

Humoral and cellular responses to histamine and pollen allergen in a skin chamber model: effect of mizolastine

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Background: Mizolastine is a new non-sedative antihistamine and antiallergic drug proven to be effective and safe in the treatment of allergic rhinitis and urticaria.

Objective: To quantitatively explore the time course of mediator release and cell recruitment during allergen challenge and the effects of mizolastine on the event, using the skin chamber model.

Methods: Twelve pollen-sensitive patients (23 ± 6 years) were included in a double-blind crossover study. Patients received 10 mg mizolastine or placebo once daily in the first 4-day period and, after a 3-week washout period, vice-versa in the crossover period. On day 4 of each period, a non-invasive in vivo skin chamber technique was used to determine the alteration of vascular permeability, mast cell mediator release, the release of soluble intercellular adhesion molecule -1 (sICAM-1) in skin sites challenged with exogenous histamine or grass pollen allergen extract, over an 8-hour period.

Results: Challenge with allergen-induced significant mast cell activation, as indicated by the release of histamine, tryptase and LTC₄, in chamber fluids 2 hours after initiation of the allergic reaction and during the following 6 hours. Both exogenous histamine and allergen induced significant vasodilatation, which was sustained during the 8-hour challenge, as indicated by the accumulation of protein in the chamber fluids. Likewise, both histamine and allergen induced the release of significant amounts of ICAM-1 throughout the 8-hour period. Mizolastine significantly inhibited the histamine- and allergen-induced extravasation (after 2 hours, $P = .003$; after 8 hours, $P = .009$; after 2 hours, $P = .044$; after 8 hours, $P = .003$ respectively) and the histamine- and allergen-induced ICAM-1 release (after 2 hours, $P = .004$; after 8 hours, $P = .05$; after 2 hours, $P = .03$ respectively).

Conclusion: Mizolastine strongly inhibited the local response to histamine in this skin chamber model with, of interest, inhibition of the release of the soluble adhesion-molecule ICAM-1.

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INTRODUCTION

Mizolastine, a benzimidazole derivative, is a potent new non-sedating an-

ti-histamine that has been proven to be effective and safe in the treatment of patients with allergic rhinitis and urticaria.^{1–3} Preclinical studies in animals have shown that its pharmacologic activity is due partly to the selective blocking of histamine H₁-receptors and partly to the inhibition of histamine release from mast cells.^{4,5} Further, mizolastine inhibits the arachidonic acid-induced cutaneous reaction, which appears to involve the 5-lipoxygenase pathway.⁶ Mizolastine administered as a single 10-mg dose to healthy volunteers inhibits a dermal histamine chal-

lenge 1 hour later, and this effect is still evident at 24 hours.⁷

Besides their H₁-receptor blocking effect, some of the new antihistamines have been shown to inhibit mediator release during allergen challenge and to modulate the recruitment and activation of inflammatory cells, especially neutrophils, in the site of an allergen challenge.⁸ Recently, several antihistamines have been shown to reduce the expression of the immunoglobulin-related cell adhesion molecule ICAM-1 on epithelial cells in the nose and eye after allergen-specific challenge in patients with pollinosis⁹ and to reduce the amount of soluble ICAM-1 (sICAM-1) recovered in nasal secretions from patients with seasonal allergic rhinitis.¹⁰

We have previously described a skin chamber model for quantification of the time course of development of an allergic reaction in vivo,¹¹ as also reported by Charlesworth et al¹² and by Atkins et al.¹³ These models have been used to explore the mechanism of action of various drugs on the allergic reaction.^{13,14} The aim of the present double-blind, crossover study was to examine the effect of mizolastine on mediator release, sICAM-1 secretion, and cellular recruitment in response to single or sustained cutaneous allergen challenges in pollen-sensitive patients.

MATERIALS AND METHODS

Subjects

Twelve patients (10 males and 2 females, mean age 23 ± 6 years) having (1) a history of seasonal allergic rhinitis during the two previous grass pollen seasons, (2) a positive immediate intradermal skin test reaction with mixed

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grass pollen extract (Laboratoires Staller-gènes, Antony, France) and (3) a high level of anti-dactyl IgE antibody determined by RAST (Pharmacia, Up-sala, Sweden) within the previous 6 months were included in the study. The study was performed outside the grass pollen season. Each subject gave informed written consent before being included. The protocol was approved by the Ethics Committee of the Hôpital Saint-Louis, Paris, France.

