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Topical azelastine has a 12-hour duration of action as assessed by histamine challenge-induced exudation of α_2-macroglobulin into human nasal airways

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Summary

Background Oral anti-histamine drugs are widely used in the treatment of seasonal allergic rhinitis. Recently, anti-histamines have become available also for topical treatment
Objective The present study, involving healthy subjects, examined the effect of topical azelastine on luminal entry of α_2-macroglobulin and symptoms evoked by repeat histamine challenges during 24 h. The effect was compared to a clinical dose of the oral anti-histamine cetirizine and to placebo treatments.
Methods Placebo and azelastine (0.254 mg per nasal cavity) were delivered as two consecutive actuations per nasal cavity using a nasal spray device. Oral placebo and cetirizine (10 mg) were given as single doses in a placebo-controlled (double-dummy), double-blind, and cross-over design. Histamine-challenges were given 1 h before treatment, and 1, 6, 9, 12, and 24 h after each treatment. The nasal mucosal surface was lavaged after each challenge. The lavage-fluid levels of α_2-macroglobulin were determined to assess mucosal exudation of bulk plasma, and nasal symptoms were scored.
Results Histamine (40–400 μg/mL) produced dose-dependent exudation and symptoms. Compared between each treatment and placebo, azelastine and cetirizine reduced the 40 and/or 400 μg/mL histamine-induced mucosal exudation of plasma from 1–12 h after treatment. In addition, cetirizine reduced the 40 μg/mL histamine-induced mucosal exudation of plasma 24 h after treatment. Differences between the two treatments were not evident regarding nasal symptoms.
Conclusion Histamine challenge-induced mucosal exudation of plasma appears to be a useful method for studies of the duration of action of antihistamines. We conclude that topical azelastine is suited for b.i.d. therapy and that neither the exudative process nor watery secretion may impede the efficacy or the duration of action of this nasal drug.

Keywords: allergy, antihistamine, rhinitis

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Introduction

Antihistamines have been used in the treatment of allergic airways disease for more than 40 yr. Recently, antihistamines such as azelastine have been introduced also for topical use in allergic rhinitis. The duration of the pharmacological effect would be a major aspect of topical antihistamines in deciding dose intervals for efficacy around the clock. Weiler *et al.* [1] have shown that a clinical dose of topical azelastine may reduce major symptoms of seasonal allergic rhinitis for up to 10 or 12 h during natural pollen exposure. In a study involving patients with seasonal allergic rhinitis out of season, Thomas *et al.* [2] have demonstrated that topical azelastine reduces histamine challenge-induced sneezes for 10 h after

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treatment, whereas the histamine challenge-induced increase in nasal airway resistance (as measured by posterior rhinomanometry) was reduced for only 1–2 h after treatment. It appears that further information on the duration of action of topical azelastine is warranted, particularly concerning its ability to counteract different pharmacological effects of histamine.

We have shown previously that repeated histamine challenges may evoke reproducible exudative effects (luminal entry of bulk plasma) over several hours [3]. The exudative action of histamine applied on the human nasal mucosa is concentration-dependent in the range 40–2000 μg/mL [4]. Hence, repeated histamine-induced mucosal exudation of plasma might be employed as a method for determination of the duration of action of antihistamine drugs. Furthermore, the plasma exudation response may be a major action of histamine because this effect brings a wide range of adhesive proteins and other biologically active peptides/proteins to the lamina propria, the basement membrane, the epithelium, and the mucosal surface of the allergic mucosa.

In the present study, we have examined the duration of the pharmacological effect of topically applied azelastine on luminal entry of plasma induced by repeated histamine-challenges. In addition, we have examined treatment effects on histamine-induced nasal symptoms. The effects of topically applied azelastine have been compared to the effect of the orally administered antihistamine drug cetirizine and to placebo.

