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The late reaction following bronchial provocation with house dust mite allergen. Dependence on arachidonic acid metabolism

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SUMMARY

The involvement of arachidonic acid metabolism in early and late bronchial reactions has been studied in four asthmatic subjects sensitive to *Dermatophagoides pteronyssinus*. Pre-treatment with either indomethacin (an inhibitor of the cyclo-oxygenase pathway) or benoxaprofen (an inhibitor of both cyclo-oxygenase and lipoxygenase pathways) failed to affect the amplitude, but did produce some foreshortening of the early response to allergen. If benoxaprofen is an effective inhibitor of SRS-A formation *in vivo*, then these observations question the role of SRS-A as a spasmogen in allergen-induced bronchospasm. Both drugs were effective inhibitors of the late reaction, implying involvement of cyclo-oxygenase products (endoperoxides, prostaglandins or thromboxanes) in the genesis of a late response to allergen.

INTRODUCTION

Inhalation of house dust mite (HDM) allergen by extrinsic asthmatic subjects commonly results in a biphasic response, with both early and late airways obstruction (McAllen, Assem & Maunsell, 1970; Warner, 1976). It is generally assumed that the acute response can be attributed to release, or generation, of autocoids, following IgE-mediated mast cell degranulation. Challenge studies using sensitized chopped lung, or isolated cell populations, have demonstrated release of a range of biologically active compounds, including histamine (Engineer *et al.*, 1978); prostaglandins, PGE2 and PGF2α (Piper & Walker, 1973); PGI2, PGD2 and thromboxanes (TxA2) (Lewis *et al.*, 1980; Schulman *et al.*, 1981); leukotrienes, LTB4, LTC4 and LTD4 (Morris *et al.*, 1980; Lewis & Austen, 1981); and PAF-acether (Bogart & Stechschulte, 1974; Knauer *et al.*, 1981). In addition to these chemically defined materials, chemotactic factors have also been detected (Atkins *et al.*, 1977).

There is no consensus as to the mechanism underlying the late onset airways obstruction to allergen. This phenomenon has been attributed variously to: immune complex deposition (Pepys & Hutchcroft, 1975); generation of high molecular weight chemotactic factors (Atkins *et al.*, 1977; Nagy, Lee & Kay, 1982); cutaneous basophil anaphylaxis (Askenase, 1977) or activation of the coagulation cascade (de Shazo *et al.*, 1979; Dahl & Venge, 1981). Alternatively, it has been proposed that unspecified mast cell products generated via either IgE (Solley *et al.*, 1976; Tannenbaum *et al.*, 1980) or IgG4 antibodies (Gwynn *et al.*, 1982) are able to produce a biphasic reaction.

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Increased arachidonic acid metabolism with production of prostaglandins, thromboxanes and leukotrienes is a feature of allergic reactions, so that it is reasonable to consider that such materials may participate in both early and late phases of allergen-induced airflow obstruction. *In vitro*, prostaglandins, thromboxane A2 and leukotrienes are potent respiratory smooth muscle spasmogens that also have inflammatory actions on lung tissue. The production of these substances via cyclo-oxygenase and lipoxygenase pathways can be inhibited by indomethacin and benoxaprofen. We have used these drugs to examine the involvement of these metabolites in allergen-induced bronchospasm of asthmatic subjects.

MATERIALS AND METHODS

A group of mild, extrinsic asthmatic subjects who gave a history of wheezing on exposure to house dust and a negative history of aspirin sensitivity were selected. All subjects gave a positive skin prick test for *Dermatophagoides pteronyssinus* extract (Bencard) and a biphasic reaction to bronchial provocation with house dust mite allergen. Bronchodilator and anti-asthma drugs were discontinued for at least 48 h prior to bronchial challenge. All subjects had a forced expiratory volume in 1 s (FEV 1) before allergen inhalation that was at least 70% of the predicted value. Informed consent was obtained from each subject and the study was approved by the Hospital Ethics Committee.

