J. Pharm. Pharmacol. 1985, 37: 587–588
Communicated January 28, 1985

Effects of isoxicam and other non-steroidal anti-inflammatory drugs on arachidonic acid metabolism by rat peritoneal leucocytes

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Inhibition of prostaglandin formation from [^{14}C]arachidonic acid by rat peritoneal leucocytes occurred with nonsteroidal anti-inflammatory drugs, their order of potency being indomethacin > piroxicam > naproxen > ibuprofen > isoxicam. At the lowest concentration tested (1 μg ml^{-1}), indomethacin markedly increased the accumulation of lipoxygenase products in the cell incubates. Naproxen, ibuprofen or piroxicam 1 or 10 μg ml^{-1} resulted in smaller increases of lipoxygenase products, and there was only a small rise with these concentrations of isoxicam.

In acute inflammation prostaglandins E_2 and I_2 interact with substances such as histamine and bradykinin to augment pain (Ferreira et al 1978) and vascular permeability (Williams et al 1983). Recently it has become clear that other metabolites of arachidonic acid, the leukotrienes and hydroxy-fatty acids produced by the action of lipoxygenases, are also important in inflammation (Samuelsson 1983). Some lipoxygenase products exhibit powerful chemotaxis (Ford-Hutchinson et al 1980), resulting in the accumulation of leucocytes, and they increase oedema formation.

Most non-steroidal anti-inflammatory drugs inhibit prostaglandin synthesis, but some such as benoxaprofen (Walker & Dawson 1979) and the experimental compound BW755C (Higgs et al 1979) also inhibit lipoxygenase activity. The effect of isoxicam, a new anti-inflammatory drug, has therefore been examined on both cyclo-oxygenase and lipoxygenase activity of rat peritoneal leucocytes, in comparison with piroxicam, indomethacin, naproxen and ibuprofen.

Methods

Rat peritoneal leucocytes were obtained from male Wistar rats, killed by exposure to diethyl ether, by washing the peritoneal cavity with heparinized phosphate buffered saline (pbs). The cell suspension was centrifuged (500*g*, 5 min) and the cell pellet resuspended in ammonium chloride solution (163 mM, 40 ml) to lyse contaminating erythrocytes. Following centrifugation, the pellet was finally resuspended in pbs (approximately 2 × 10^6 cells ml^{-1}, measured by a Coulter counter). Aliquots of the leucocyte suspension (700 μl) were pre-incubated (37 °C, 15 min) with 200 μl of pbs solution alone or containing 1, 10 or 100 μg of a drug. This was followed by further incubation at 37 °C for 5 min with calcium ionophore A23187 (0·5 μg) and [1-^{14}C]arachidonic acid (0·1 μCi, 1·7 nM) in a final volume of 1 ml. Enzymic activity was terminated with methanol : formic acid (1 ml : 10 μl), after which the eicosanoids were extracted with diethyl ether (3 ml × 2) and evaporated to dryness. These extracts were chromatographed using silica gel thin layer plates (Eastman Kodak Ltd) developed in hexane–ether–acetic acid (40 : 60 : 3) together with the authentic standards PGE_2, leukotriene B_4 (LTB_4), 5-hydroxyeicosatetraenoic acid (5-HETE), and arachidonic acid. Autoradiographs were prepared (Eastman Kodak Ltd, NS-2T, 10-day exposure), the TLC tracks cut into segments, and the radioactivity determined in a scintillation counter.

Results

Rat peritoneal leucocytes stimulated by the calcium ionophore A23187 metabolized [^{14}C]arachidonic acid via both the cyclo-oxygenase and lipoxygenase pathways. By comparison with authentic standards, some of the products were characterized as prostaglandins (determined collectively), LTB_4 and 5-HETE. The mean percentage incorporations of ^{14}C into metabolites in the controls in two series of experiments were: prostaglandins 21·8 ± 1·4%, LTB_4 3·0 ± 0·5% and 5-HETE 3·5 ± 0·5% (n = 17).

All drugs concentration-dependently reduced the formation of prostaglandins (Table 1). The order of potency was indomethacin > piroxicam > naproxen > ibuprofen > isoxicam. Unlike the other drugs tested, indomethacin 1 μg ml^{-1} markedly increased the formation of both LTB_4 (286% increase), and 5-HETE (488%). At 1 μg ml^{-1} isoxicam or piroxicam had no effect on LTB_4 accumulation while a modest increase occurred with ibuprofen (18%, $P < 0·02$). The highest concentration of all drugs reduced incorporation of ^{14}C into LTB_4. 5-HETE accumulation tended to increase with 1 μg ml^{-1} of each drug tested, the probability of this effect being $P < 0·02$ with naproxen, ibuprofen and piroxicam (47, 41 and 36% increase respectively), while with isoxicam the increase was 16% ($P < 0·05$). The highest concentration of naproxen, ibuprofen or piroxicam (100 μg ml^{-1}) resulted in small inhibitions of 5-HETE accumulation while isoxicam had no effect.

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Table 1. Effect of non-steroidal anti-inflammatory drugs on the accumulation of [^{14}C]cyclo-oxygenase and lipoxygenase products in incubates of rat peritoneal leucocytes.