Methods

Study design

The study was of a placebo-controlled (double-dummy), double-blind, and cross-over design. Subjects were treated on three occasions, i.e. with active nasal spray (azelastine) and placebo tablets, with placebo nasal spray and active tablets (cetirizine), and with placebo nasal spray and placebo tablets. The wash-out time was 2 weeks. Placebo and drug treatments were given as single doses and histamine-challenges were given 1 h before and 1, 6, 9, 12, and 24 h after each treatment. The nasal mucosal surface was lavaged after each challenge and the lavage-fluid concentrations of α_2-macroglobulin were determined as an index of mucosal exudation of plasma. Furthermore, baseline and histamine-induced nasal symptoms were scored after each challenge.

Subjects

Twelve healthy subjects, 23–28 yr of age (mean age 25 yr), received histamine-challenges and placebo and drug treatment. The subjects had no history of general, allergic or recent nasal disease, and no history of recent drug treatment. The study was approved by the local ethics committee, and informed consent was obtained.

Nasal pool challenge and lavage technique

A nasal pool device was used for concomitant histamine-challenge and lavage of the nasal mucosa [4]. The nasal pool device is a compressible plastic container equipped with a nasal adapter. The adapter is inserted into one of the nostrils and the container is compressed by the sitting subject leaning forward in a 60° flexed neck position. The nasal pool fluid is then instilled in one of the nasal cavities and maintained in contact with a large and reproducible area of the mucosal surface for a determined period of time. When the pressure on the device is released, the fluid returns into the container. In the present study the total volume of the nasal pool fluid was 14 mL.

Drug treatment and histamine challenges

The topical placebo and azelastine (0.254 mg per nasal cavity) drug solutions were delivered as two consecutive actuations per nasal cavity using a nasal spray device (0.254 mg azelastine corresponds to 0.280 mg azelastine hydrochloride). Oral placebo and cetirizine (10 mg) were given as single oral doses. Using the nasal pool device, isotonic saline and histamine (40 and 400 μg/mL) were employed as challenge and lavage solutions 1 h before, and 1, 6, 9, 12 and 24 h after each treatment. The solutions were given consecutively at each time point, and each solution was maintained in the unilateral nasal cavity for 10 min. The lavage fluids were centrifuged (325 ***g***, 10 min, 4°C) and samples were obtained from the supernatant and frozen (−20°C) to await analysis of α_2-macroglobulin.

Analysis of α_2-macroglobulin

The lavage fluid levels of α_2-macroglobulin were measured using a radioimmunoassay sensitive to 7.8 ng/mL. Rabbit anti-human α_2-macroglobulin (Dakopatts, Copenhagen, Denmark) was used as anti-serum and standard human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human α_2-macroglobulin (Cappel-Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or sample) was mixed with anti-serum before adding goat anti-rabbit anti-serum (Astra Draco, Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and inter-assay coefficients of variation are between 3.8–6.0% and 3.1–7.2%, respectively.