Bronchial provocation technique. The bronchial provocation tests were carefully standardized according to the method of Robertson *et al.* (1974). After an initial control inhalation of normal saline, house dust mite allergen (Bencard SDV No. 3 solution, 3·3 mg/ml) in saline was administered via a Wright's nebulizer containing 5 ml of solution, fitted to an AMBU face mask and driven by compressed air at 7 l/min. Identical conditions were used on each occasion and the nebulizer output was checked by periodic weighing before and after use. Bronchial provocation was performed at the same time of day on each occasion and the tests were separated by intervals of at least 1 week. Allergen was administered during tidal breathing for periods of 1–2 min. On the first visit, a threshold dose was determined by increasing the allergen concentration by five-fold increments until a fall of at least 20% in the FEV 1 value during the early phase of bronchospasm was recorded. The lowest concentration of allergen was that concentration which produced a skin wheal, on prick testing, of less than 3 mm diameter.

Drug treatment. After the threshold dose of allergen had been determined, bronchial provocation tests were repeated on four subsequent occasions, following pretreatment with either indomethacin (25 mg orally, 1 h before allergen), benoxaprofen (600 mg orally, 11 h before allergen), both drugs at the same doses, or placebo. This design necessitated placebos both for benoxaprofen and for indomethacin. Treatments were single blind and were allocated according to a four by four Latin Square. The allergen dose for each patient was kept constant, as was the nebulizer output and inhalation period on each subsequent occasion. Baseline FEV 1 values were measured using a dry bellows spirometer (Vitalograph) taking the maximal of three successive estimates. Subjects were only studied if basal FEV 1 values were within 15% of previous observations for that subject. Measurements were made at 10 min intervals over 40 min prior to allergen inhalation; then at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min, with hourly recording for up to 8 h after allergen provocation. Blood was taken for determination of plasma concentrations of indomethacin and benoxaprofen at the time of allergen administration and also during late reactions. The plasma was separated immediately and stored at −20°C until assayed for benoxaprofen (Chatfield & Woodage, 1978) and indomethacin (Skellern & Salole, 1975).

Analysis of results. For statistical analysis, three phases of the response have been studied; firstly the fall in FEV 1 during the early reaction; secondly the average FEV1 during the recovery phase between (2–4 h); and thirdly the average FEV 1 during the late reaction (5–7 h). These results were evaluated, using a one way analysis of variance by ranks and the Wilcoxon test for pairs. As an index of both severity and duration of asthma, a cumulative index has been adopted. Measurement of the area under the curve that depicts the reduction of the FEV 1 values below mean pre-challenge FEV 1, gives the cumulative reduction in lung function in litre hours (Fig. 1). The early reaction was measured over the first hour and the late reaction over the period 3–7 h taking the pre-allergen FEV

