

Effect of astemizole on antigen-mediated histamine release from the blood of patients with allergic rhinitis

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The main objective of this study was to test the effectiveness of astemizole *in vitro* in blocking the release of histamine from blood of patients with allergic rhinitis. The results of this investigation indicated that astemizole inhibited allergen-mediated histamine release from blood basophils of patients with this allergic disorder. The inhibition by astemizole (33–156 μmol) was immediate, requiring no pre-incubation of the cells, and was dose-dependent, with maximal inhibition of about 91%. The relatively high potency of astemizole in inhibiting the immunologic release of histamine may provide an additional measure in the treatment of allergic rhinitis with this H_1 -receptor antagonist.

Clinical aspects

It is known that some antihistaminic drugs may exercise an inhibitory effect on histamine release. In the present study, we have examined the effect of astemizole at a concentration range of 33 to 156 μmol on allergen-mediated histamine release from patients with allergic rhinitis. The results of this study showed that astemizole significantly inhibited the release of histamine after challenging the blood samples with common environmental allergens. Our data suggest that the beneficial effect of astemizole in treatment of allergic disease may be due not only to its antihistaminic property, but also to a direct inhibition of chemical mediator release from blood basophils and mast cells.

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Astemizole (Hismanal, Janssen Pharmaceutica) is an orally potent, long-acting H_1 -receptor antagonist, devoid of central, sedative, and peripheral anticholinergic effects (1). Clinical studies have shown it to be clearly superior to placebo in the treatment of allergic rhinitis (2–4).

In addition to blocking H_1 receptors, many antihistamines have the capacity *in vitro* and *in vivo* to stabilize mast cells and basophils, and to inhibit histamine release (5, 6). Agents capable not only of antagonizing histamine-mediated responses, but also of effectively inhibiting the release of allergic mediators, are more valuable when treating human allergic disease. To determine whether astemizole might exert effective inhibitory action in human tissues, we utilized a newly developed histamine-dependent allergy blood test (7) to examine the influence of astemizole *in vitro* on allergen-mediated histamine release from the blood of patients with allergic rhinitis.

Material and methods

Twenty-five patients ranging in age from 12 to 55 years were included in the study. We obtained informed consent. Diagnosis of their allergic rhinitis was determined by history (i.e. symptoms of nasal itching, obstruction, discharge, sneezing, watering and itching of eyes), nasal smear, and a positive skin test. Patients excluded from this study were those pregnant, lactating, on steroids (local or systemic), on desensitization therapy, or with liver/renal disease.

The skin tests were performed with the same antigens used for *in vitro* histamine release. The concentrations used for skin testing were 1000 PNU/ml for house dust, dog dander, and tree pollen, and 100 PNU/ml for house-dust mite, cat dander, ragweed, and grass pollen. For skin tests, 0.02 ml of allergen was injected at each site on the upper arm with a disposable syringe, and reactions read at

15 min. Skin-test grading was based on measurements of the greatest diameter of wheal following the usual criteria: negative (site no different from diluent control), 3+ (8–12 mm of wheal), and 4+ (> 12 mm wheal).

Venous blood (10 ml) was drawn into evacuated tubes containing sodium heparin (143 USP units/tube). The blood was studied immediately or kept at 4°C up to 48 h.

Histamine release reactions were carried out in the presence and absence of varying concentrations of astemizole (33, 65, 131, 196 µmol). To each of 12 × 75 mm plastic tubes were added 0.5 ml of Tris-albumin buffer, the allergens (in PNU/ml) (Category I: house dust/house-dust mite, 200:5; Category II: cat/dog dander, 60:1000; and Category III: trees/grasses/ragweed, 150:150:100) and 0.5 ml of heparinized blood. Control tubes were prepared by mixing buffer with blood alone. The tubes were incubated in a shaking water bath for 60 min at 37°C. This was followed by centrifugation at 500 × *g* for 10 min. The plasma was separated and stored at –70°C until analysis. Samples for determination of total blood histamine were prepared by heating 0.5 ml of whole blood with 1.5 ml of 0.05 mol/l phosphate buffer (pH 7.9) in a boiling water bath for 20 min. After centrifugation at 15000 × *g* for 30 min, the supernatant was removed and stored at –70°C until analysis.

