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Investigation of the anti-allergic activity of azelastine on the immediate and late-phase reactions to allergens and histamine using telethermography

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Summary

Background Due to the interest in azelastine's diverse modes of action, this study investigated its effects on immediate and late-phase cutaneous allergic reactions using visual methods and telethermography.

Objective The aim of the study was to investigate the effect of azelastine on the immediate and late-phase skin reactions using both planimetric evaluation of weal and erythema and a telethermographic technique.

Methods The study was a double-blind crossover study; medication consisted of one tablet per day for 7 days of either placebo or azelastine 4 mg. Eight allergic patients were assessed on five occasions: prior to treatment, at the end of the first 7-day treatment, after a 21-day washout period, following the second 7-day treatment period and finally following a 2–6 week washout period. Skin prick tests with timothy grass and intradermal tests with Alternaria allergens were performed on the patients' back. In addition, patients were tested with intradermal histamine as a positive control.

Surfaces of weal, erythema and infiltration were calculated using computerized planimetry at 0, 20, 40 and 60 min, and 3, 6 and 8 h. Thermographic images were recorded and the thermographic area and the increase in average temperature (ΔT) were calculated.

Results The coefficient of variation within baseline reactions ranged from 3 to 32% for weal and erythema and from 5 to 25% for thermographically recorded reactions. The stronger the reaction, the more constant the baseline was. Treatment with azelastine (4 mg/os once daily) inhibited immediate reactions to allergens by 65% (range 55–74) and to histamine by 68% (range 47–82). The late-phase reactions to allergens were less well defined and showed larger individual differences in the degree of inhibition caused by azelastine, they were inhibited by 49% (range 32–67). Late-phase reactions to histamine were less intense and could only be detected with thermography; only thermographic units showed a decrease (26%) in response to azelastine.

Conclusion This study has confirmed azelastine's histamine-blocking activity. In addition, the late-phase results suggest that azelastine has anti-inflammatory activity. The reproducibility and sensitivity of the thermographic results confirm the usefulness of this technique in immunopharmacology.

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Introduction

This study was performed to investigate the effects of the anti-allergic drug azelastine on immediate and late-phase cutaneous allergic reactions, assessed by traditional methods (evaluation of weal and erythema areas) and also by a new thermographic technique. An additional aim of the study was to further validate this new thermographic method which has been used previously in the investigation of inflammatory joint diseases [1] and sport injuries [2] and

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has only recently been introduced to immunopharmacology [3–5].

The usual method used to quantify immediate allergic skin reactions is to mark weal and erythema reactions with a pen, transfer them with scotch tape to paper, and assess the surface of the reactions using planimetry [6]. This technique, however, only measures the reaction in a two dimensional way. Furthermore, late-phase reactions characterized by tissue infiltration are more diffuse and less well defined in this way. It is now possible to evaluate inflammatory skin reactions in a three-dimensional way, using infrared thermographic cameras and software capable of assessing the development of the reaction [4–6].

The relation between weal and erythema areas measured by classical planimetry in millimetres^2 and the thermographic area in millimetres^2, as well as the intensity of the reaction expressed as thermographic units (area times average rise in temperature) have been extensively described and analysed on a large number of reactions [5]. In absolute terms, the thermographic area is about three to five times larger than the visible erythema area and about 20–25 times larger than the visible weal area (Figs 1 and 2) [5].

Azelastine is an anti-allergic drug which is marketed as a nasal spray in most major European countries for treatment of allergic rhinitis (tradenames: Allergodil®, Rhinolast® and others). Azelastine has proven histamine receptor blocking activity and also inhibits the release of histamine and leukotriene C_4 from mast cells in *in vitro* studies [7]. Because of the interest in azelastine's multifaceted mechanisms of action, our aim was to assess its effect on both the early and late-phase allergic reaction. The immediate allergic reaction is triggered by allergen interacting with tissue mast cells carrying specific immunoglobulin (Ig) E. The late-phase reaction then follows a few hours later. Various mediators, particularly leukotrienes and cytokines, produced by activated mast cells or T lymphocytes may be responsible for the cellular infiltration and the secondary release of mediators that characterize the late-phase reaction [8].

