Inhalation challenge with specific grass pollen antigens in asthmatics and the effect of lodoxamide tromethamine

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ABSTRACT Highly purified species-specific grass pollen antigens were used for inhalation bronchial challenge in 12 patients with atopic asthma. This was found to result in reproducible experimental asthma. Lodoxamide tromethamine was administered by inhalation in two different doses, 0·01 mg and 0·1 mg, to each patient, in double-blind, random, placebo-controlled manner, 15 minutes before pollen inhalation. It was found to have a significant protective action at both doses (p < 0·05), but it produced troublesome side effects at the higher dose of 0·1 mg.

Inhibition of the release of mediators from mast and basophil cells is an attractive therapeutic concept. These cells lie in the bronchial lumen and submucosa and release of their preformed mediators induced by inhalation of specific allergen is a probable early stage in the development of allergen-induces bronchoconstriction.

Sodium cromoglycate was reported in 1967 to inhibit experimental asthma. Its mechanism of action is still unclear but it is thought to include a mast-cell-stabilising effect with inhibition of mediator release.

Lodoxamide tromethamine, a dioxamic acid derivative chemically unrelated to sodium cromoglycate, is a new drug which appears to act on the mast cell. It is known to be more potent than sodium cromoglycate in inhibiting passive cutaneous anaphylaxis in the rat and to prevent experimental bronchoconstriction in sensitised Rhesus monkeys.

Bronchial challenge testing with allergens has been found difficult to perform in a reproducible manner. Although this has partly been due to technical problems, the lack of allergens standardised in terms of biological activity has been a serious deficiency. We have chosen the highly standardised grass pollen antigens (Spectralgen, Pharmacia) for use in challenge studies to control this variable.

The present study was carried out to determine whether lodoxamide has any effect on the development of bronchoconstriction in experimental allergic asthma.

Methods
Sixteen adult asthmatic patients were admitted to the study and all gave informed consent. Thirteen were men, with ages ranging from 19 to 44 years (mean 25·5 years). None was a smoker and all had mild asthma, of at least five years' duration, which had never led to admission to hospital. All were atopic as shown by multiple positive responses to skin prick tests to a variety of common allergens. In addition, all 16 had seasonal hay fever and pollen asthma. All took regular salbutamol and beclomethasone inhalations and four also took sodium cromoglycate by Spinhaler. The subjects did not deviate by more than 15% from their ideal weight and had no serious coexisting disease. The study took place out of the grass pollen season.

Species-specific skin prick tests with pollens of *Lolium perenne* (rye grass), *Phleum pratense* (Timothy grass), *Secale cereale* (cultivated rye), and *Holcus lanatus* (velvet grass or Yorkshire fog grass) were performed, and the species producing the largest wheal response was used for that particular patient throughout the study. In 14 of the subjects this proved to be Timothy grass, and in the other two it was velvet grass. Specific IgE was demonstrated in serum in every subject by the radioallergosorbent test (RAST).

To establish a safe initial dose of antigen for bronchial inhalation challenge, skin prick tests were repeated with increasing dilutions of the appropriate pollen extract. The concentrations used ranged from 1 BU/ml to 10 000 BU/ml (1000 BU/ml gives the same average response as 0·1% histamine chloride in skin prick testing of allergic patients); 10 000
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BU/ml is the recommended concentration for routine skin prick tests. The starting concentration of inhalant was the highest concentration producing no more than a 2-mm wheal response.

Patients took no medication in the 24 hours before each of the inhalation challenges. Pollen extracts were diluted in saline and serum albumin diluent (Pharmacia) and were delivered in nebulised form with a Wright nebuliser driven by compressed air at a pressure of 20 lb/in² (138 kPa). This generates droplets that are all smaller than 10 μm. The standard inhalation technique consisted of five slow breaths from functional residual capacity to total lung capacity.

Each patient attended on four occasions, each separated by at least seven days. On each of these occasions baseline measurements of forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and peak expiratory flow rate (PEFR) were made. If the subject deviated by more than 15% from his predicted normal no further study took place on that day.

The first or diagnostic visit was intended to establish whether the patient developed bronchoconstriction after inhalation of the appropriate pollen extract. After the baseline measurements of FEV₁, FVC, PEFR, pulse rate, and blood pressure, the subject took five inhalations of the diluent control solution and the measurements were repeated after 10 minutes. If the FEV₁ did not fall by more than 10% pollen challenge was performed. Five breaths of the nebulised starting concentration extract were taken and the pulmonary function measurements were repeated after 10 minutes. Provided that the FEV₁ had not fallen by more than 20% from the post-diluent control value the next highest concentration of pollen extract was given, and pulmonary function tests were performed after 10 minutes as before. This procedure was continued until the FEV₁ had fallen by 20% or more, but in no case was the concentration of 10 000 BU/ml exceeded. At this point the inhalations were stopped and bronchoconstriction was reversed with inhaled salbutamol, 200 μg.