The study was conducted according to a double-blind, randomized, cross-over design. Subjects received either mizolastine 10-mg tablets (Sanofi-Synthelabo France) or an identically appearing placebo tablet once daily for 4 days in the first treatment period. This was followed by a 3-week wash-out period before they received the crossover treatment during the second treatment period.

Skin Chamber Technique

The skin chamber method was performed as previously described (Michel et al).¹¹ In brief, on day 3, immediately after taking the study drug, four blisters (0.8 cm diameter base) were raised on the patient's right and left forearms by vacuum suction. Four hours after taking the study drug, the blister fluids were collected for determination of mizolastine and protein content. Simultaneously, a blood sample was drawn for measurement of the plasma drug level. Over the subsequent 2-hour period, the roof of each blister was taken off aseptically and the diameter of the resulting dermal window was measured. Macrolon skin chambers were then applied on the dermal windows obtained. The skin chambers were filled with 1 mL of neutral medium [Hanks balanced salt solution (HBSS)]. This was left in place overnight in order to obtain a steady state of permeability of the vascular network underlying the chambers.

The next day (day 4), 30 minutes before taking the morning dose of the study drug, the chamber fluids were renewed with freshly prepared HBSS. Immediately after taking the drug (at 0

hours), 1 mL of each of the following substances was introduced into the chambers: chamber 1, HBSS (diluant control chamber); chamber 2, histamine (1 $\mu\text{g/mL}$); chamber 3, Staller-gènes grass pollen extract (0.01 IR = Index of Reactivity), for the 2-hour "single" allergen challenge period; and chamber 4, grass pollen extract (0.01 IR), for the "sustained" 24-hour allergen challenge period. Fluids from chambers 1, 2, and 4 were collected and replaced by identical, freshly prepared solutions every 2 hours during the following 8 hours (ie, H2, H4, H6, and H8). After the allergen-containing fluid was collected from chamber 3 at H2, it was replaced by freshly prepared HBSS at 2, 4, 6, and 8 hours. The chamber fluids replaced at H8 were then left in place overnight. On day 5, 24 hours postchallenge, these chamber fluids were recovered for leukocyte counts and the chambers were then removed just before taking the study drug.

The chamber fluids recovered at H2, H4, H6, and H8 were centrifuged and the supernatants were stored at -20°C until assayed for the following substances: histamine (by immunoassay—Immunotech, Marseille, France), except for the fluid from chamber 2; tryptase (radio immunoassay—Pharmacia, Sweden); total protein (Lowry method); leukotriene LTC_4 (radio immunoassay—Amersham, UK); and sICAM-1 (immunoassay—R & D Systems, UK). Results of assays on samples recovered at H2 were considered to represent the early response. Results at H2, H4, H6, and H8 were combined and the resulting sum was considered to represent the late-phase response to the single and the sustained challenge.

Inflammatory cells in chambers fluids collected at H24 (day 5) were examined after centrifugation and staining with trypan blue using Malassez slides. Cells were cytocentrifuged onto two sets of coverslips. The coverslips were air-dried and stained with May-Grunwald-Giemsa stain for leucocyte differential counts or acidic toluidine blue for basophil counts.

Drug Concentration Assays

The plasma and blister fluid mizolastine concentration in samples collected on day 3. It was taken 4 hours after taking the morning dose of the study was measured by gas chromatographic analysis.¹⁵

Statistical Analysis

The objective of the study was to compare the effects of mizolastine versus placebo on pharmacologic and cellular events occurring in the early and late phases of an IgE-dependent reaction after single (2 hour) and sustained (24 hour) allergen challenges. The results of the individual subjects' measurements of histamine, tryptase, sICAM-1, protein, and LTC_4 present in skin chamber fluids were pooled for the analyses. Results during the drug treatment were compared with those during placebo treatment, for 2 hours and for the first 8 hours (the sum of values Σ 8h).

Results are expressed as mean $\pm$ standard deviation (SD). Analyses were performed using an ANOVA model including as main factors sequences, period, and treatment. The frequency effect was tested using patient (sequence) as the error term. The statistical significance of differences was estimated using a paired test. Significance was accorded at the 5% level.

RESULTS

Drug Concentration in Plasma and Blister Fluid

The concentration of mizolastine in the plasma and in the blister fluid 4 hours after the administration of the drug on the third day of treatment was $201 \pm 33 \mu\text{g/L}$ (range 152 to $310 \mu\text{g/L}$) and 283 ± 88 (range 107 to $376 \mu\text{g/L}$), respectively. No mizolastine was detected in either plasma or blister fluids during administration of the placebo.