Methods

Eight patients were selected who presented a clear-cut seasonal inhalation allergy (rhinitis and/or asthma) and positive immediate and late-phase allergic skin reactions to timothy grass and/or *Alternaria tenuis*. Patients were assigned to the two treatment groups according to a predefined randomization code. The study was conducted double-blind; medication consisted of one tablet per day for 7 days of either placebo or azelastine. Following the first washout period, patients received the alternative medication for a further 7 days. At the end of each medication period, drug packages were collected to assess compliance, in addition patients were questioned about the intake of their medication.

Patients were assessed prior to treatment (first visit), at the end of the first 7-day treatment period (second visit), after a 21-day drug-free washout period (third visit), following the second 7-day treatment period (fourth visit) and finally following a 2–6-week washout period (fifth visit).

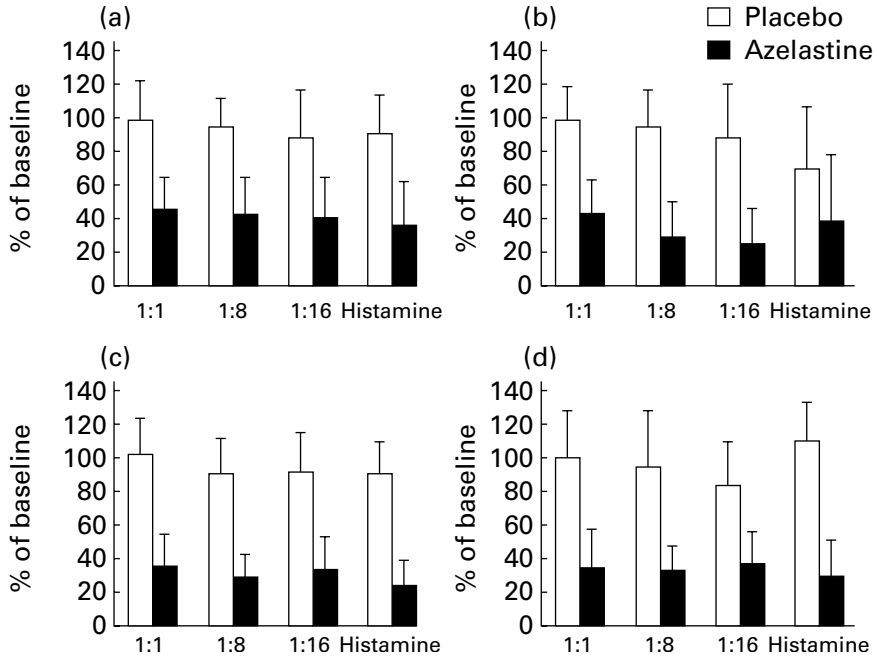

Fig. 1. Mean inhibition of the immediate reactions to allergen and histamine, expressed as percentage of baseline (mean and SD). (a) Thermographic area, (b) thermographic units, (c) erythema, and (d) weal

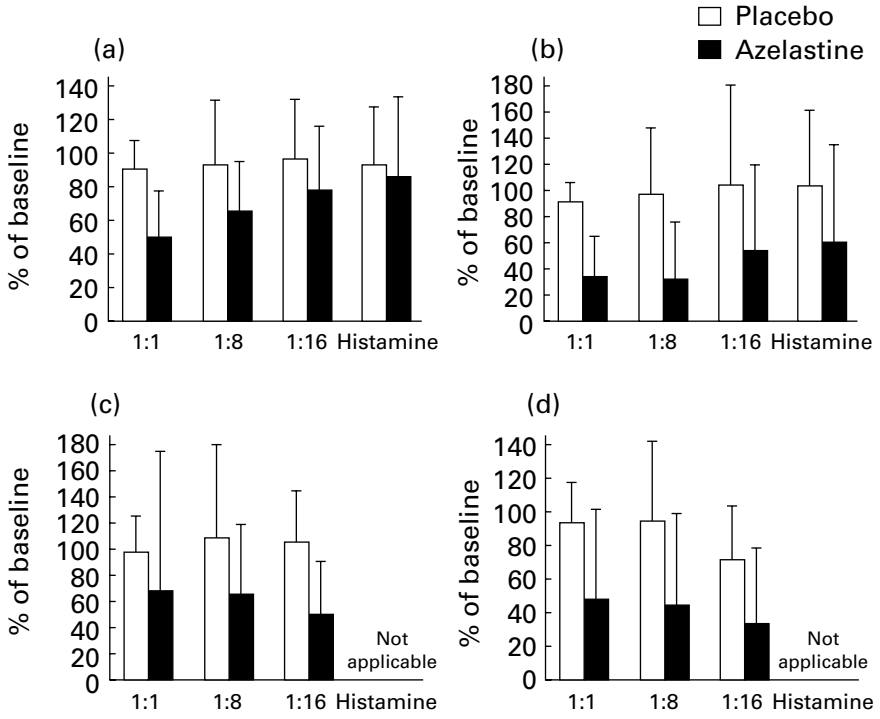

Fig. 2. Mean inhibition of the late-phase reactions to allergen and histamine, expressed as percentage of baseline (mean and SD). (a) Thermographic area, (b) thermographic units, (c) erythema, and (d) infiltration