The concentration of histamine released in plasma (see above) was measured by a radioenzymatic technique according to Faraj et al. (8). The method is based on the use of purified enzyme histamine-*N*-methyltransferase (HNMT), isolated from rat kidney, to transfer a radioactive methyl group from the cofactor (3*H*-methyl)-*S*-adenosyl-L-methionine (SAM) to an endogenous histamine acceptor molecule in the sample to form the radioactive metabolite (3*H*-methyl)-*N*-histamine. This is followed by selective extraction of the liberated metabolite and its subsequent quantification by liquid scintillation counting. The lower level of sensitivity is 65 pg histamine/sample; intra- and interassay coefficients of variation are 5% and 8%, respectively.

The percentage of histamine released from 1 ml of blood by the allergen mixture in each of the three categories was determined from the following formula: $100 \times A/B = \% \text{ histamine release}$, where *A* = concentration of histamine in plasma following the incubation of 1 ml of whole blood with allergens, minus concentration of histamine in plasma following incubation of 1 ml of blood alone; *B* = concentration of total histamine obtained from 1 ml of boiled blood minus blank sample.

The percent (%) inhibition of allergic histamine release by astemizole was calculated by the following formula: $\text{inhibition (\%)} = (A - P)/A \times 100$, where *A* = % of histamine release in absence of drugs;

P = histamine release in the presence of the drug. The line of best fit was calculated from the concentration-effect curve of astemizole for each of the allergens in the three categories, and the concentration inhibiting histamine release by 50% (*IC*₅₀) was estimated.

Statistical analysis

The results obtained were expressed as the arithmetic mean and standard deviation (± SD). Student's *t*-tests were performed on the data to assess their statistical significance. Values of *P* < 0.05 were accepted as representing significant differences.

Results

Clinical observations

Of the 25 patients evaluated in this study with allergic rhinitis, 13, 7 and 18 exhibited 3+ to 4+ skin-test reactivity to allergens in categories I (house dust/house-dust mite), II (cat/dog dander), and III (trees/grasses/ragweed). In agreement with our previous report (7), there was a significant correlation between allergen-mediated histamine release (in % of total blood histamine) from whole blood (Fig. 1) and skin testing among these patients.

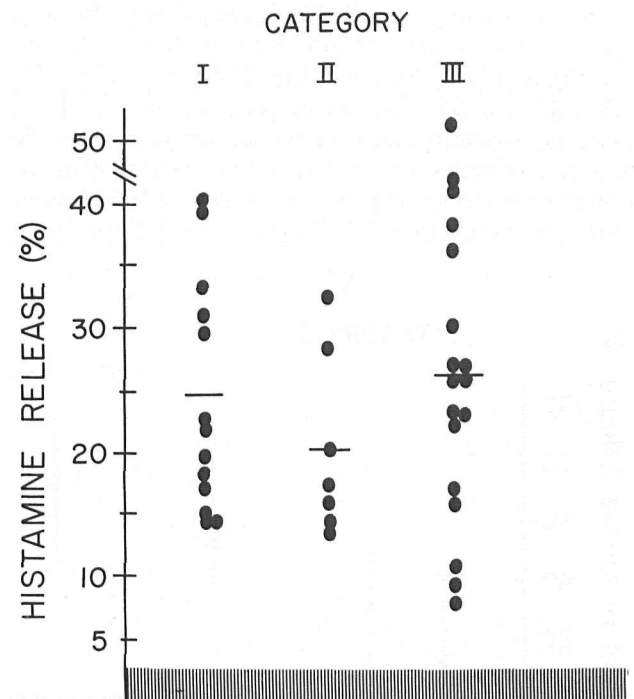


Fig. 1. Allergen-mediated histamine release (in % of total blood histamine) from whole blood of 25 patients with allergic rhinitis following incubation of their blood with common allergens in 3 major categories (category I, house dust/mite; category II, cat/dog; category III, trees/grasses/ragweed). Shaded area below 5% histamine release represents the release of histamine by nonatopic subjects.

Table 1. Inhibitory activity of astemizole against allergen-induced histamine release from blood basophils of 25 patients with allergic rhinitis (mean $\pm$ SD)

Allergen	Astemizole μ mol	Histamine release %	Inhibition %	IC ₅₀ μ mol
Category I (n=13) house dust/mite	0	24.2 $\pm$ 9.4		133 $\pm$ 57
	33	21.0 $\pm$ 10.3	13.3 $\pm$ 15.46	
	65	17.3 $\pm$ 7.0	30.4 $\pm$ 12.2	
	131	11.0 $\pm$ 7.0	53.2 $\pm$ 15.0	
	196	6.3 $\pm$ 4.2	73.0 $\pm$ 16.5	
Category II (n=7) cat/dog dander	0	20.0 $\pm$ 7.4		100 $\pm$ 45
	33	14.3 $\pm$ 2.3	16.5 $\pm$ 13.50	
	65	11.7 $\pm$ 1.5	36.0 $\pm$ 7.7	
	131	6.6 $\pm$ 2.8	66.3 $\pm$ 5.8	
	196	4.3 $\pm$ 1.5	77.5 $\pm$ 3.64	
Category III (n=18) trees/grasses/ragweed	0	26.0 $\pm$ 12.3		84 $\pm$ 51
	33	17.8 $\pm$ 11.0	33.1 $\pm$ 18.8	
	65	15.0 $\pm$ 8.6	46.4 $\pm$ 16.7	
	131	13.4 $\pm$ 8.3	58.0 $\pm$ 12.5	
	196	7.7 $\pm$ 6.1	74.6 $\pm$ 13.0	

