

The effect of a histamine synthesis inhibitor on the immediate nasal allergic reaction

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We studied the effect of α -fluoromethyl histidine, an irreversible histamine synthesis inhibitor, on the immediate nasal reaction to antigen challenge in a double-blind, placebo controlled, randomized, parallel study using 13 subjects. The patients received either active drug 100 mg twice daily or placebo, for 3 weeks. A nasal allergen challenge was performed before and after at weekly intervals. Symptoms at challenge were assessed and the levels of histamine, TAME-esterase activity and kinins were measured in nasal lavages before and after antigen challenge. Skin tests were also performed at weekly intervals. In addition, the urinary excretion of the main histamine metabolite, tele-methylimidazole acetic acid, was measured before and after 3 weeks of treatment. The active treatment induced 60% reduction in histamine levels in the lavage fluids before and after antigen challenge, as well as a reduction in the histamine levels in the lavage fluids before and after antigen challenge, as well as a reduction in the main urinary histamine metabolite. However, no reduction was found in nasal symptoms obtained after antigen challenge. The levels of kinins and TAME-esterase activity were not significantly reduced.

Key words: alpha fluoromethyl histidine; allergen challenge; histamine; histamine synthesis inhibition; nasal challenge; nasal lavage.

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CLINICAL ASPECTS

Although it was possible to reduce selectively the release of histamine occurring in response to nasal allergen challenge during prolonged treatment with a histamine synthesis inhibitor, no reduction of induced symptoms was found. This observation, combined with a significant number of side effects, makes this an unlikely therapeutic approach. These results suggest that mediators other than histamine are important in the induction of the signs and symptoms of allergic rhinitis.

Histamine is considered to be one of the major mediators of nasal allergic reactions. This statement is supported by the findings that nasal challenge with histamine elicits all the symptoms of nasal allergy (16) and that antihistamines effectively ameliorate hay fever. The latter has been questioned because, although the main action of the classical antihistamines is considered to be through a blockade of histamine receptors, these compounds possess other ef-

fects: anticholinergic activity (15) and mediator release blocking properties (10, 12). Since the exclusive role of histamine has been questioned during the last decade, interest has focused on other putative mediators of allergic reactions, such as leukotrienes, prostaglandins, kinins, serotonin, platelet activating factor and a variety of neuropeptides (2, 7, 8, 11, 13, 17-19, 21). Another approach in studying the role of histamine is to selectively reduce the amount of

histamine available for release. Knowing that histamine is synthesized intracellularly from histidine via the actions of the enzyme histidine decarboxylase, one can block the generation of histamine with α -fluoromethyl histidine, an irreversible inhibitor of this enzyme (4, 9).

The aim of the present investigation was to see whether it was possible to alleviate the symptoms of nasal allergy by a selective reduction of the amount of histamine available for release.

PATIENTS AND METHODS

Study design

The study was a double blind, placebo controlled, parallel group comparative clinical trial. Nasal challenges and skin tests were performed before, and at weekly intervals during the 3-week study. The 24 h excretion of the main urinary histamine metabolite, telemethylimidazole acetic acid, was measured before and after 3 weeks of treatment.

The study comprised 13 subjects (7 men and 6 women) aged 22 to 47 years (mean age 31.5 years) who had a history of strictly seasonal allergic rhinitis due to birch and/or grass pollen. All subjects had a positive history of allergic rhinitis, a positive skin prick test and a positive nasal challenge to the relevant allergen. The patients were fully informed of the aims and the methods of the present investigation, and the study was approved by the Ethics Committee at the University of Göteborg.

Skin tests. Intradermal skin tests were performed in a serial manner until a wheal diameter of 10 mm was obtained. Ten-fold increasing doses of timothy or birch pollen extracts were used (Pharmalgen®, Pharmacia, Uppsala). The last dose was then repeated weekly during the study, and the diameters of the induced wheal were measured.

Nasal challenge procedure. The technique used for the nasal challenge has previously been described in detail (13). Briefly (see Fig. 1), the

protocol involved prechallenge lavages with saline solution to reduce to a stable baseline the levels of cell-free mediators that are present initially in nasal secretions. Then 0.1 mg/ml of oxymetazoline hydrochloride (Nezeril®, Draco, Sweden) was sprayed into the nose (two sprays of 50 μ l/nostril) to maintain nasal patency during the allergic reaction and thereby allow the collection of nasal secretions. It has been shown previously that this dose of oxymethazoline does not affect mediator release (13). Subsequently, two challenges with diluent (the same as for pollen extracts) were employed to control for the effect of the delivery system. Thereafter, serial challenges with 10-fold increasing doses of antigens were performed. Lavages with saline solution were collected 10 min after all challenges. In addition, a follow-up lavage was performed 20 min after the last antigen dose. The levels of histamine, kinins and TAME-esterase in the nasal lavages were measured.