Skin prick tests with timothy grass (Allergopharma, 50 000 BE/mL solution) and intradermal tests with the *Alternaria tenuis* allergens (Allergopharma, Reinbek, Germany, 500 S BE/mL) were performed on the patients' back. Allergens were used at full concentration and at 1 : 8 and 1 : 16 dilutions. In addition, all patients were tested with intradermal histamine as a positive control (concentration 0.1 mg/mL, 1 : 10 000). Doses injected intradermally were 0.03 mL.

Surfaces of weal and erythema were taken as scotch tape imprints and calculated using computerized planimetry. For late-phase reactions where no weal is apparent, palpable infiltration was delineated by pen and recorded together with erythema. Thermographic images were recorded at 0, 20, 40 and 60 min, and 3, 6 and 8 h using an infrared camera (NEC, Tokyo, Japan) capable of recording temperature increments of 0.1 °C and a Thermoflir (Kriens, Switzerland) thermographic analytical system which allows pictures to be taken and stored at predetermined intervals. Thermographic investigations were conducted in a room controlled at 21 °C. Patients were allowed to adapt to this temperature for 30 min before testing was performed. The distance between the camera and the patient's back was maintained at 400 mm. Two parameters were analysed, the thermographic area and the increase in average temperature (ΔT) in the area involved. This allowed an evaluation of the thermographic reaction in arbitrary thermographic units (TU) which are calculated by multiplying the increase in average temperature of the afflicted area (ΔT) by the area size expressed in square millimetres ($TU = \text{area} \times \Delta T$) [4,5]. Statistical analysis was performed using the 'Systat' Programme (Microsoft), χ^2 and student *t*-tests; a *P*-value of $P < 0.05$ was considered significant.

This investigation was conducted outside the pollen season from January to April. The study was approved by the ethics committee of the Medical Faculty of the University of Bern. Each patient gave informed consent to participate in the study.

Results

All patients showed strong positive skin test reactions to one or both allergens. With the exception of a few patients being RAST negative to Alternaria, all patients had strong positive RAST tests as well. Demographic and diagnostic data are given in Table 1. All patients complied with the drug regimen and treatment schedule.

To assess reproducibility of the methods, the results obtained at the three drug-free assessments were taken as a baseline to determine variability of the reactivity of the skin over time. The resulting coefficients of variation obtained with the thermography indicate that this method is at least as reproducible as the classical eye-evaluated parameters of weal and erythema [5] and is more sensitive. For immediate reactions, the overall mean baseline and placebo coefficient of variation in percentage are similar for all four parameters measured: thermal area, 18.8; thermal units, 17.1; erythema area, 18.2 and weal area, 13.9. For late-phase reactions, results are similar: thermal area, 14.1;

Table 1. Demographic and diagnostic data

Patient number	Age (years)	Sex	Diagnosis	Allergen	RAST	Skin test	Total IgE (kU/L)
1	28	M	Seasonal rhinitis	Timothy	+(3)	+++	228
2	43	M	Seasonal rhinitis, asthma	Timothy	+(3)	+++	56
3	29	M	Seasonal rhinitis, asthma	Alternaria	+(3)	+++	221
4	41	F	Seasonal rhinitis, asthma	Alternaria	−(0)	+++	35
5	22	M	Seasonal rhinitis, asthma	Timothy	+(4)	+++	346
6	19	M	Seasonal rhinitis, asthma	Alternaria	−(0)	+++	112
				Timothy	+(4)	+++	
7	41	F	Seasonal rhinitis, asthma	Alternaria	−(0)	+++	558
				Timothy	+(3)	+++	
8	20	M	Seasonal rhinitis, asthma	Timothy	+(3)	+++	257

thermal units, 20.8; erythema area, 21.6 and infiltration area, 22.2. The stronger the reaction, the more constant the baseline was.