When it was established that the subject had bronchial sensitivity to the pollen, he was admitted to the study proper. Twelve of the 16 patients were selected for further study. The aim was to determine whether a single inhaled dose of lodoxamide given 15 minutes before pollen inhalation began would protect asthmatic subjects from antigen-induced bronchoconstriction. Three treatments were tested: 0-1 mg and 0-01 mg lodoxamide tromethamine and placebo aerosol. They were prepared in identical pressurised inhalers and administered in randomised, Latin square, double-blind fashion. All patients received each drug dose and the placebo on one occasion only.

The studies on the three treatment-dosing days were performed in identical fashion to that on the preliminary diagnostic day, except that the trial preparation was taken after the pulmonary function tests that followed 10 minutes after the diluent control inhalation. The pollen challenge was started 15 minutes after inhalation of the trial preparation. For each patient the same antigen concentration was used to begin each bronchial challenge, and as before the end-point was a fall in FEV₁ of 20% or more. Beta-2-sympathomimetic agonists were given to reverse the bronchoconstriction at the end of the trial inhalation.

Blood samples were taken before each treatment trial day and also one week after completion of the study. Measurements of cholesterol, creatinine, glucose, urea, and electrolyte concentrations; full blood counts; and liver function tests were performed. A midstream urine specimen was taken on each occasion for microscopy and culture.

Patients were not admitted to hospital for the study but were warned of possible late reactions and given appropriate instructions. Subjects measured their PEFR at home thrice daily for the seven days after each bronchial challenge.

We arbitrarily defined a single breath inhalation of 1 BU/ml strength pollen extract as 1 inhalation unit, so that five breaths of 50 BU/ml, for example, constitutes 250 inhalation units. Since during the bronchial challenge patients inhaled nebulised extracts at fixed time intervals, we expressed the total pollen dose taken in terms of cumulative inhalation units. For each subject and for each challenge percentage fall in FEV₁ was plotted arithmetically on the ordinate and log-cumulative inhalation units were plotted on the abscissa. The provocative dose of pollen causing a 20% fall in FEV₁ (PD₂₀-FEV₁) was calculated by the method of Chai et al. A straight line was drawn through the points bracketing a 20% fall in FEV₁. From the point at which this line intersects the 20% level a vertical is dropped to the abscissa and log PD₂₀-FEV₁ (fig 1) is read from the scale. To ensure accuracy, this manoeuvre was performed mathematically by electronic calculator. Where pulmonary function did not fall below 80% of the post-diluent FEV₁, the last cumulative antigen dose was taken to estimate log PD₂₀-FEV₁. This occurred on two occasions, in a single patient. Thus for every drug study day in every patient we were able to determine log PD₂₀-FEV₁.

Any protective effect of the study drug would therefore be reflected in a higher log PD₂₀-FEV₁ than was obtained on the placebo day. Data were analysed by a three-factor analysis of variance, with
patients, treatments, and order of administration of treatments as the factors. The effects of the two dose levels of lodoxamide were compared by means of Duncan's multiple range test.

**Results**

Skin wheal responses to the recommended extract concentrations ranged from 4 mm to 21 mm (mean 11 mm). Total serum IgE levels ranged from 52 to 7425 units/ml, where 1 unit is equivalent to 2.5 ng IgE.

Four subjects who were originally considered for entry into the study were found to have no bronchoconstrictor response to pollen inhalation. All had positive responses to skin prick tests but these were rather smaller than those of the study group, the wheals ranging from 4 to 8 mm, with lower RAST scores for specific pollen (average 2). Of the 12 subjects selected for further study, six had a RAST score of 3 (high level of IgE antibodies) and six had a score of 4 (very high level). The four apparently non-responding subjects presumably lie towards the less responsive end of the range of bronchial hyperreactivity, and would therefore require higher inhaled concentrations of allergen than were used in this study before change became evident.

Log PD$_{20}$-FEV$_1$, values for each drug treatment, placebo, and diagnostic day, with mean values for the two drug treatment days, are shown in figure 2. As expected, there was a considerable range of sensitivity to grass pollen in the group as a whole. In one subject the log PD$_{20}$-FEV$_1$, values fall well beyond the range for the remaining 11 subjects. He developed an upper respiratory tract infection after the diagnostic and placebo treatments—in his case placebo was the first of the three trial inhalations.

We found good reproducibility of the airways response within subjects. Log PD$_{20}$-FEV$_1$, values in each subject for the diagnostic day and for the placebo day did not vary significantly: 0.5 > p > 0.4 (Student's paired t test). The log PD$_{20}$-FEV$_1$, values for the two dose levels of lodoxamide tromethamine differed significantly from those for the placebo days (p < 0.05). We found no significant difference between the two dose levels in terms of protective effect.

