammation[9,10] and graft rejection[11]. Endotoxin was shown to bind primarily to the endothelial surface of blood vessels[12] and to affect circulating cells[13].

The present paper describes experiments in which the effect of levan administration on the toxicity of endotoxin was tested on mice and rats.

Materials and methods. Endotoxin (E.coli 0111:B4 lipopolysaccharide W Difco) was dissolved in pyrogen-free 0.85% NaCl solution. Animals were injected i.p. or i.v. with 0.2 ml of solution containing the desired amounts of endotoxin. *Aerobacter levanicum* levan was prepared according to Hestrin et al.[14] with consequent alkaline ethanol precipitation in order to free the levan from endotoxin[9]. A 5% levan solution was administered to mice i.p. in amounts of 10 or 25 mg per mouse and 50 mg per rat. As the batch of levan used in these experiments caused death in some animals, they were pretreated with small doses (5–10 mg) of levan on a number of alternate days before the endotoxin injection. Balb/c mice of both sexes, 20–25 g in weight, and Charles River female rats, 200–250 g in weight, were used. The rats were sensitized to endotoxin by i.p. injection of 10 mg of lead acetate dissolved in 0.2 ml of 0.85% NaCl solution together with the endotoxin[15]. 3 animals of each experimental group and 3 controls were killed by neck dislocation and their lungs and kidneys taken for histological examination.

Results. In repeated experiments on over 100 animals, levan injected together with or after the endotoxin had no beneficial effect on survival. The protective effect of levan was obvious, however, in animals started on levan *before* the injection of endotoxin.

Protective effect of levan on endotoxin toxicity in levan pretreated mice

Group	Endotoxin per 20-g mouse	Levan i.p.	Mortality within 20 h	Mortality within 24–26 h
1	0.8 mg	–	9/11	11/11
2	0.8 mg	10 mg	3/8	4/8
3	0.8 mg	25 mg	0/8	0/8
4	1.2 mg	–	10/10	10/10
5	1.2 mg	10 mg	4/10	7/10
6	1.2 mg	25 mg	4/10	9/10