Oral azelastine, 4 mg once daily, had a marked inhibitory effect on the immediate reactions to allergens and to histamine in all eight patients. This inhibition varied between 38 and 84% for thermographic area, 42–90% for thermographic units, 28–86% for erythema area and 15–91% for weal area. Results (means and standard deviations) are presented graphically in Fig. 1. The late-phase reactions show larger individual differences in the degree of inhibition caused by azelastine and the parameters measured are not equally affected. Thermographic units which reflect not only the size of the reaction but also its intensity were markedly inhibited by azelastine even where the reaction was small. By contrast, erythema area was small in most patients in the late-phase reaction and inhibition by azelastine was less evident in some patients. Palpable infiltration was evident only at the highest allergen concentration; all patients with a sizeable late-phase reaction also showed a marked inhibition by azelastine. Late-phase reaction results (means and SD) are presented graphically in Fig. 2; it can be seen that treatment with placebo causes no deviation from baseline. An example of thermographic recording of the reaction and of its inhibition by azelastine is shown in Fig. 3.

Inhibition of the immediate positive control reactions to histamine is also shown in Fig. 1. Clear-cut inhibition of the immediate reaction to histamine under azelastine treatment was observed in all patients with all parameters. By comparison, there is practically no inhibition of immediate reactions to histamine under placebo treatment. Late-phase reactions to histamine are less intense and could only be detected by thermography (Fig. 2). These reactions were less inhibited by azelastine than reactions to allergens. In a number of patients, the histamine-induced late-phase reaction is not markedly impaired in terms of size; however, there is more inhibition in terms of thermographic units and hence of the inflammatory reaction.

The allergen-induced reactions and their inhibition by azelastine and placebo are shown graphically in Fig. 3. This shows the results of patient 7 at the highest allergen concentrations. The course and intensity of the immediate and late-phase reactions are similar for the three baseline reactions and the reaction observed after placebo. This is particularly true for both the thermographic parameters and less so for visually recorded erythema. Azelastine had a marked inhibitory effect at all times on the immediate reaction. The inhibition of the late-phase reaction by azelastine is evident for the thermographic parameters but the differences in erythema area and infiltration are less impressive.

Discussion

The results reported here confirm the sensitivity of computerized telethermography in the kinetic evaluation of cutaneous reactions to allergens. This study has permitted the evaluation of the less well-defined late-phase inflammatory reaction which has not previously been feasible using less sensitive techniques. This work has confirmed previous findings that the reaction to histamine may extend beyond the initial immediate phase [5].

As described in detail elsewhere [5], only the telethermography permits detection of late-phase reactions to histamine, which are not detectable by the naked eye. The skin reaction to allergens involves many pathophysiological steps which are different from the reaction to histamine. The relationship between erythema area and thermographic (inflammation) area is not the same for histamine-induced and allergen-induced reactions (Fig. 3) [5]. The allergen-induced inflammatory area is markedly larger, with respect