Symptom score

Nasal symptoms, i.e. sneezes, secretion and blockage, were

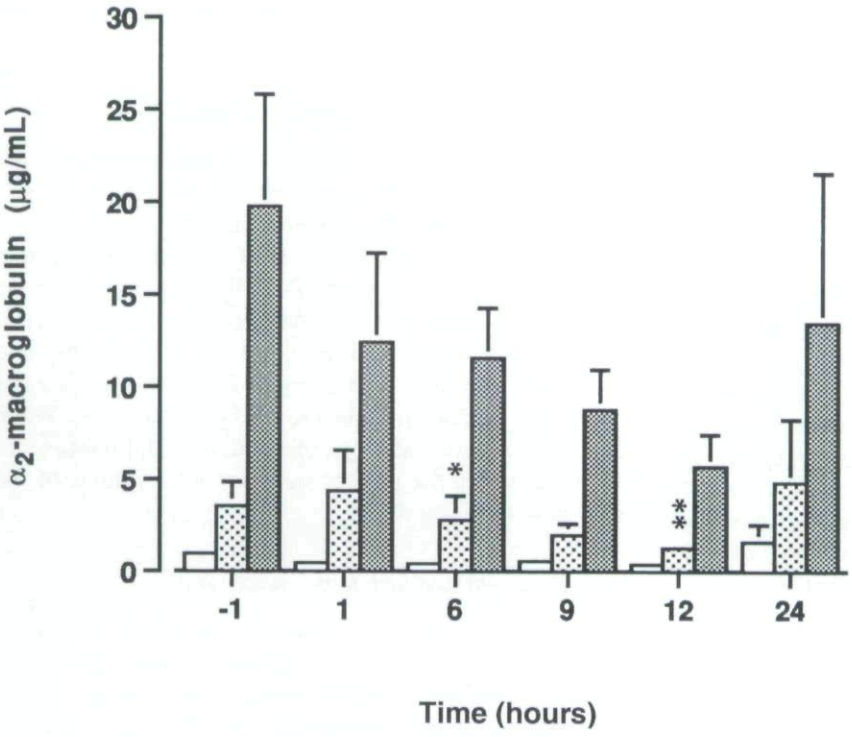


Fig 1. Histamine produced dose-dependent mucosal exudation of bulk plasma (α_2-macro-globulin) 1 h before treatment and 1–24 h after placebo treatment. The response to 40 μg/mL histamine decreased with time (Friedman $P < 0.05$). The reduction was significant at 6 and 12 h after treatment compared with before treatment. (Comparisons between levels obtained before treatment and 1–24 h after treatment, $*P < 0.05$, $**P < 0.01$.) Although mean values were reduced by repeat histamine challenge, there was no significant tachyphylaxis to 400 μg/mL histamine (Friedman $P > 0.05$). □ = control saline; ▦ = histamine (40 μg/mL); ▨ = histamine (400μg/mL).

scored by the subjects immediately after each challenge using a symptom score: No = 0, mild = 1, moderate = 2, severe = 3 symptoms.

Statistics

Differences in histamine-induced nasal symptoms and α_2-macroglobulin levels between the placebo and the two antihistamine treatments were analysed by the Wilcoxon signed rank (WSR) test. Differences in nasal symptoms and α_2-macroglobulin levels within each treatment group were analysed by the Friedman test and the WSR test. A P-value less than 0.05 was considered significant. Data are presented as mean ± SEM.