Release of Mediators and Proteins in Skin Chambers

In skin chambers containing HBSS (control), the concentrations of histamine in the 0 to 2-hour sample and in the 0 to 8-hour summed sample were low, and no tryptase was detected. Likewise, only minimal amounts of

proteins, sICAM-1, and LTC₄ were detected in these samples (Figs 1–3).

Histamine. During the placebo treatment period, within the first 2 hours of allergen challenge, a significant amount of histamine was released in comparison to the chamber without allergen (56.0 ± 41.4 versus 1.4 ± 1.7 ng/mL/cm², $P = .0007$, $n = 12$). Two-hour allergen-induced histamine release during mizolastine administration (69.3 ± 46.0 ng/mL/cm², $n = 12$) was not significantly different from that which occurred during the placebo treatment period.

As we had previously reported,¹¹ with sustained allergen challenge, histamine release continued at a lower rate during the subsequent 6 hours, leading to the release of a total of 79.9 ± 50.4 ng/mL/cm² ($n = 12$) over the 8-hour period. Mizolastine did not modify this sustained histamine release significantly (data not shown). Additional evidence of allergen-induced activation of tissue mast cells was provided by the results of the tryptase assay at the sites exposed to allergen for up to 8 hours. No effect of mizolastine on tryptase release was observed in comparison to placebo at 2 hours: 68.0 ± 62.4 ng/mL/cm²; over 2 to 8 hours: 97.8 ± 75.2 ng/mL/cm² ($n = 6$); comparison of allergen versus placebo, NS.

Protein diffusion. During placebo treatment, there was a significant increase in the protein concentration in chambers exposed to either histamine or allergen for 2 hours as compared with the control chamber (Fig 1). Over 8 hours, significant protein exudation was also measured in both histamine and allergen-containing chambers. Mizolastine administration markedly inhibited the effect of histamine on the protein extravasation (after 2 hours: $P = .003$ and after 8 hours: $P = .009$), reducing it to nearly control levels. It likewise significantly inhibited the action of allergen on protein extravasation (after 2 hours: $P = .044$ and after 8 hours: $P = .003$).

sICAM-1 release. During placebo treatment, both histamine and allergen induced the release of significant

amounts of sICAM-1 at 2-hour sites and there was continued accumulation of sICAM-1 in the chamber fluid during the 8 hours of sustained exposure to these agonists (Fig 2). Mizolastine compared with placebo significantly inhibited the release of sICAM-1 in sites challenged with histamine for 2 hours ($P = .004$) or for 8 hours ($P = .05$). It also significantly inhibited sICAM-1 in sites challenged with allergen for 2 hours ($P = .03$), but the difference between the mizolastine treatment period and the placebo treatment period in sites challenged for 8 hours (35.2 ± 16.0 versus 45.0 ± 28.1 ng/mL/cm²) did not reach statistical significance.

LTC₄ release. During placebo treatment, both histamine and allergen challenge induced significant release of LTC₄ observed at 2 hours, and the levels were even greater after the 8th hour of challenge with both stimuli (Fig 3). Compared with the placebo period, a decrease in LTC₄ release was observed after mizolastine administration in chambers containing histamine during both early and late reactions. The difference was not statistically significant because of large individual variations.

Inflammatory Cells in Chamber Fluids and Adhering to the Base of Chamber Sites at 24 Hours

The overall number of leukocytes was similar at 24 hours in all the chambers

(data not shown). Although most all the cells were neutrophils ($\geq 95\%$) in both HBSS and histamine chambers, eosinophils were detected after histamine ($\leq 1\%$) and allergen challenge (2.5% to 5%). The remaining leukocytes (1% to 2.5%) were monocytes or lymphocytes. No basophils were seen in any of the chamber fluids. Mizolastine did not alter this pattern in this model.

DISCUSSION

The purpose of the present study was to examine the potential inhibitory effects of mizolastine, a new histamine H₁-receptor antagonist, on the pharmacologic and cellular events in the early and the late-phase allergic reactions using a skin chamber model in pollen-sensitive patients.

The concentration of mizolastine in plasma recovered 4 hours after administration of the drug on the third day of the treatment period was within the therapeutic range previously reported for this drug,¹⁵ indicating the subjects' complete compliance with the treatment regimen.