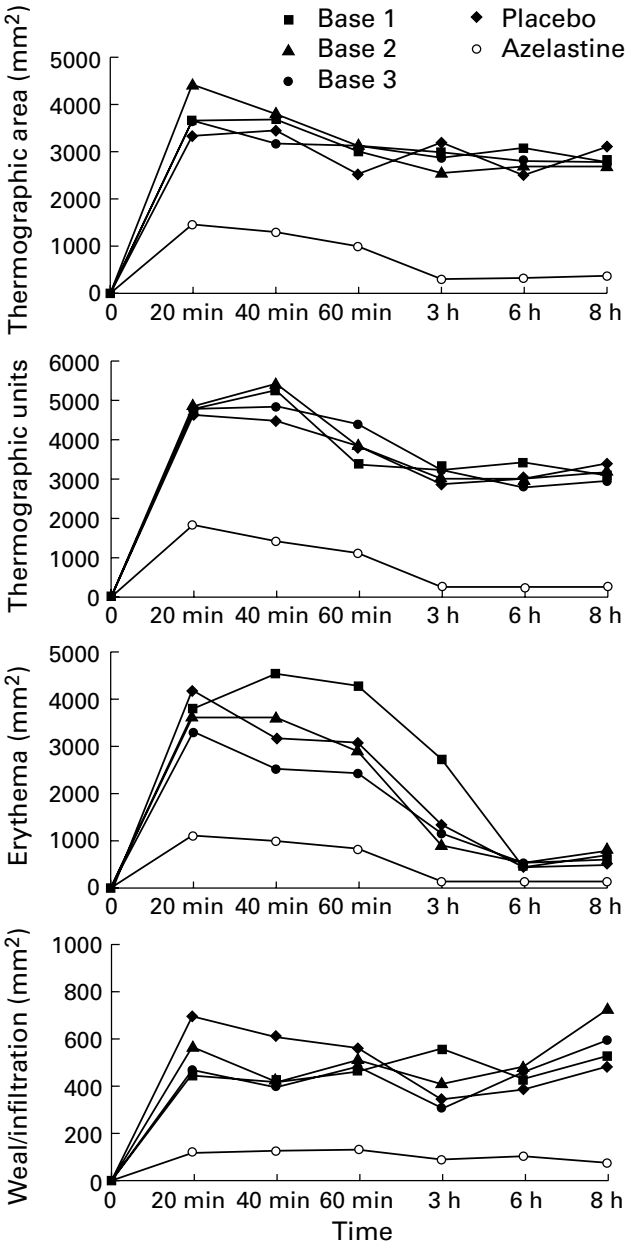

Fig. 3. Example of the thermographic recording of the reaction to allergen (1 : 1) and its inhibition by azelastine (patient 7)

to erythema area, than the histamine-induced reaction. This is most likely due to the fact that many inflammatory factors other than histamine are involved in the allergen-induced reaction. The precise mechanism of the thermographically-apparent late-phase reaction to histamine in some patients is not clear at this time. Since, in contrast with allergen-induced late-phase reactions, no infiltration is palpable, the histamine-induced late-phase reaction is likely to be due to vasomotor rather than cellular infiltration mechanisms. Suitable histopathological examination has not yet been performed.

Telethermographic technique permits measurement of both the size, intensity and kinetics of skin inflammation reactions. The heat measured on the surface area is an expression of a three dimensional inflammatory reaction. Future investigations of the kinetics of the late-phase reaction using this objective technique should increase our understanding of the mediators and mechanisms involved.

The current study has confirmed the histamine-blocking effects of azelastine on the cutaneous allergic response. In addition, azelastine's effect on the late-phase reaction has highlighted the anti-inflammatory property of this drug. A similar effect has been demonstrated previously in atopic asthmatics in significantly inhibiting the late bronchoconstrictor response to inhaled allergen [9].

References

1 Da Silva M, Kyle V, Hazelman B, Salisbury R, Page TP, Wright PA. Assessment of inflammation in the rheumatoid knee joint: correlation between clinical, radio isotopic and thermographic methods. Ann Rheum Dis 1986; 45:277–80.

2 Giani E, Rocchi L, Tavoni A, Montanan M, Garagiola U. Telethermographic evaluation of NSAIDS in the treatment of sports injuries. Med Sci Sports Exercise 1989; 21:1–6.

3 de Weck AL, Derer T, Bischoff SC, Takafuji S. The effect of terfenadine on the immediate and late-phase reactions mediated by Immunoglobulin E. Int Arch Allergy Immunol, 1993; 101:326–32.

4 Staub A, Summermatter U. The use of telethermography for quantitative assessment of inflammatory skin reactions. Dissertation, University of Bern, 1992.

5 de Weck AL, Derer T. Critical evaluation of the use of skin tests and cellular tests in standardisation of allergens. In: Kurth R, Haustein D, eds. Regulation Control and Standardization of Allergenic Extracts, Proceedings from Paul Ehrlich Institut. Stuttgart: G. Fischer-Verlag, 1994; 87:89–114.

6 de Weck AL, Gluck U, Derer T. Thermographic analysis of allergic reactions in the skin. Allergy Clin Immun News 1990; 2:7–10.

7 Chand N. Antagonism of histamine and leukotrienes by azelastine in isolated guinea pig ileum. Agents Actions 1986; 19:164–8.

8 de Weck AL, Stadler BM, Dahinden CA. New perspectives in the modulation of allergic inflammation. Int Arch Allergy Appl Immunol 1989; 90:17–21.

9 Rafferty P, Ng WH, Phillips G et al. The inhibitory actions of azelastine hydrochloride on the early and late bronchoconstrictor responses to inhaled allergen in atopic asthma. J Allergy Clin Immunol 1989; 84:649–57.