Results

Reproducibility of control saline and histamine challenges

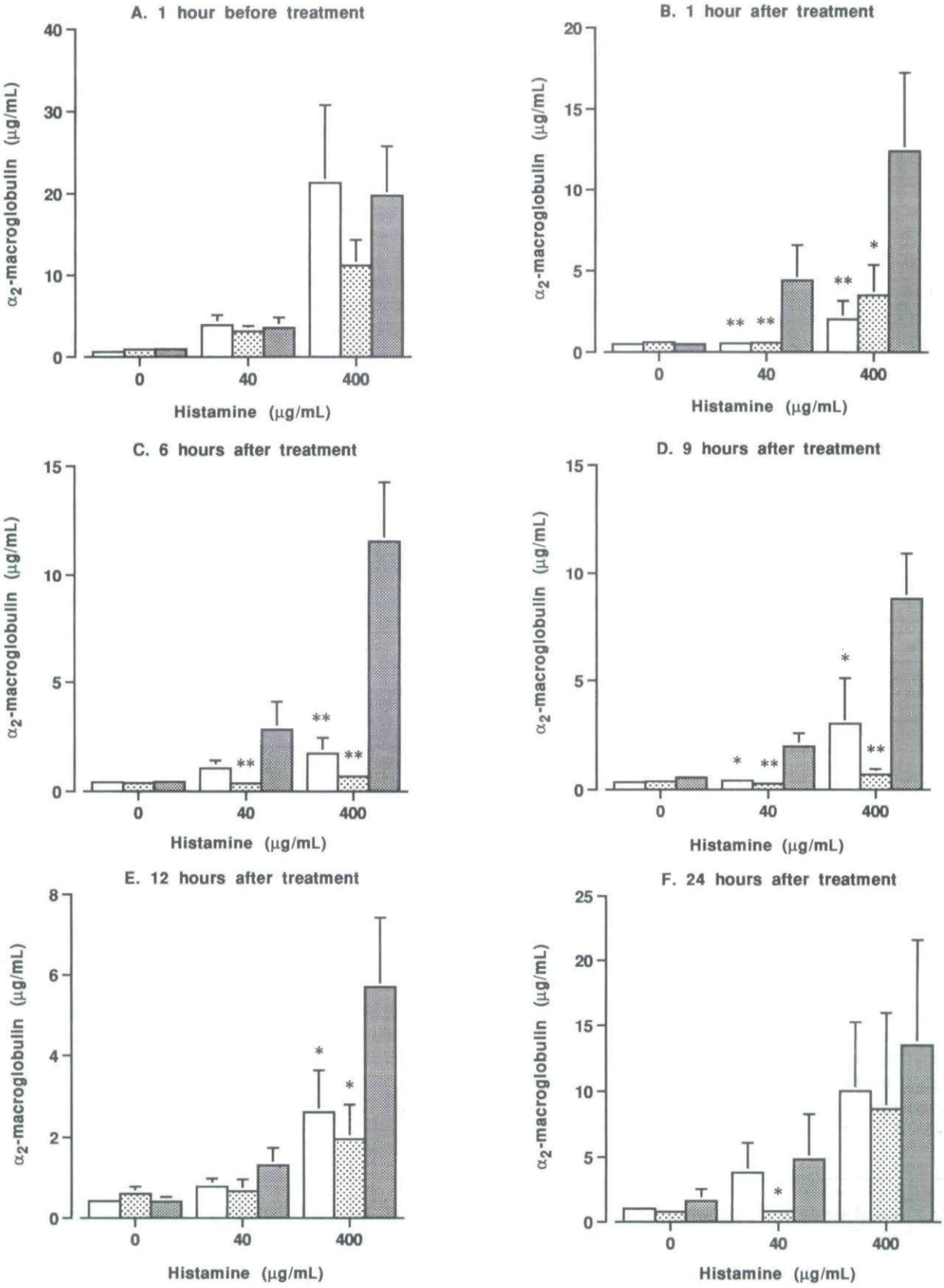
The mean of the intra-individual coefficients of variation calculated on lavage fluid levels of α_2-macroglobulin after challenges with control saline, histamine (40 μg/mL) and histamine (400 μg/mL), respectively, on three occasions before treatment were 0.71, 0.61 and 0.71. The mean of intra-individual coefficients of variation calculated on lavage fluid levels of α_2-macroglobulin after challenges with control saline, histamine (40 μg/mL) and histamine (400 μg/mL), respectively, at the six challenge series before and after placebo were 0.69, 0.86 and 0.84. The latter coefficients are influenced by any degree of tachyphylaxis to histamine and by any effects of topical and oral placebo.

Effect of treatment on mucosal exudation within the placebo group

The response to 40 μg/mL histamine in the placebo group decreased with time (Friedman $P < 0.05$). The reduction was significant at 6 and 12 h after treatment compared with before treatment (WSR $P < 0.05$ and 0.01, respectively) (Fig. 1), confirming a certain degree of tachyphylaxis to histamine [5]. Because of this tachyphylaxis in the plasma exudation response to histamine after placebo treatment, the primary comparisons in the present study are performed between each treatment and placebo at each time point. Although mean values were reduced by repeat challenge, there was no statistically significant tachyphylaxis to 400 μg/mL histamine (Friedman $P > 0.05$).

Effect of treatment on mucosal exudation between the treatment groups

There were no differences in lavage fluid levels of α_2-macroglobulin after control saline and histamine challenge, respectively, between the treatment groups and placebo 1 h before treatment. The azelastine-induced reduction in



histamine (40 μg/mL) mucosal exudation was significant when compared with placebo at 1 and 9 h after treatment (Fig. 2B and D) and the reduction in histamine (400 μg/mL) mucosal exudation was significant compared with placebo at 1, 6, 9, and 12 h after treatment (Fig 2B, C, D and E). The cetirizine-induced reduction in histamine (40 μg/mL) mucosal exudation was significant compared with placebo at 1, 6, 9, and 24 h after treatment (Fig. 2B, C, D and F) and the reduction in histamine (400 μg/mL) mucosal exudation was significant compared with placebo at 1, 6, 9 and 12 h after treatment (Fig. 2B, C, D and E).