Mediator assays. Histamine was measured with a spectrofluorometric assay sensitive to 1 ng/ml, kinins with a radioimmunoassay sensitive to 20 pg/ml, and TAME-esterase activity with a radiochemical assay. Additional information can be obtained in the references provided (13, 17, 20).

Measurement of urinary histamine metabolites. Urine was collected for 24 h, once before treatment and once after 3 weeks of treatment. The urine was analyzed for the main histamine metabolite, telemethylimidazole acetic acid (MeImAA), by HPLC as described earlier (5). During the day of urine collection the patients ate a standardized diet with a low histamine content (6).

Assessment of symptoms. A detailed symptom score sheet was used during the challenge procedure. In addition to counting the number of sneezes, a six-point scale from 0 to 5, with 0 = symptoms and 5 = severe symptoms, was used for assessing the following symptoms: nasal stuffiness, nasal secretion and nasal itchiness. The mean value of these three symptoms was calculated as a composite nasal symptom score. Possible side effects were also noted.

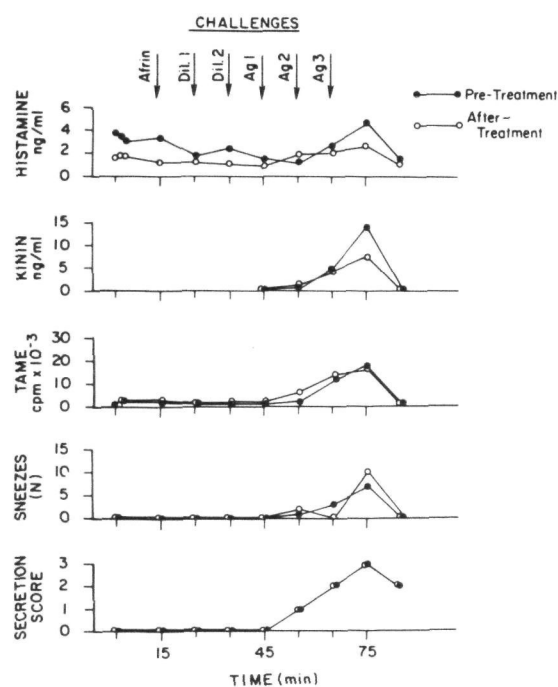


Fig. 1. Nasal challenge protocol with results of mediator measurements and symptoms in one patient before and after 3 weeks of active treatment. After treatment the amount of histamine released was lower throughout the challenge procedure.

Drug. The patients recieved either active drug or placebo for the 3-week study period. The active drug was 100 mg of α -fluoromethyl histidine b.i.d. The corresponding placebo tablets were identical in appearance and taste and contained only the vehicle. A complete blood chemistry (ESR, HB, WBC, differential count blood glucose, creatinine, ALAT, ASAT, albumin electrolytes), urineanalysis and ECG were made before and after the treatment period.

Statistics. To ensure whether active treatment induced any reduction in the levels of mediators (histamine, TAME-esterase and kinins) recovered at peak challenge, and analysis of variance and covariance for repeated measurements was made. In this evaluation the 3-weekly observations after treatment, in relation to their pretreatment values were compared for the active and placebo treated patients. The same analysis was also employed for the levels of histamine in the first prechallenge lavage. Further analysis between the pretreatment and 3-week post treatment mediator levels for the

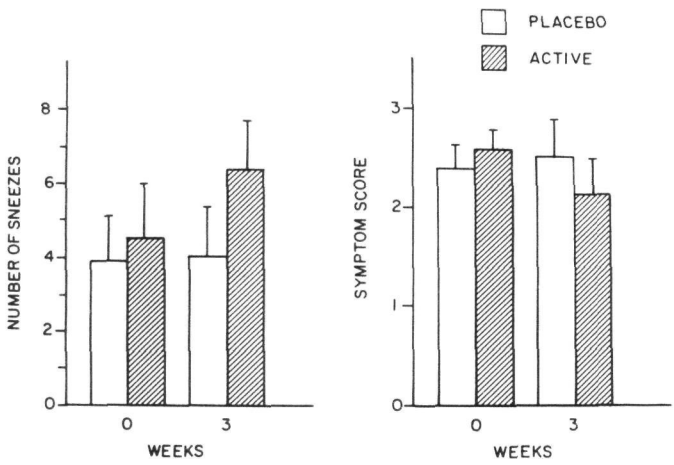


Fig. 2. Symptoms obtained at peak challenge. No statistically significant changes were found.

same peak and prechallenge parameters as above, symptoms, skin test, and urinary levels of the histamine metabolite was made with Student's t-test, for paired samples (before and after), and non-paired samples (active versus placebo treated patients).