During the placebo treatment period, sustained histamine challenge in the skin chambers, used as a positive control for the antihistaminic effect of the study drug, induced the expected vasodilatation as indicated by the significantly greater level of protein in fluid from these chambers compared to

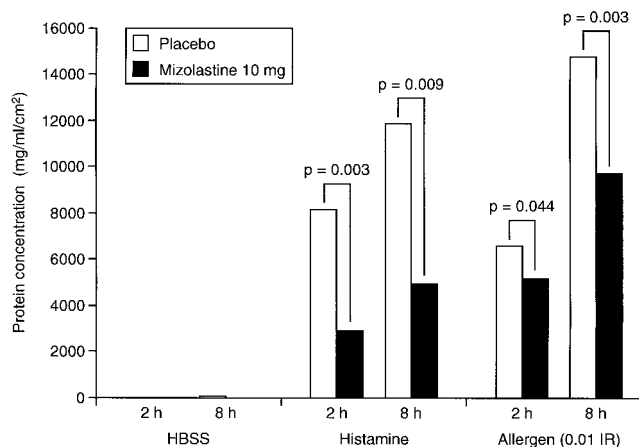


Figure 1. Mean of protein concentration ($\mu\text{g/mL/cm}^2$) in chamber fluids after control, histamine, and sustained grass pollen challenge during placebo and mizolastine treatment.

fluid recovered from chambers containing buffer only. This histamine effect was maximal during the first 2 hours, two-thirds of the protein accumulating between 0 and 8 hours. Allergen challenge likewise induced vasodilation, but the kinetics were quite different compared to the vasodilation induced by histamine. Allergen challenge resulted in a low 2-hour lasting vasodilatation but there was significant vasodilatation with the 8-hour sustained allergen challenge as compared with control HBSS chambers. The difference in the kinetics of the response to histamine and allergen could be explained by the fact that the concentration of exogenous histamine in chambers challenged was more than 10-fold greater compared with the level produced in allergen-containing chambers. Mizolastine was observed to significantly reduce the protein concentration in chamber fluids, in both histamine and allergen-challenged chambers.

The accumulation of significantly greater amounts of histamine and tryptase in the 2-hour allergen-challenge chambers compared with HBSS-containing chambers (data not shown) confirms the local and immediate activation of mast cells. Further, our present results also imply that mast cells (and possibly basophils) continue to respond to allergen during sustained challenge, with a long lasting release of histamine. This release and the mast cell secretion of tryptase were not modified by a 4-day intake of mizolastine.

Allergen-induced activation of mast cells and basophils induces the liberation of products of arachidonic acid metabolism, including LTC₄, during ongoing cutaneous allergic responses.¹⁶ In the present study, LTC₄ release was greater with sustained challenge than with single challenge in both histamine and allergen containing chambers, in agreement with the report of Talbot et al.¹⁶ We found that LTC₄ continued to be released during the sustained allergen challenge and this can be explained by continued stimulation of the tissue mast cells and/or secretion of LTC₄ from incoming leukocytes, including eosinophils and

monocytes,¹⁷ since both these cells are present at the blister base and in the dermis underlying the chambers.

Compared with HBSS, both histamine and allergen induced the release of significant amounts of sICAM-₁ into the skin chamber fluid, and this response was observed over the 8 hours of sustained challenge continued. The source of this cell adhesion molecule is most likely endothelial cells of the local vasculature.¹⁷ ICAM-₁ is typically expressed constitutively at a low level on unstimulated endothelial cells and it is also found on a variety of nonvascular cell types, including epithelial cells in the nose and conjunctivae, al-

though it is not expressed constitutively on these cell types.⁹ Surface ICAM-₁ is released from endothelial cells into the extracellular space as a result of proteolytic cleavage.¹⁸ Under the influence of various cytokines, for example, IL-1 and TNF α (both of which can be secreted by allergen-activated mast cells¹⁹), endothelial cells express increased amounts of ICAM-₁ on their surface.²⁰ Furthermore, in response to histamine, endothelial cells can redistribute adhesion molecules from cytoplasmic storage granules to their surface within minutes.¹⁷ Histamine may therefore be one of the most potent factors responsible for the in-