Effect of treatment on nasal symptoms between the treatment groups

The treatment with azelastine as well as cetirizine reduced the histamine-induced sneezes and secretion but not blockage when symptoms between the treatment groups and placebo were compared (Table 1 A–C). There were no differences for either symptom between the treatment groups before treatment. When comparing the differences between azelastine and ceterizine, respectively, and placebo, azelastine as well as cetirizine significantly reduced the sneezes after histamine (400 μg/mL) 1, 9 and 12 h after treatment (Table 1A). Azelastine reduced the secretion after histamine (40 μg/mL) 1 h after treatment as well as after histamine (400 μg/mL) 12-12 h after treatment (Table 1B). Cetirizine reduced the secretion after histamine (400 μg/mL) and 24 h after treatment as well as after histamine (40 μg/mL) 9 h after treatment.

Discussion

The present study, involving healthy subjects, has demonstrated that a single dose of topical azelastine significantly reduces nasal histamine challenge-induced mucosal exudation of α_2-macroglobulin for 12 h. In addition, this drug with a similar duration of action reduced the sneezing and the secretory responses evoked by the histamine challenges. Topical azelastine was generally comparable to oral cetirizine concerning effect and duration of action.

In allergic rhinitis, the acute response to allergen challenge is dependent on histamine, and treatment with anti-histamines have marked anti-allergy effects in allergen challenge models [6–8]. Histamine challenge, similar to acute allergic airway conditions, produces several symptoms along with mucosal exudation of plasma. As expected [9], mucosal exudation of α_2-macroglobulin, contrasting measurements of symptoms, was a sensitive and graded response suited for quantitative measurements (this study). Histamine at the lower concentration (40 μg/mL) thus produced significant mucosal exudation of plasma in the absence of symptoms, and at the larger concentration (400 μg/mL) it produced an approximately 20-fold increase in lavage fluid levels of α_2-macroglobulin compared to baseline. The mean exudative response to histamine was reduced by repeated challenges (seen with both dose levels but statistically significant only with the lower dose). Such a reduction has previously been recorded in the nose [5] and may relate to a tachyphylaxis that may occur with histamine-type mediators at the level of microvascular permeability regulating cells [10]. The primary comparison was, therefore, made between each treatment and placebo.

The anti-exudative effect and its duration was 12 h for both drugs. Cetirizine appeared at least as potent as azelastine with somewhat superior inhibition at 6 and 9 h after treatment. Cetirizine also was the only drug that showed some efficacy after 24 h and then exclusively against the low concentration of histamine. These data may need confirmation, particularly since they differed from the 12-h findings. The present findings suggest that around the clock anti-exudative effects require administration twice daily of either topical azelastine or oral ceterizine.

The present observations on symptoms confirm and extend the work by Thomas *et al.* [2] who observed a 10-h duration of action of topical azelastine on histamine-induced sneezing. Thomas *et al.* [2] further reported that topical azelastine only had effect for 2 h on histamine challenge-induced increase in nasal airway resistance. In accordance, the present study demonstrated little effects in general on nasal blockage: Only a non-significant tendency ($P = 0.06$) was recorded at 1 h after topical azelastine treatment. In addition, this study recorded a 12-h duration of effect of secretion by azelastine. This may reflect a true pharmacological inhibition although the recording of 'subjective' secretion could have been somewhat influenced by the repeated nasal lavage procedures.

A potentially more important aspect concerns the fact that azelastine had a long duration of action despite the lavages since these could have removed the topically applied drug. Indeed, the antihistaminic efficacy of azelastine at 1 h before the first lavage had been carried out was generally not

Fig 2. Effects of topical azelastine, oral ceterizine and placebo on histamine challenge-induced mucosal exudation of bulk plasma (α_2-macroglobulin). Treatments were given at time point zero and challenges with control saline histamine (40 and 400 μg/mL) were given 1 h

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