RESULTS

One individual stopped treatment after 2 days due to extreme tiredness (active treatment), thus leaving 12 individuals for the study. Five subjects had received active drug and seven had received placebo. The treatment did not result in any change in skin test reactions. Mean diameter before active treatment was 8.2×7.2 (mm $\times$ mm) and after 3 weeks of treatment 7.6×6.8 (mm $\times$ mm) (N.S.). An example of the results from the nasal challenge procedure in one individual is shown in Fig. 1. The group mean data for symptoms score and number of sneezes before and after 3 weeks of treatment are shown in Fig. 2. Changes observed due to treatment are not significant.

Although the initial challenge showed no difference between the active and placebo group for the histamine levels in the lavage fluid, the analysis of variance and covariance for repeated measurements revealed a statistically significant post treatment difference between active and placebo treatment for the histamine levels in the lavage fluid recovered at the start of the chal-

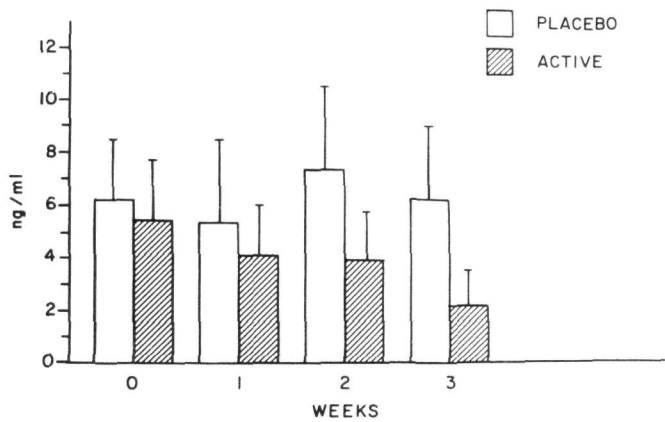


Fig. 3. Histamine levels (mean + SEM) at peak challenge (after highest antigen dose). A significant reduction as compared to placebo ($P < 0.05$) was seen after 3 weeks of active treatment.

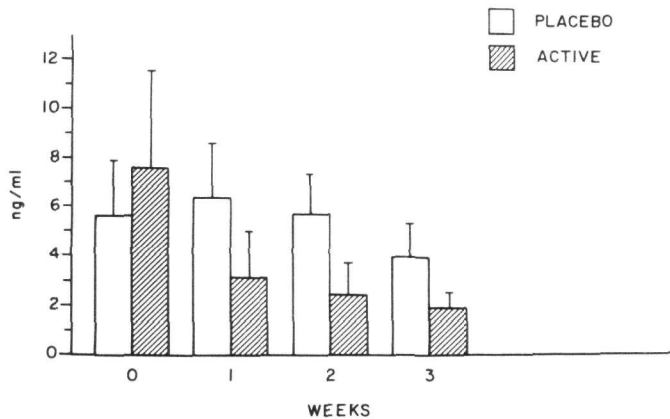


Fig. 4. Kinins (mean + SEM) in nasal lavages at peak challenge (after highest antigen dose). No statistically significant differences were found.

challenge procedure (pre 1) ($P < 0.05$) and at the peak of the allergen induced reaction (highest antigen dose) ($P < 0.05$). The same analysis revealed no statistically significant difference between active and placebo treatment for the levels of kinins and TAME-esterase at peak challenge. Further analysis was made using Student's test for the comparison between day 0 and the last day of treatment as described above. The baseline (pre 1) histamine level in the actively treated patients was reduced from 5.4 ± 2.5 ng/ml (mean $\pm$ SEM) to 1.9 ± 0.5 ng/ml (mean $\pm$ SEM). The corresponding values for the placebo treated patients were 4.6 ± 1.3 ng/ml (mean $\pm$ SEM) before and 5.0 ± 1.0

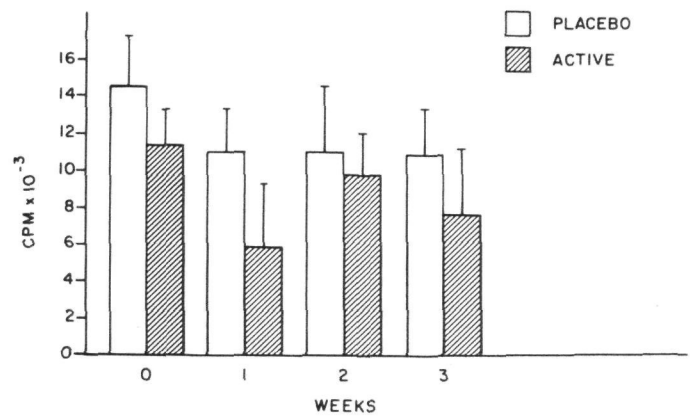


Fig. 5. TAME-esterase activity (mean + SEM) in nasal lavages at peak challenge. No statistically significant differences were found.

