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Inhibition of the release of slow-reacting substance of anaphylaxis by inhibitors of lipoxygenase activity

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Recent evidence has suggested that both 5-hydroxy-6-glutathionyl-eicosatetraenoic acid (leukotriene C) and its probable degradation product 5-hydroxy-6-cysteinyl-glycine eicosatetraenoic acid (leukotriene D) contribute to the biological activity hitherto referred to as slow-reacting substance of anaphylaxis (SRS–A) (Orning et al 1980; Morris et al 1980b). These compounds are believed to derive from 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) produced from the oxygenation of arachidonic acid by a lipoxygenase enzyme (Samuelsson et al 1979).

The possibility of a pivotal role for lipoxygenase activity with regard to SRS–A production has received indirect support from experimental results using non-steroidal anti-inflammatory drugs (NSAIDs). In general, these drugs enhance SRS–A release (Walker 1973; Engineer et al 1978), and it has been suggested that this effect is related to inhibition of prostaglandin (PG) biosynthesis which in turn controls SRS–A production. More recent evidence, however, with drugs such as steroids and eicosatetraynoic acid which inhibit both PG biosynthesis and SRS–A production, has suggested that the enhancement observed with NSAIDs may be due to diversion of arachidonate down a lipoxygenase mediated pathway (Burka & Flower 1979; Hitchcock 1978; Morris et al 1980a).

Benoxaprofen, a new anti-inflammatory agent, has recently been reported to inhibit the production of hydroxy-acid derivatives of arachidonic acid by rabbit PMN lipoxygenase enzymes (Walker & Dawson 1979), whilst possessing relatively low PG cyclo-oxygenase inhibitory activity (Cashin et al 1977). This communication compares the effect of benoxaprofen with those of BW755c, another lipoxygenase inhibitor (Higgs et al 1979), and with two potent PG cyclo-oxygenase inhibitors, indomethacin and piroxicam (Carty et al 1980) on the formation of both lipoxygenase products and SRS–A. The effect of nordihydro-guaiaretic acid (NDGA) a free radical scavenger and known lipoxygenase inhibitor (Hamberg 1976) was also assessed.

Inhibition of lipoxygenase activity was demonstrated using intact elicited rabbit peritoneal polymorpho-nuclear leucocytes (PMNs) as previously described (Walker & Dawson 1979). Briefly, isolated cells were exposed to calcium ionophore, A23187, in the presence of [1-^{14}C]arachidonic acid after 10 min preincubation with or without compounds at the concentrations indicated. Incubations were terminated after 5 min and the acidic lipids extracted and subjected to radio-t.l.c. and quantitation as previously described.

The release of SRS–A from guinea-pig chopped lung was assessed as described elsewhere (Dawson & Sweatman 1980). Briefly, lungs excised from previously sensitized guinea-pigs were perfused with Tyrode solution and chopped into small cubes. Samples of tissue were incubated with or without drug for 5 min before challenge with ovalbumin. Supernatants were removed after 15 min incubation at 37 °C and bio-assayed for SRS–A activity using a mepyraminized guinea-pig ileum preparation. Compounds were tested at the highest concentrations used for direct antagonism of the 'in house' guinea-pig SRS–A standard.

Both benoxaprofen and BW755c inhibited hydroxy-acid and SRS–A formation in a concentration dependent fashion. NDGA, however, while effectively inhibiting the rabbit lipoxygenase, failed to modify the production of SRS–A except at the highest concentration used. Lower concentrations of the drug resulted in non-concentration dependent potentiation (Table 1).

At concentrations higher than those required to inhibit PG biosynthesis, both indomethacin and piroxicam inhibited SRS–A release but not hydroxy-acid formation. At lower concentrations indomethacin potentiated both SRS–A and hydroxy-acid formation, whereas piroxicam did not. None of the drugs examined affected the response of the ileum to guinea-pig SRS–A standards.