Side effects were frequently encountered. Side effects associated with the inhalation of pollen were experienced by four of the 12 subjects and consisted of nocturnal sleep disturbance, morning dipping of PEFR, early morning wheeze, and exercise-induced asthma. These symptoms lasted for up to one week. The side effects associated with inhalation of lodoxamide tromethamine were a sensation of generalised "heat" and nausea. Symptoms started five to 10 minutes after inhalation and faded within an hour. The effect appeared to be dose related: eight of the 12 subjects experienced this at the 0.1-mg dose level and two at the 0.01-mg dose level, but no subject reported it after placebo.

With the exception of raised eosinophil counts and serum IgE levels, the results of laboratory investigations remained normal in all patients throughout the study period.
Discussion

The use of highly purified species-specific grass pollen antigens enables inhalation bronchial challenge tests to be performed in a safe and reproducible fashion. If conclusions are to be drawn about drug-induced modulation of the allergic airways response satisfactory reproducibility is essential. We believe that the use of antigens with standardised biological activity makes this easier to achieve.

The results of this study show that lodoxamide tromethamine when given in a single inhaled dose exerts a significant protective effect on the development of bronchoconstriction induced by grass pollen in a group of 12 patients with atopic extrinsic asthma.

Although chemically unrelated to sodium cromoglycate, lodoxamide is thought to have a similar "mast-cell-stabilising" effect that contributes to its biological activity. It has been shown to be some 2500 times more potent than sodium cromoglycate in inhibiting passive cutaneous anaphylaxis in the rat. The same workers also found that lodoxamide had no primary bronchodilator or mediator-antagonist activity, and did not raise intracellular levels of cyclic AMP. When given by inhalation lodoxamide protects sensitised rhesus monkeys from bronchoconstriction induced by Ascaris suum antigen.

There are discrepancies between reports of the effects of lodoxamide compounds on skin responses. Two studies, both using intradermal testing, found no effect of lodoxamide, given either orally or by inhalation, on histamine-induced wheals. In one of these studies, however, inhibition of allergen-induced whealing was found and in the other none. The fact that one group used lodoxamide ethyl rather than lodoxamide tromethamine may be relevant. A further study, using intradermal premedication with various concentrations of lodoxamide, reported reduction in the cutaneous response to allergen, again without any effect on histamine wheals.

Recently lodoxamide, unlike sodium cromoglycate, has been shown to block eosinophil chemotaxis in vitro in response to various stimuli. Lodoxamide has been shown to reduce the severity of exercise-induced asthma and allergen-induced asthma.

As with sodium cromoglycate, the precise mode or modes of action of lodoxamide are not completely understood. It may "stabilise" mast-cell membranes by modulating calcium ion movements across the membrane, although over a five-fold to 10-fold range of concentrations it can be stimulatory or inhibitory in this respect.

The duration of the protective effect of lodoxamide on allergen bronchial challenge was recently investigated. The maximum effect was found to be at 15 minutes after dosing, with little effect after eight hours. Our study used a fixed interval of 15 minutes between administration of the drug and the start of allergen challenge, although in some subjects 60 minutes had elapsed by the time the final concentration of pollen extract had been given. Since for each subject the initial pollen concentration was the same for the three study days, any decay of the effect of lodoxamide with time would not vary from one study day to another.

The interval between study days is an important consideration. The non-specific bronchial hyperreactivity, which is a hallmark of the asthmatic state, is known to be temporarily increased by inhalation of specific allergens either experimentally or by natural exposure—for instance, in pollen-sensitive asthmatics during the grass pollen season. In the highly artificial setting of experimental exposure this increased non-specific bronchial hyperreactivity is known to persist for at least a week and the design of studies should take this into account. This has not always been considered in previous studies. Increased bronchial hyperreactivity induced by pollen exposure was manifest in four subjects in our study who developed nocturnal symptoms, morning dipping, and exercise-induced asthma. The mechanism of this remains obscure. If these subjects were rechallenged with antigen before sufficient time had elapsed for this increased bronchial hyperreactivity to subside an exaggerated sensitivity and a misleadingly low log \(PD_{20-\text{FEV}}\) value would be recorded. Upper respiratory tract infections are known to produce increased non-specific bronchial hyperreactivity and therefore increased bronchial responsiveness to endogenous agents released after specific antigen challenge in sensitised individuals. This explains our findings in the subject who developed an upper respiratory tract infection during the study.

Sodium cromoglycate is known to protect to a significant degree against the immediate bronchoconstrictor response to specific antigen in a laboratory setting but its overall efficacy in clinical allergic asthma in adults has been rather disappointing. It remains to be seen whether lodoxamide tromethamine has any advantage in this respect, and long-term outpatient studies are in progress.

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