Drug μg ml^{-1}	PGs	LTB_4	5-HETE
Isoxicam			
1	79·3 ± 4·2^{d}	105·0 ± 5·4	115·7 ± 7·9^{a}
10	37·1 ± 4·6^{d}	85·1 ± 6·4	113·3 ± 7·9
100	14·3 ± 2·3^{d}	70·2 ± 6·7^{c}	103·7 ± 10·6
Piroxicam			
1	46·6 ± 11·0^{d}	100·2 ± 8·6	136·3 ± 12·9^{b}
10	12·5 ± 1·7^{d}	77·3 ± 9·5^{a}	120·2 ± 17·2
100	7·7 ± 1·1^{d}	52·4 ± 6·2^{d}	79·6 ± 8·2^{a}
Indomethacin			
1	38·2 ± 2·9^{d}	386·1 ± 51·6^{d}	587·6 ± 47·4^{d}
10	7·7 ± 1·2^{d}	93·2 ± 27·8	100·7 ± 9·6
Naproxen			
1	48·6 ± 4·0^{d}	111·5 ± 9·0	147·4 ± 11·2^{b}
10	24·9 ± 3·1^{d}	111·3 ± 17·0	150·3 ± 8·2
100	12·7 ± 3·0^{d}	39·7 ± 4·9^{d}	73·4 ± 4·1^{b}
Ibuprofen			
1	75·0 ± 4 2^{c}	117·9 ± 3·5^{b}	140·6 ± 4·1^{c}
10	39·0 ± 2·3^{d}	106·1 ± 10·8	136·7 ± 13·6^{a}
100	12·2 ± 1·2^{d}	49·3 ± 4·5^{d}	80·2 ± 7·5

Results are expressed as % control, and analysed by Student's *t*-test for paired data. n = 17 for isoxicam, 11 for piroxicam, 6 for other drugs. *P* values; $^{a} < 0{\cdot}05$, $^{b} < 0{\cdot}02$, $^{c} < 0{\cdot}005$, $^{d} < 0{\cdot}001$.

Discussion

Rat resident peritoneal leucocytes stimulated with the calcium ionophore A23187 converted [^{14}C]arachidonic acid to prostaglandins, LTB_4 and 5-HETE, and into some other products that were not characterized. The amounts of these compounds can vary with the ratio of monocytes to polymorphonuclear cells (PMNs) since monocytes produce mainly prostaglandins (Humes et al 1977) while PMNs form substantial amounts of lipoxygenase products (Borgeat et al 1976). Perhaps changes in the cell ratios may explain the small differences in amounts of product accumulation that occurred in our two series of experiments which were performed a few months apart.

The formation of prostaglandins was greatly inhibited by all the drugs tested. However, indomethacin differed from the other drugs in the effects on 5-HETE and LTB_4 formation by rat peritoneal leucocytes. It markedly increased the formation of the lipoxygenase products, possibly by stimulating lipoxygenase activity (Siegel et al 1979), and/or by removing the inhibitory effect of prostaglandins (Malmsten 1984). A diversion of substrate metabolism from the cyclo-oxygenase pathway (Higgs et al 1980) is unlikely, since there was an excess of exogenous precursor. Piroxicam, naproxen or ibuprofen 1 μg ml^{-1}, also increased the accumulation of at least one lipoxygenase product, whereas isoxicam 1 or 10 μg ml^{-1} had only a small effect. Isoxicam was therefore the only drug that inhibited cyclo-oxygenase with comparatively little increase in the accumulation of 5-lipoxygenase products. If isoxicam acts similarly at sites of human inflammation, and if the ensuing production of lipoxygenase products is deleterious, isoxicam could have a therapeutic advantage over drugs that may increase lipoxygenase activity.

We thank the Wellcome Trust and NATO for support.

REFERENCES

Borgeat, P., Hamberg, M., Samuelsson, B. (1976) J. Biol. Chem. 251: 7816–7820

Ferreira, S. H., Nakamura, M., Castro, M. S. A. (1978) Prostaglandins 16: 31–37

Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., Smith, M. J. H. (1980) Nature 286: 264–265

Higgs, G. A., Flower, R. J., Vane, J. R. (1979) Biochem. Pharmacol. 28: 1959–1961

Higgs, G. A., Eakins, K. E., Mugridge, K. G., Moncada, S., Vane, J. R. (1980) Eur. J. Pharmacol. 6: 81–86

Humes, J. L., Bonney, R. J., Pelus, L., Dahlgren, M. E., Sadowski, S. J., Kuehl, F. A. J. R., Davies, P. (1977) Nature 269: 149–151

Malmsten, C. (1984) Prostaglandins and Leucotrienes 1984. IVth International Washington Symposium, USA Abstract 15

Samuelsson, B. (1983) Adv. Prostaglandin, Thromboxane and Leukotriene Res. 11: 1–13

Siegel, M. I., McConnel, R. T., Cuatrecasas, P. (1979) Proc. Natl. Acad. Sci., USA, 76: 3774–3778

Walker, J. R., Dawson, W. (1979) J. PHarm. Pharmacol. 31: 778–780

Williams, T. J., Jose, P. J., Wedmore, C. V., Peck, M. J., Forrest, M. J. (1983) Prostaglandin, Thromboxane and Leukotriene Res. 11: 33–37