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Effects of a 5-lipoxygenase inhibitor, REV-5901, on leukotriene and histamine release from human lung tissue in-vitro

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REV-5901, α-pentyl-3-(2-quinolinylmethoxy)benzene-methanol, is an arylmethylphenyl ether derivative which inhibits 5-lipoxygenase activity of leukocytes. Its effects on the release of leukotrienes, induced by antigen and calcium ionophore, from human lung tissue in-vitro have been examined. At 1 and 10 μM it significantly inhibited the release of leukotrienes induced by both stimuli. At 10 μM it also inhibited antigen-induced histamine release. These results suggest that REV-5901 may be useful in clinical disorders such as asthma in which leukotriene release may be involved.

Metabolites of arachidonic acid, produced via the 5-lipoxygenase pathway possess a wide range of biological actions which suggest that they may be involved in immediate hypersensitivity and inflammatory reponses (Lewis & Austen 1981). Considerable evidence has accumulated to implicate leukotrienes in the pathophysiology of various lung diseases including asthma (O'Driscoll & Kay 1982). In particular, the sulphidopeptide leukotrienes (LTs), leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) are potent bronchoconstrictors in man (Barnes et al 1984). Furthermore, these LTs induce airway hyper-responsiveness, which is a fundamental abnormality in asthma (Boushey et al 1980), in normal humans (Barnes et al 1984).

One approach to developing a better treatment for asthma has been to synthesize new compounds which inhibit the 5-lipoxygenase pathway. REV-5901, α-pentyl-3-(2-quinolinylmethoxy)benzene-methanol is an arylmethylphenyl ether that inhibits 5-lipoxygenases in rat and human leukocytes in-vitro with respective EC50 values of 0·16 and 5 μM (Coutts et al 1985). Furthermore, it demonstrates selectivity for 5-lipoxygenases, being inactive against 12-lipoxygenase from rat platelets and cyclooxygenase from sheep seminal vesicles in concentrations of 100 and 200 μM, respectively (Coutts et al 1985).

The present experiments were designed to determine the effects of REV-5901 on the release in-vitro of leukotriene and histamine from human lung tissue, induced by immunological and non-immunological stimuli.

Materials and methods

Human lung tissue was obtained from operative specimens resected from patients with carcinoma of the lung after approval had been obtained from the Human Ethics Review Committee of the University of Sydney. Lung fragments were prepared, sensitized and challenged as described previously (Armour et al 1981). Briefly, the tissue was washed free of blood, cut into small fragments (approximately 3 mm^3) then incubated overnight in serum with a high titre of specific immunoglobulin E (IgE) to *Dermatophagoides pteronyssinus*. The following day, the fragments were repeatedly washed in warm Tyrode's solution, divided into repli-

* Correspondence.

cates (approximately 250 mg wet weight) resuspended in Tyrode's solution then challenged with a freeze-dried extract of *D. pteronyssinus* (200 Noon units mL^{-1}) at 37 °C. After 15 min the reaction was terminated by plunging the incubation tubes into ice.

For challenge with the calcium ionophore (A23187) the fragments did not require overnight sensitization with IgE (see Armour et al 1981). Replicates were resuspended in Tyrode's solution to which A23187 (9·6 μM final concentration) or the vehicle (1% ethanol) was added and the incubation was terminated after 45 min.

The release of leukotrienes into the supernatant fluid was determined by bioassay using longitudinal strips of guinea-pig ileum (see Armour et al 1981). The responses of the assay tissues were calibrated with synthetic LTD_4 so that the net release of leukotrienes was expressed as picomole equivalents of LTD_4. In some experiments small volumes of supernatant were stored at −70 °C for subsequent determination of leukotriene content by radioimmunoassay (RIA) using New England Nuclear Kit for LTC_4. Samples were read from the linear portion of the standard curve from 0·16–2·56 pmol mL^{-1}. Histamine was assayed by an automated fluorimetric method (see Armour et al 1981) and the histamine released into the supernatant was expressed as a percentage of the original tissue histamine.

REV-5901 was dissolved initially in 50% ethanol in 0·9% NaCl to prepare a stock solution 1 mM from which further dilutions were made using 0·9% NaCl such that the final concentration of ethanol was not greater than 0·5%. This concentration of ethanol had no inhibitory effect on mediator release. To determine the effects of REV-5901, lung fragments were pre-incubated for 15 min at 37 °C with various concentrations of the drug before addition of antigen or A23187.

The effect of each concentration of REV-5901 on the release of leukotrienes and histamine was expressed as a percentage of control release in the absence of any drug and the significance of any difference from control release was determined by using Student's *t*-test.

Results

In ten lung specimens, the antigen-induced release of leukotrienes was 1·13 ± 1·08 pmol equivalents LTD_4 mL^{-1} (mean ± s.e.m.) and the percentage histamine release was 28·5 ± 2·7. REV-5901 (1 and 10 μM) caused a dose-related inhibition of leukotriene release, determined by bioassay (Fig. 1). The antigen-induced release of histamine was also significantly inhibited by the higher concentration (10 μM) of REV-5901 (Fig. 1).