Inhibition of allergen-mediated histamine release from whole blood by astemizole

Astemizole produced a concentration-dependent inhibition of histamine release from the blood basophils of patients with allergic rhinitis as a result of the incubation of the sample with common allergens in three major categories (Table 1, Figs. 2–4). The IC₅₀ (μ mol) for astemizole in inhibiting allergen-mediated histamine release for categories I–III were 133 $\pm$ 57, 100 $\pm$ 45, and 84 $\pm$ 51, respectively (Table 1). However, at concentrations below 25 μ mol, astemizole was not effective in inhibiting the release of histamine *in vitro* following the incubation of blood samples with environmental allergens. The inhibitory ef-

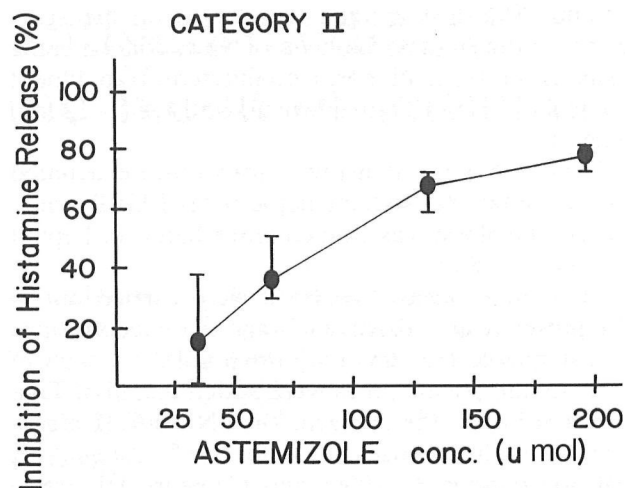


Fig. 3. Dose-response curve of astemizole inhibition of category II antigen-induced histamine release from blood basophils of patients with allergic rhinitis.

fects of astemizole on allergen-mediated histamine release from blood of patients with allergic rhinitis did not vary significantly regardless of its addition to same blood sample immediately (0 min) or after pre-incubation, for 10 or 30 min prior to allergen challenge.

Discussion

Histamine release from blood basophils represents a useful *in vitro* model for the study of reagenic hypersensitivity because of the shared biochemical pathways of IgE-triggered allergic mediator release in blood basophils and in tissue mast cells (9, 10). The purpose of this study was to use this model of allergic disease in order to assess the effectiveness of

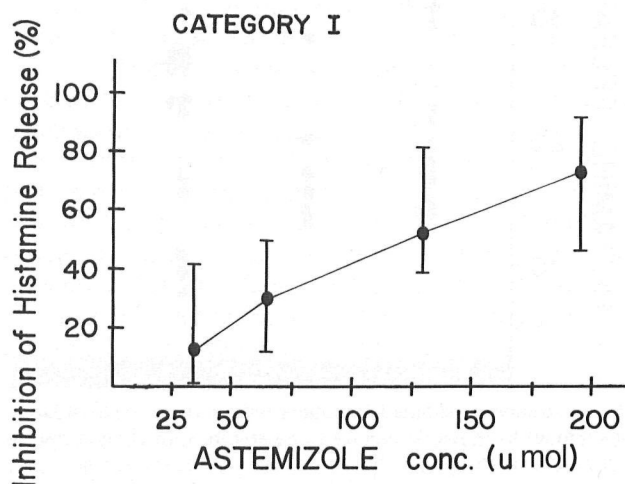


Fig. 2. Dose-response curve of astemizole inhibition of category I antigen-induced histamine release from blood basophils of patients with allergic rhinitis.