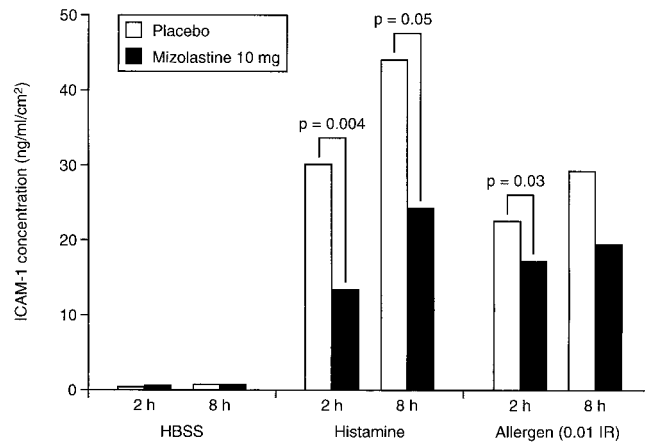


Figure 2. Mean of soluble ICAM-1 concentration (ng/mL/cm²) in chamber fluids after control, histamine, and single grass pollen challenge during placebo and mizolastine treatment.

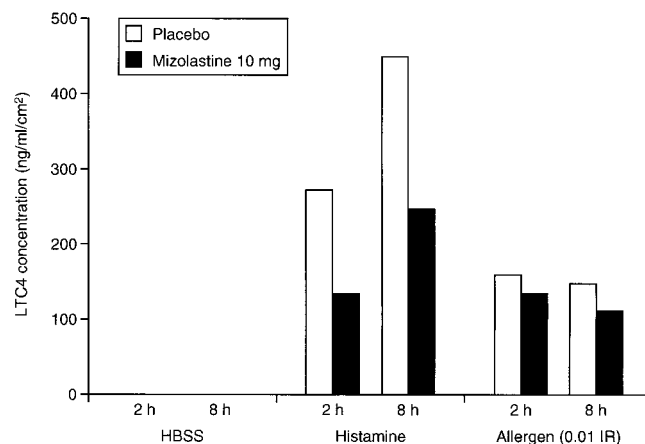


Figure 3. Mean of LTC₄ concentration (ng/mL/cm²) after control, histamine, and single grass pollen challenge during placebo and mizolastine treatment.

crease in sICAM-1 that we observed in the histamine-challenged and allergen-challenged chamber fluids.

During the mizolastine treatment period, there was a significant decrease in the level of sICAM-1 in fluids from the histamine-challenged and allergen-challenged chambers (although more marked in the former than in the later) compared with the levels detected during the placebo treatment period. Previous reports have shown that administration of cetirizine or loratadine to allergic patients inhibits the expression of ICAM-1 on nasal epithelial cells^{21,22} and reduces the release of sICAM-1 in nasal secretions.¹⁰ Canonica has stated, however, that cetirizine has no effect on ICAM-1 expression by human endothelial cells.⁹

In our *in vivo* model of human cutaneous allergic reactions, mizolastine does inhibit the release of ICAM-1, most likely coming from *endothelial* cells, partly through its H₁ antagonist activity.

On the other hand, allergen-activated mast cells as well as the inflammatory cells that enter the site of an allergic reaction are the source of cytokines, including interleukins and TNF- α .^{23,24} These are substances that can stimulate endothelial cells to up-regulate the expression of ICAM-1. Beside histamine, cytokines coming from allergen-activated mast cells, as well as from incoming leukocytes, could have stimulated endothelial cells to overexpress ICAM-1. Mizolastine administration may therefore act by modulating this cascade of events to lead ultimately to reduced solubilization of ICAM-1.

As we have reported previously,¹¹ the large majority of the cells recruited into HBSS and histamine chamber 24 hours after the initial challenge were neutrophils ($\geq 99\%$ in most cases). Although the proportion of eosinophils in fluid from chambers containing either HBSS or histamine was less than 1%, the fluids from allergen chambers contained significantly more eosinophils the proportion being, on average, from 2.5% to 5%.

Cell adhesion molecules including ICAM-1 are necessary for cell recruitment, which includes primary adhesion (rolling), firm adhesion and trans-endothelial migration.²⁵ Indeed, firm adhesion of intravascular leukocytes to the vascular endothelium has been shown to be mediated by leukocyte integrins interacting with endothelial cell surface adhesion molecules such as ICAM-1 and VCAM-1.^{17,25}

In conclusion, mizolastine, a newly developed second generation antihistamine, when administered to pollen-allergic patients at a dose of 10 mg daily for 4 days, effectively inhibited the action of histamine and allergen on the local vasculature in a skin chamber model allowing study of the early and late phase allergic reaction. Although it did not significantly inhibit the activation and secretory response of cutaneous mast cells in this model, mizolastine treatment was associated with significant decreased levels of the soluble adhesion molecule ICAM-1 and proteins in response to sustained antigen and exogenous histamine *in vivo* challenge.

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