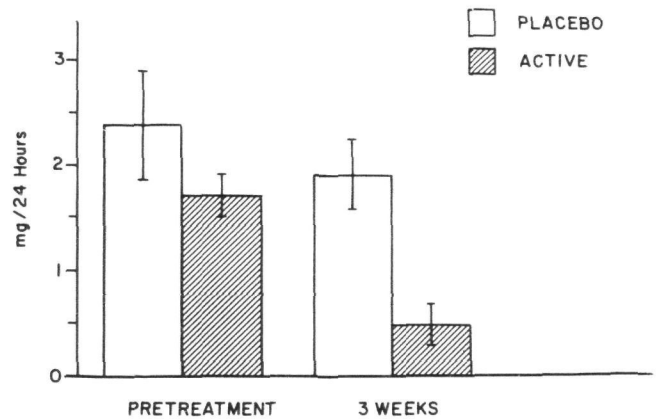


Fig. 6. 24 h excretion of methyl imidazole acetic acid (MeImAA) (mean + SEM). The reduction seen in the actively treated group is statistically significant ($P < 0.05$) and is consistent with the findings for histamine in the nasal lavages.

ng/ml (mean $\pm$ SEM) after treatment. The difference between the actively and placebo treated patients on day 21 was significant ($P < 0.05$). The difference between actively and placebo treated patients on day 21 was also significant for the peak histamine levels ($P < 0.05$) which are shown in Fig. 3. Any changes for the other mediator measurements which are shown in Figs. 4 and 5 are not significant, although the kinin levels are approaching significance ($P = 0.08$) in the comparison between prevalues (day 0) and 3-week values for the actively treated group.

The measurements of the main urinary hista-

mine metabolite are shown in Fig. 6. A similar reduction of the histamine metabolite excretion ($P < 0.05$) as for the histamine levels in the lavage fluid was found.

Severe tiredness was a side effect observed in four of the five actively treated patients, in addition to the drop out. One of these five also complained of impotency which was reversed after treatment stopped. No changes in any of the blood parameters, urinalysis and ECG were found.

As the majority of patients in the present study suffered annoying side effects, an attempt to overcome this problem was to study the drug at a reduced dosage. The same study was repeated using only half the daily dose (100 mg daily). This reduction abolished the incidence of side effects (unpublished observation) but did not reduce the levels of histamine in lavage fluids before challenge (baseline) or after challenge.

DISCUSSION

To investigate the role of mediators in allergic rhinitis, a novel approach to reducing the amount of histamine reaching the receptors sites following a nasal antigen challenge was undertaken. Instead of using a competitive histamine antagonist, an effort was made to reduce the amount of histamine available for release through blockage of its synthesis. Despite the fact that the histamine storage capacity is unknown, we achieved a 60 % reduction of this mediator after 3 weeks of treatment with α -fluoromethyl histidine. This reduction did not influence the symptoms obtained at nasal challenge or the generation of TAME-esterase activity and kinins. Our data suggest a 3-week half-life of histamine stores in the nasal mucosa. This is consistent with the estimated half-life of the histamine in basophils (14). An equal reduction (60 %) in the histamine content of nasal lavages and urinary histamine metabolites was noted after 3 weeks treatment. Whether further reduction can be obtained by more prolonged treatment with α -fluoromethyl histidine treatment remains to be clarified, although such a prolonged treatment for a seasonal disease seems unjustified.

The increase in the levels of histamine in nasal lavage after nasal challenge has been questioned (3). This study supports our previous observation by modulating the level of histamine in nasal lavage in clinically equivalent nasal challenges.

Our results raise questions with regards to the action of antihistamines. If they function solely as antagonists, it is unlikely that the concentration in the nasal mucosa after an orally administered drug antagonises more than 60 % of the released histamine, particularly at the site of degranulation. Thus antihistamines probably function clinically by other mechanisms as well (9, 11, 14).