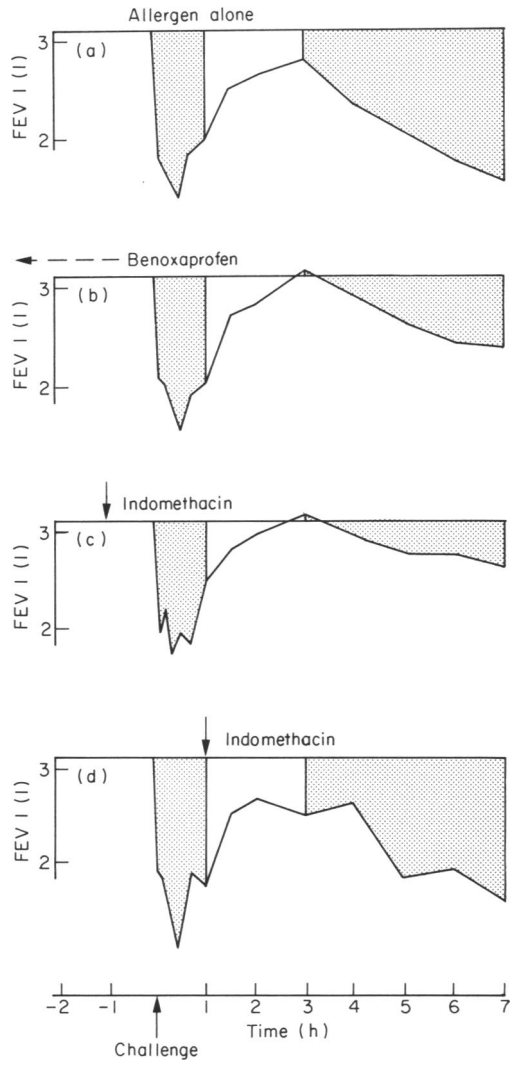

Fig. 1. Bronchial responses to house dust mite allergen following: (a) placebo treatment, (b) benoxaprofen, (c) indomethacin and (d) indomethacin, 1 h after challenge. Shaded areas (0–1 h; 3–7 h) depict the cumulative reduction in FEV 1 expressed in litre hours, relative to the mean pre-challenge FEV 1 value depicted as a solid horizontal line on each graph.

1 values as the baseline for calculation. By comparing the drug treatment data with the placebo pre-treatment challenges, a percentage inhibition for each drug was calculated for both early and late phases of bronchospasm.

RESULTS

Bronchial provocation tests were undertaken on four subjects (three male and one female) aged 17–36 years (mean age: 25·3 years). All except one had chronic asthma from childhood. Two had been taking bronchodilators only, one had been treated with disodium cromoglycate and one had required inhaled beclomethasone for control of asthma. All these drugs were stopped at least 2 days before bronchial provocation. All subjects gave positive skin tests to house dust mite (mean wheal

Table 1. Percentage inhibition of allergen-induced bronchospasm*

Treatment (*n*)	Early reaction (0–1 h)	Late reaction (3–7 h)
Indomethacin (4)	33·3 ± 25·4	90·8 ± 27·5
Benoxaprofen (4)	12·5 ± 45·6	80·5 ± 47·1
Indomethacin + Benoxaprofen (2)	39·5 ± 27·5	71·5 ± 11·5

* Inhibition by drug treatment calculated from the product of intensity and duration of bronchospasm, during the specified period, as a percentage of product of intensity and duration of bronchospasm observed following placebo treatment.

Table 2. The effect of indomethacin and benoxaprofen on bronchial response to house dust mite

	Mean FEV 1 values (litres) $n = 4$							
Placebo:	Control	†	Recovery	*	Early reaction	†	Late reaction
	3·06		2·72		2·46		2·10
Indomethacin:	Recovery		Control		Late reaction	†	Early reaction
	2·75		2·69		2·58		2·33
Benoxaprofen:	Recovery		Control		Late reaction	†	Early reaction
	2·71		2·69		2·68		2·32

Comparison between adjacent columns indicates significance at 1% (*) and 0·1% (†) levels.

diameter 10 mm, range, 7–14 mm). In addition, all gave positive skin prick tests to other common allergens. None were cigarette smokers.

Fig. 1a shows a typical recording of FEV 1 during bronchial provocation. The tests proved very reproducible for each subject and exhibited similar intensity of early bronchospasm on each occasion. Benoxaprofen (Fig. 1b) and indomethacin (Fig. 1c) had no noteworthy effect on the amplitude of the fall in FEV 1 during early bronchospasm. Some foreshortening of the response was evident when the response was assessed by reference to its intensity/duration product (area under the curve) for the first hour. This accounts for the weak inhibition detected during the early reaction (Table 1). The most striking finding was marked inhibition of the late reaction, in terms of the amplitude of airways obstruction. The time course of the late reaction appeared unaltered, although recordings were not continued beyond 9 h after bronchial provocation. Combination of benoxaprofen with indomethacin proved no more effective than either drug alone.

Plasma levels of the drugs at the time of allergen inhalation were 44·0–65·3 μg/ml (mean 51·3 μg/ml) for benoxaprofen and 0·9–5·4 μg/ml (mean 3·3 μg/ml) for indomethacin. Blood taken during the late reaction, 6 h after allergen inhalation, gave levels of 47–49 μg/ml (mean 48 μg/ml) for benoxaprofen and 2·0–4·5 μg/ml (mean 3·3 μg/ml) for indomethacin.