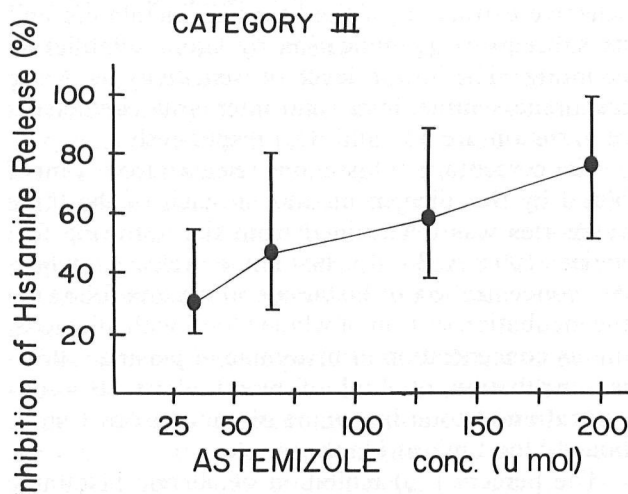


Fig. 4. Dose-response curve of astemizole inhibition of category III antigen-induced histamine release from blood basophils of patients with allergic rhinitis.

astemizole in inhibiting the release of histamine from blood basophils of patients with allergic rhinitis. This property of astemizole is important, as it is widely accepted that the release of chemical mediators by mast cells and/or basophils occurs early in the pathophysiology of allergic reaction (11).

Our data indicated that astemizole inhibited allergen-stimulated histamine release from blood basophils of patients with allergic rhinitis. The inhibition of astemizole (33–156 μmol) was immediate, requiring no pre-incubation of the cells, and was dose-dependent, with maximal inhibition of about 91%. This inhibition of allergen-mediated blood basophil histamine release by astemizole appears to be similar to the previously reported inhibition of IgE-mediated histamine release from human basophils (12), rabbit basophils (13), and rat peritoneal mast cells (14) by the antiallergic agent azelastine. Upon comparison with other H_1 antihistamines, astemizole was found to be a more potent inhibitor of allergic histamine release *in vitro* than azatadine (6, 15) and ketotifen (16). The mechanism of this inhibition remains unknown. It has been postulated that the interaction with H_1 cell surface receptors causes an increase in intracellular cyclic adenosine monophosphate, thereby inhibiting the release of allergic mediators (15).

Whether or not the inhibition of allergic mediator release by astemizole observed *in vitro* contributes to its therapeutic effect *in vivo* remains unclear. The 33 to 196 μmol concentrations of astemizole found to significantly inhibit allergen-mediated histamine release, as observed in these experiments, are greater than the plasma concentrations of 10 to 23 nmol found during therapy with astemizole (17). Due to astemizole's ability to bind irreversibly to H_1 receptors (18), the relationship of serum levels to clinical benefit is unclear. This could make the concentration of astemizole in relevant tissues, such as skin, nasal, and respiratory mucosa, much higher than those in plasma, and mediate drug effects in tissues not predicted by plasma concentrations. Indeed, Howarth et al. (17) noted that with an oral dose of 10 mg daily for 8 weeks, astemizole almost completely inhibited the skin wheal response to 50 $\mu\text{g}/\text{ml}$ of histamine and attenuated the wheal response induced by intradermal antigen by 50%. Such an effect lasted for at least 2 weeks after the end of the therapy. Interestingly, discrepancies between concentrations achieved in plasma and the concentrations effective *in vitro* have been noted for other antihistamines. Daniels & Temple (19) reported inhibition of antigen-induced histamine release from human lung fragments by 10 $\mu\text{mol}/\text{l}$ of azatadine, whereas plasma concentrations of the drug achieved therapeutically were reported to be approximately 50 nmol/l. Moreover, evidence in support of good

agreement between *in vitro* and *in vivo* experimental models of the allergic reaction was provided recently by Naclerio et al. (5), who showed that in patients with allergic rhinitis, terfenadine not only antagonized the H_1 activity of released histamine during the immediate allergic reaction, but also, at currently prescribed doses, inhibited the release of histamine. Further, Zimmermann et al. (20) studied the effect of treatment of the newly developed antiallergic compound picumast dihydrochloride on antigen-induced histamine release from blood leukocytes of patients with allergic obstructive airway disease. A significant decrease in allergen-mediated histamine release from leukocytes could be seen after treatment with this drug.

In summary, the present data provided evidence that astemizole *in vitro* can inhibit allergic histamine release from blood basophils of patients with allergic rhinitis. It remains an open question whether treatment with astemizole at currently prescribed therapeutic doses suppresses allergen-mediated histamine release from the blood of patients with allergic rhinitis that correlates with efficacy. If this is the case, premedication with astemizole would markedly affect the allergic response by reducing mediator release from mast cells and blood basophils in conjunction with antagonism of H_1 receptors.

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