Thus, we have shown that reducing the amount of histamine available for release in the immediate response to nasal challenge with antigen did not alter the symptoms of the response. If the degree of reduction in histamine release (60 %) can be considered physiologically significant, then our studies appear to question the central role of histamine in the acute response to nasal allergen challenge. Conversely, the amount of histamine which was released (40 %), as compared to control, may be sufficient to induce nasal symptoms. This concept is supported by data indicating that 1–2 % histamine release is sufficient to cause greater than 50 % maximal contraction of airways (2). On the other hand, the overlapping properties of the various inflammatory mediators may eliminate the predominance of any one mediator. Our studies do not preclude a role for histamine as either a immunoregulator or as a participant in the inflammatory response following allergen challenge.

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REFERENCES

1. Adams, G. K. III. & Lichtenstein, L. M.: In vitro studies of antigen-induced bronchospasm: Effect of antihistamine and SRS-A antagonist on response of sen-

- sitized guinea pig and human airways to antigen J. *Immunol.* 122, 558-562, 1979.
2. Creticos, P. S., Peters, S. P., Adkinson Jr, N. F. et al.: Peptidoleukotriene release after antigen challenge in patients sensitive to ragweed. *N. Engl. J. Med.* 310, 1626-1630, 1984.
3. Davies, R., Corrado, O. J., Änggård, A. & Borum, P.: Rhinitis and asthma. In Mygind, M. & Weeke, B. (eds.): *Clinical aspects of allergic and vasomotor rhinitis*. pp. 65-79. Munksgaard, Copenhagen, 1986.
4. Garbarg, M., Barbin, G., Rodergas, E. & Schwartz, J. C.: Inhibition of histamine synthesis in brain by alfa-fluoromethylhistidine, a new irreversible inhibitor: in vitro and in vivo studies. *J. Neurochem.* 35, 1045-1052, 1980.
5. Granerus, G., & Wass, U.: Urinary excretion of histamine, methylhistamine and methylimidazoleacetic acid in mastocytosis: Comparison of new HPLC methods with other present methods. *Agents Action* 14, 341-345, 1984.
6. Granerus G.: Urinary excretion of histamine, methylhistamine and methylimidazoleacetic acid in man under standardized dietary conditions. *Scand J. Clin. Invest. Suppl.* 104, 59-68, 1968.
7. Jackson, R.: Prostaglandin E as a nasal constrictor in normal human volunteers. *Curr. Ther. Res.* 50, 165-169, 1970.
8. Karim, S. M. M. & Adakian, P. G.: Effects of topical prostaglandins on nasal patency in man. *Prostaglandins Med.* 3, 33-37, 1978.
9. Kollonitsch, J., Patchett, A. A., Marburg, S. et al.: Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature* 274, 906-908, 1978.
10. Lichtenstein, L. M. & Gillespie, E.: The effect of H₁ and H₂ antihistamines on "allergic" histamine release and its inhibition by histamine. *J. Pharmacol. Exp. Ther.* 192, 441-450, 1975.
11. Lundblad, L.: Protective and vascular effects in the nasal mucosa elicited by activation of capsaicin-sensitive nerve fibers. Thesis, University of Stockholm, Sweden, 1984.
12. Naclerio, R. M., Meier, H. L., Kagey-Sobotka, A., Norman, P. S. & Lichtenstein, L. M.: In vivo model for evaluation of topical antiallergic medication. *Arch. Otolaryngol.* 110, 25-27, 1984.
13. Naclerio, R. M., Meir, H. L., Kagey-Sobotka, A. et al.: Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.* 128, 597-602, 1983.
14. Parwarech, M. R.: *The human blood basophil. Morphology, origin, kinetics, function and pathology*. Springer, Berlin, 1976.
15. Pearlman, D. S.: Antihistamine, pharmacology and clinical use. *Drugs* 12, 258-277, 1979.
16. Pipkorn, U.: Budesonide and nasal histamine challenge. *Allergy* 37, 359-363, 1982.
17. Pipkorn, U., Karlsson, G. & Bake, B.: The effect of platelet activating factor on the human nasal mucosa. *Allergy* 39, 141-145, 1984.
18. Proud, D., Togais, A., Naclerio, R. M. & Lichtenstein, L. M.: Kinins are generated in vivo following nasal airway challenge with allergen. *J. Clin. Invest.* 72, 1678-1685, 1983.
19. Shaw, R. J., Fitzharris, P., Cromwell, O., Wardlaw, A. J. & Kay, A. B.: Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB₄ in allergic rhinitis. *Allergy* 40, 1-6, 1985.
20. Siraganian, R. P.: An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* 57, 383-394, 1974.
21. Tønnesen, P. & Mygind, N.: Nasal challenge with serotonin and histamine in normal persons. *Allergy* 40, 350-353, 1985.

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