In one subject, bronchial challenge was repeated on two subsequent occasions when benoxaprofen or indomethacin was given orally, after the early phase of bronchospasm had become established. In both instances, the late reaction was unaffected by drug treatment (Fig. 1d), even though drug levels in plasma collected at the onset of the late response indicated adequate absorption (44 μg/ml for benoxaprofen and 1·1 μg/ml for indomethacin).

Table 2 shows the mean FEV 1 values arranged in rank order for each drug pre-treatment. With placebo plus indomethacin and placebo plus benoxaprofen pre-treatment, the mean pre-challenge

FEV1 was significantly higher than the early reaction values, the 2–4 h recovery values and the late reaction. With indomethacin and/or benoxaprofen, the FEV 1 recovery values were not significantly lower than the pre-treatment values of FEV 1. However, each drug produced a pronounced inhibition of the late reaction, such that there was no significant difference between FEV 1 during the pre-treatment phase and during the period assigned to the late reaction. This contrasts with the significant difference between pre-treatment FEV 1 values and values recorded during the early phase of bronchospasm.

DISCUSSION

Neither indomethacin nor benoxaprofen inhibited the early bronchoconstrictor response to house dust mite inhalation in sensitized individuals. The lack of effect of indomethacin confirms the observations previously described by Smith (1975). These observations suggest that the cyclo-oxygenase products, prostaglandins and thromboxanes, although known to be released *in vitro*, have little action as airway smooth muscle spasmogens in the early response to allergen. The lack of efficacy of benoxaprofen questions also the importance of leukotrienes as spasmogens in the early phase of the response, since benoxaprofen, at therapeutic concentrations, inhibits allergen-induced SRS-A generation from animal and human chopped lung (Boot *et al.*, 1982).

On the other hand, therapeutic concentrations of either indomethacin or benoxaprofen, alone or together, inhibited the late response to inhalation of house dust mite. The inhibition was substantial and was observed on each of the 10 occasions that these non-steroidal anti-inflammatory drugs were administered prior to allergen challenge. Rank sum analysis revealed a highly significant difference between late responses following placebo and following non-steroidal anti-inflammatory drug pre-treatment; discriminant analysis confirmed that these responses could be assigned into two groups. Although the present study was limited to four patients in whom the effects of four treatments, placebo, indomethacin, benoxaprofen and a combination of indomethacin and benoxaprofen were compared; Joubert & Viljoen, (1982) have also reported that indomethacin inhibited the late onset bronchial response in 11 of 13 asthmatics who showed delayed responses to allergen inhalation. It is likely that the inhibitory effect of these non-steroidal anti-inflammatory drugs on the late bronchospasm is due to cyclo-oxygenase inhibition, as this is a property common to both indomethacin (Shen, 1979) and benoxaprofen (Dawson, 1980). On those two occasions when non-steroidal anti-inflammatory drugs were administered after expression of the early response, indomethacin and benoxaprofen failed to inhibit the late response, even though both drugs were present in concentrations sufficient to inhibit cyclo-oxygenase activity at the time of onset of the late response. Such observations imply that a transient phase of arachidonic acid metabolism at, or soon after, inhalation of allergen is mandatory for expression of a delayed onset response.

The delayed component of allergen-induced bronchospasm has long attracted clinical interest, for it may be followed by persistent hyperreactivity of the airways, reminiscent of clinical asthma (Cockcroft *et al.*, 1977). Hitherto, only glucocorticosteroids and disodium cromoglycate like drugs have been shown to inhibit the late response (Booij-Noord, Orie & de Vries, 1971); non-steroidal anti-inflammatory drugs can now be included in this category. Although the present study has been concerned with the effect of non-steroidal anti-inflammatory drugs on allergic responses of human lung, analogous observations have been reported for other allergic responses. Thus, in the skin, indomethacin inhibited the erythematous but not the oedematous component of the late skin reactions to injected allergen, an effect which is likely to be mediated by prostaglandins (Dorsch & Baur, 1980). In the gut, non-steroidal anti-inflammatory drugs have been reported to be dramatically effective in certain individuals with food allergy (Youlten, 1980), raising the possibility that, in these situations, a transient phase of cyclo-oxygenase activity may also be pre-requisite for a clinically evident allergic response.

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