The results described provide direct evidence for the involvement of a lipoxygenase pathway in the biosynthesis or release of SRS–A. Benoxaprofen appears to be a slightly more potent inhibitor on the lung system than on the PMNs (IC50 lung, 10^{-5} M; IC50 PMN, 6×10^{-5} M, Walker & Dawson 1979). BW755c, a structural analogue of phenidone, inhibits PG cyclo-oxygenase and horse platelet lipoxygenase activity at similar concentrations (Higgs et al 1979). In the present study the drug has been found to be inhibitory for both

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Table 1. Effect of lipoxygenase inhibitors, benoxaprofen, BW755c and NDGA on the production of 5-HETE, 5,12-diHETE and SRS-A 5-HETE and 5,12-diHETE produced by rabbit PMN lipoxygenase attack on [1-^{14}C] arachidonic acid in the presence of 0·5 μg ml^{-1} ionophore (A23,187). Percentage conversion of added label was 14·9 ± 1·0 to 5-HETE and 28·3 ± 2·6 to 5,12-diHETE (mean ± s.e.m.) in the challenged control. Unchallenged controls gave conversions of 2·9 ± 0·5 and 5·3 ± 0·8 (mean ± s.e.m.) to 5-HETE and 5,12-diHETE respectively. All results are combined from 3 experiments, n = at least 6. SRS-A is expressed as the mean % change from control challenged guinea-pig lung from 2 replicate experiments.

Drug concn (M)	% change from control 5-HETE	5,12-diHETE	SRS-A
Benoxaprofen			
6×10^{-5}	31·3↓**	48·4↓**	64·3↓**
3×10^{-5}	15·1↓	10·1↓	62·8↓**
10^{-5}	4·2↓	3·3↑	56·1↓**
6×10^{-6}	ND		25·8↓*
BW755c			
$1·5 \times 10^{-4}$	91·7↓**	98·0↓**	61·5↓**
10^{-4}	46·1↓**	50·0↓**	47·5↓**
5×10^{-5}	24·2↓*	3·0↓	33·5↓*
$1·5 \times 10^{-5}$	3·0↑	13·8↑	14·0↓
NDGA			
3×10^{-5}	100·0↓**	100·0↓**	47·5↓**
10^{-5}	100·0↓**	100·0↓**	10·0↑
3×10^{-6}	42·6↓**	50·5↓**	62·0↑**
10^{-6}	23·0↓*	16·5↓*	50·0↑**
3×10^{-7}	0	6·5↓	ND

*$P < 0·05$, ** $P < 0·005$. ND = Not determined.

SRS-A and hydroxy-acid production at similar concentrations (IC50 10^{-4} M). The lack of effect of NDGA on SRS-A production compared to its activity on the rabbit PMN enzyme may be due to poor access to the lung enzymes or may be of some, as yet unclear, significance. Other workers have reported the need for high concentrations of drug to inhibit lung lipoxygenase activity (Hamberg 1976; Morris et al 1980b).

The inhibition of SRS-A production by the two cyclo-oxygenase inhibitors was only observed at much higher concentrations than those achieved therapeutically or required for inhibition of PG biosynthesis. The expected potentiation observed with lower concentrations of indomethacin was not great but was in agreement with levels of potentiation reported in the literature (Burka & Flower 1979; Hitchcock 1978; Morris et al 1980a). The non-dose related potentiation of product formation in both test systems may reflect an independence of lipoxygenase activity and substrate concentration.

The reduction of SRS-A release observed at the highest concentration of indomethacin and piroxicam does not appear to be related to lipoxygenase inhibition and is presumably due to some other action of the compounds, or may reflect some toxicity.

Table 2. Effect of PG cyclo-oxygenase inhibitors, indomethacin and piroxicam on the production of 5-HETE, 5,12-diHETE and SRS-A.

Drug concn (M)	% change from control 5-HETE	5,12-diHETE	SRS-A
Indomethacin			
10^{-4}	24·1↑	20·0↑	36·0↓**
3×10^{-5}	16·7↑	24·0↑	4·0↓
10^{-5}	25·0↑*	16·0↑	63·0↑**
3×10^{-6}	28·3↑*	25·0↑	35·5↑*
10^{-6}	11·6↑	28·0↑*	
Piroxicam			
10^{-4}	6·7↓	5·0↑	48·0↓**
2×10^{-5}	5·0↑	3·1↓	16·5↑
10^{-5}	6·7↑	21·8↑	4·0↑

Results as described for Table 1.
*$P < 0·05$, ** $P < 0·005$.

The discrepancies noted above may well reflect the lack of knowledge concerning the basic mechanism of action of the NSAIDS. However, the results support the the suggestion that drugs which inhibit lipoxygenase activity reduce the production or release of SRS-A on immunological challenge.

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