Effect of benoxaprofen on release of slow-reacting substances from human lung tissue in vitro

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Summary

1. Macroscopically normal human lung tissue was obtained from operative specimens removed for lung cancer and challenged with antigen or calcium ionophore. The release of histamine and slow-reacting substances was measured by fluorimetric and bioassay techniques respectively.

2. Benoxaprofen, a drug with inhibitory effects on the lipoxygenase and cyclo-oxygenase pathways, caused a dose-related reduction of release of slow-reacting substances without affecting histamine release.

3. These results with human lung tissue in vitro suggest that benoxaprofen may be used to investigate the role of slow-reacting substances in experimental and clinical asthma.

Key words: benoxaprofen, lipoxygenase inhibitor, human lung, slow-reacting substances.

Abbreviation: SRS, slow-reacting substance.

Introduction

For many years, slow-reacting substance of anaphylaxis (SRS-A) has been regarded as a putative mediator of allergen-provoked asthma because it is released from human lung tissue in vitro after antigen challenge [1] and it causes contraction of isolated preparations of human bronchi [2, 3] and human peripheral lung [4]. Furthermore, the increased vascular permeability induced by SRS-A [5] may contribute to the mucosal oedema which is an important feature of clinical asthma [6]. Since a slow-reacting substance (SRS) which is indistinguishable from SRS-A is released from human lung tissue after non-immunological stimulation with the calcium ionophore A23187, it is possible that these substances may be involved in non-allergic asthma [7].

The leukotrienes, which are lipoxygenase products of arachidonic acid, have been identified recently as the active moieties in SRS-A and SRS [8, 9]. Therefore, drugs which inhibit the lipoxygenase pathway may reduce leukotriene release from the lungs. Since benoxaprofen has been found to inhibit lipoxygenase and cyclo-oxygenase activity in rabbit polymorphonuclear leucocytes and in guinea-pig lung tissue [10], the present experiments were conducted to determine the effects of benoxaprofen in human lung tissue.

Materials and methods

The technique for preparation, sensitization and challenge of human lung fragments is described elsewhere [11]. Briefly, macroscopically normal chopped lung tissue was incubated overnight at room temperature in human serum. Lung for challenge with A23187 was incubated in serum from normal subjects and serum with a high titre of immunoglobulin E to Dermatophagoides pteronyssinus (determined by the radioallergosorbent test) was used for passive sensitization of the lung for antigen challenge. The next day, washed lung fragments (approximately 250 mg replicates) were challenged either with A23187 (final concentration 5 μg/ml; 9.6 × 10⁻⁶ mol/l) or D. pteronyssinus extract (final concentration 200 Noon units/ml). After incubation at 37°C for 45 min (A23187) or 15 min (antigen) the
release of SRS and SRS-A into the supernatant was determined by bioassay with guinea-pig ileal strips and compared with a laboratory standard of partially purified SRS-A [11]. Preliminary experiments were optimal for the release of histamine, SRS and SRS-A. The laboratory standard was calibrated by bioassay with chemically synthesized leukotriene E₄ (LTE₄) and 1 unit was equivalent to $1.3 \times 10^{-10}$ mol of LTE₄. The histamine content of the supernatant was measured by a modified fluorimetric method [7] and expressed as a percentage of the original tissue content after correction for spontaneous release. The composition of Tyrode solution was as follows (mmol/l): NaCl, 136.8; KCl, 2.7; MgSO₄, 1.1; NaH₂PO₄, 0.5; CaCl₂, 1.8; NaHCO₃, 11.9; glucose, 5.3.

To determine the effects of benoxaprofen, lung fragments were pre-incubated at 37°C with dilutions of benoxaprofen (final concentrations $10^{-6}-10^{-4}$ mol/l) in sodium chloride solution (154 mmol/l) for 15 min before addition of antigen or ionophore. In each experiment the release of SRS or SRS-A and histamine in the presence of benoxaprofen was compared with control release from lung fragments challenged with A23187 or antigen and results were expressed as a percentage of these control values.

**Results**

In experiments with antigen challenge of lung tissue obtained from six patients, the release of SRS-A ranged from 0.34 to 4.12 unit/dm³ and the mean release of histamine was 40.9% (SEM 5.5; n = 6) of original tissue content. With calcium ionophore, the release of SRS ranged from 1.1 to 2.0 units/ml and the mean release of histamine was 47.1% (SEM 7.6; n = 5). The contractile effects of SRS and SRS-A on guinea-pig ileum were abolished by FPL55712 [12] in a concentration of $5 \times 10^{-6}$ mol/l.

The effects of benoxaprofen on antigen-induced and ionophore-induced release of mediators are shown in Fig. 1. In concentrations of $10^{-6}-10^{-4}$ mol/l, benoxaprofen caused a dose-related statistically significant inhibition of SRS-A release without significantly altering histamine release. Benoxaprofen did not antagonize the action of the laboratory standard of SRS-A on guinea-pig ileum and A23187 (9.6 $\times$ $10^{-6}$ mol/l) did not contract the bioassay tissues.

**Discussion**

The present experiments have shown that benoxaprofen, an inhibitor of the lipoxygenase pathway [10] caused a dose-related inhibition of SRS and SRS-A release from human lung tissue in vitro. Since the leukotrienes, which are lipoxygenase products of arachidonic acid [13], have been identified as the constituents of SRS [9] and SRS-A [8] these results indicate that benoxaprofen inhibits the lipoxygenase pathway in human lung tissue. Benoxaprofen at concentrations of $3 \times 10^{-5}$ mol/l reduced prostaglandin synthesis in enzyme preparations.
of ram seminal vesicles [14]. However, if this inhibitory effect on cyclo-oxygenase activity occurred in human lung tissue it would not account for the reduction in SRS-A release, since selective cyclo-oxygenase inhibition with indomethacin enhances the release of SRS-A [15]. Our findings are supported by the results of preliminary experiments from another laboratory reporting that one concentration of benoxaprofen (3 x 10^{-5} mol/l) caused an average inhibition of 58% of control SRS-A release in two lung specimens [16].

In normal subjects, the plasma concentrations of benoxaprofen exceeded 10^{-4} mol/l for more than 15 h after oral ingestion of 600 mg tablets [17], indicating that concentrations of benoxaprofen which caused significant inhibition of the lipoygenase pathway in vitro may be achieved in vivo.

Other drugs currently available for the treatment of asthma, such as β-adrenoceptor agonists, theophylline and cromoglycate, inhibit the release of both SRS-A and histamine [18, 19]. In contrast, benoxaprofen has a selective inhibitory effect on SRS-A release without reducing histamine release. These findings in vitro suggest that benoxaprofen may be used to investigate the role of SRS-A in experimental and clinical asthma.

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References