In nine human lung specimens, the A23187-induced release of leukotrienes was 3·2 ± 1·2 pmol equivalents LTD_4 mL^{-1} and the percentage histamine release was 32·1 ± 4·6. REV-5901 (1 and 10 μM) inhibited leukotriene release in a dose-related manner without affecting histamine release (Fig. 1). These concentrations of

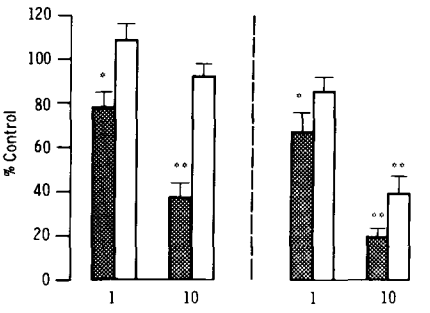

FIG. 1. Effects of REV-5901 (1 and 10 μM) on the release of leukotriene (shaded columns) and histamine (open columns) from human lung fragments challenged with A23187 (left panel, n = 9) and antigen (right panel, n = 10). Vertical bars denote s.e.m. $^*P < 0·02$, $^{**}P < 0·001$ compared with control release.

Table 1. Effects of REV-5901 (1 and 10 μM) on antigen-induced release of leukotrienes (expressed as % control), determined by bioassay and RIA.

	1 μM		10 μM	
	Bioassay	RIA	Bioassay	RIA
Mean	67·3	81·8	19·5	24·7
s.e.m.	8·6	19·9	3·4	8·2
n	10	7	10	7

REV-5901 did not affect the responses of the bioassay tissues to synthetic LTD_4.

In experiments where lung supernatant was also submitted to RIA, the mean value for leukotriene release was 10·2 ± 3·8 pmol LTC_4 mL^{-1} (n = 9). There was a significant correlation between the values obtained by bioassay and by RIA ($r = 0·77$, $P < 0·02$). The results with RIA also showed a dose-related inhibition of leukotriene release by REV-5901 (1 and 10 μM) similar to that which was demonstrated with bioassay (Table 1). There was a significant correlation between the determination of leukotriene inhibition by bioassay and by RIA ($r = 0·79$, n = 18, $P < 0·001$).

Discussion

The present experiments have shown that the 5-lipoxygenase inhibitor REV-5901, at concentrations of 1 and 10 μM caused a dose-related inhibition of leukotriene release from human lung tissue in-vitro, following activation with either immunological (*D. pteronyssinus*) or non-immunological (calcium ionophore) stimuli. Reduced release of leukotrienes in the presence of REV-5901 was shown with both bioassay and RIA and there was significant correlation between the values obtained with the two methods, a finding reported by others (Aharony et al 1983). The bioassay system measures the net contractile activity of the sulphidopeptide leukotrienes (LTC_4, LTD_4 and LTE_4). LTB_4 is not detected because it does not contract strips of guinea-

pig ileum (Ford-Hutchinson et al 1982). Since the RIA depends upon an antibody to LTC_4 with 55, 8 and 0·01% cross reactivity with LTD_4, LTE_4 and LTB_4 respectively, it will also be a measure of net sulphidopeptide leukotriene release. Thus, the inhibitory activity of REV-5901 against 5-lipoxygenases, which has previously been found in rat and human leucocytes (Coutts et al 1985) also occurs in human lung tissue. This in-vitro evidence of 5-lipoxygenase inhibition suggests that REV-5901 may be therapeutically useful in asthma provoked by antigen and stimuli such as exercise and cold air which are thought to release chemical mediators (Lee et al 1982).

Whereas REV-5901, at the higher concentration, inhibited antigen-induced histamine release, it did not significantly affect histamine release induced by A23187. Similar inhibitory activity against immunological histamine release has been demonstrated in experiments with human basophils (Coutts et al 1985). This differential effect on histamine release in human lung tissue confirms that there are different mechanisms underlying activation by antigen and by calcium ionophore as previously suggested (Lichtenstein 1975). Evidence supporting this notion was obtained in previous studies with human lung which showed that β-adrenoceptor agonists inhibited antigen, but not A23187-induced histamine release, implying that ionophore stimulation is not susceptible to modulation by cyclic AMP (Hughes et al 1983).

The mechanism by which a 5-lipoxygenase inhibitor could suppress not only leukotriene release but also histamine release requires further investigation. In human basophils, it is evident that 5-lipoxygenase products facilitate immunological histamine release (Peters et al 1981) but experiments have failed to demonstrate a similar mechanism in human lung tissue (Vardey et al 1984).

Irrespective of the cellular mechanisms responsible, it is likely that a drug which inhibits the release of both leukotrienes and histamine may be a useful anti-allergic drug. However, caution should be exercised in attributing efficacy in clinical studies solely to inhibition of 5-lipoxygenase products because inhibition of histamine release may also contribute to any beneficial effects observed.

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