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Inhibition of mediator release during the early reaction to antigen

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A nasal lavage model and a new filter paper disk model have been used to measure biologic and physiologic responses to antigen challenge in patients with allergic rhinitis. Pretreatment of subjects with cetirizine reduced the number of sneezes induced by antigen challenge but did not significantly reduce levels of histamine or prostaglandin D_2 in a double-blind, placebo-controlled trial with the lavage model. Pretreatment with 60 or 300 mg of terfenadine did significantly reduce levels of histamine, kinin, albumin, and TAME-esterase activity in a double-blind, placebo-controlled study with this model. Again with the nasal lavage model, a double-blind, placebo-controlled comparison of pretreatment with 60 mg of terfenadine or 10 mg of loratadine showed that both agents significantly reduced sneezing. Both drugs also lowered levels of antigen-induced histamine and TAME-esterase, but only terfenadine did so significantly. In a double-blind, placebo-controlled study, the new disk method showed that terfenadine reduced sneezing but not nasal congestion in eight patients with allergic rhinitis. Terfenadine significantly reduced the weight of nasal secretions on both sides of the nose and significantly reduced histamine on the ipsilateral side. (J ALLERGY CLIN IMMUNOL 1992;90:715-9.)

Key words: Allergic rhinitis, antigen, histamine, mast cell, cetirizine, loratadine, terfenadine

We have used a lavage technique for several years at Johns Hopkins to study the response to antigen challenge. In this nasal model a metered-dose inhaler is used to spray antigen into the subject's nose so that a reproducible dose can be delivered to the nasal mucosa. The response is monitored by nasal lavage. The subject closes off the nasopharynx; 10 ml of saline solution is then placed into the nasal cavity. After 10

Abbreviations used

H_1 : Histamine type 1 receptor
PGD₂: Prostaglandin D₂
LTC₄: Leukotriene C₄
PNUs: Protein nitrogen units

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seconds, the subject brings forth secretions and saline solution into a plastic receptacle. The recovered fluids are then processed for the measurement of mediators.

When antigen is sprayed into the nasal mucosa, it stimulates nasal mast cells to release histamine, and that released histamine then stimulates nerves to cause

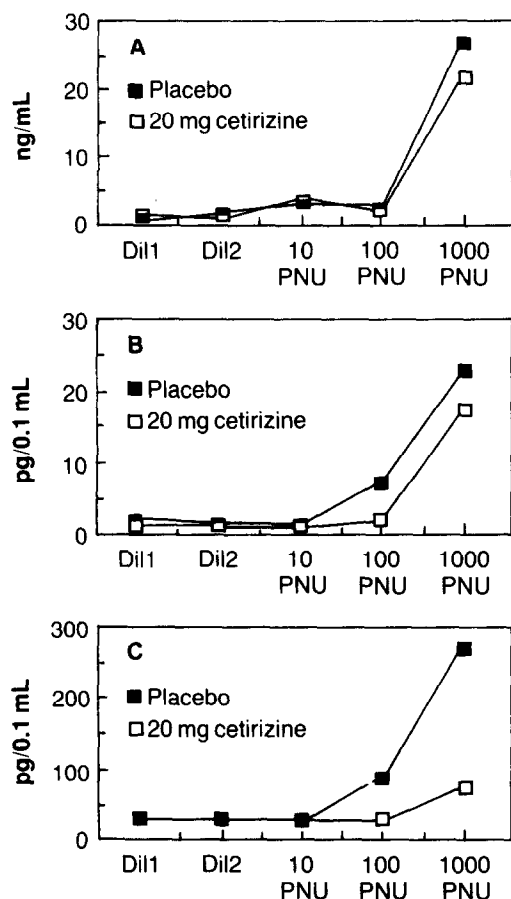


FIG. 1. Antigen challenge after pretreatment with 20 mg of cetirizine daily for 2 days or with placebo produced equivalent amounts of **A**, histamine and **B**, PGD₂ in nasal lavage of 10 patients with seasonal allergic rhinitis. However, the amount of **C**, LTC₄ recovered after pretreatment was significantly reduced in those pretreated with cetirizine compared with those given placebo. *Dil*, Diluent for antigen extract. (From Naclerio R, et al. The effect of cetirizine on early allergic response. *Laryngoscope* 1989; 99:596-9.)

sneezing and dilates blood vessels to cause vascular permeability. That process has been confirmed by several studies, which are summarized here.

EFFECT OF CETIRIZINE ON ANTIGEN CHALLENGE

We used this technique to assess the effect of cetirizine on the early response to nasal challenge with antigen in a double-blind, crossover study.¹ Ten subjects were allergic to either ragweed or grass, and the study was conducted outside their allergy season. They received premedication with 20 mg of cetirizine once a day for 2 days before the challenge.

As the dose of antigen increased, histamine was released, subjects began sneezing, and TAME-esterase was released. Using chromatographic studies, David Proud in our group showed that about 80% of

this TAME-esterase was plasma kallikrein complexed to α_2 -macroglobulin. Approximately 20% was mast cell tryptase, and a very small percentage was glandular kallikrein. The plasma kallikrein- α_2 -macroglobulin is a large molecule with a molecular weight of approximately 900,000. Its appearance in nasal secretions represents a large vascular leak.

Patients who received pretreatment with 20 mg of cetirizine or placebo daily in the 2 days before the challenge produced equivalent concentrations of histamine in the recovered lavage (Fig. 1, *A*). Likewise, no significant differences occurred in the amount of released PGD₂ (Fig. 1, *B*), the major cyclooxygenase product of mast cells; this finding supports the fact that mast cells were equally stimulated during the challenge. As expected, pretreatment with cetirizine dramatically reduced the number of sneezes and the vascular leak induced by antigen challenge.

In this study the amount of LTC₄ released into the lavage after treatment was significantly reduced (Fig. 1, *C*). This finding was somewhat surprising in that LTC₄ is known to originate in mast cells and histamine and PGD₂ were not reduced. It is conceivable that this LTC₄ could come from a source other than mast cells or that the mast cells were affected in a differential manner, but we do not have a definite explanation for this finding.

EFFECT OF TERFENADINE ON EARLY ALLERGIC RESPONSE

Using this model, our group has also studied the effect of terfenadine on the early response to nasal challenge.² In a double-blind, placebo-controlled crossover study, we evaluated 12 subjects who were allergic to ragweed or grass. This study was done outside their allergy season. To try to achieve a steady-state concentration of this H₁ antihistamine, we administered 60 mg of terfenadine twice daily (the standard dose), 300 mg of terfenadine twice daily, or placebo for 1 week before the challenge.

The subjects were challenged sequentially with antigen and histamine. Pretreatment with either dosage of terfenadine significantly blocked histamine-induced sneezing (Fig. 2, *A*) and vascular permeability (Fig. 2, *B*) compared with placebo. Unlike cetirizine, however, terfenadine at a dosage of 60 mg twice daily also significantly reduced the amount of histamine recovered from the nasal lavage compared with placebo ($p = 0.07$) (Fig. 3). Terfenadine at a dosage of 300 mg twice daily reduced the amount of histamine recovered even more.

Terfenadine at either 60 or 300 mg also significantly reduced levels of kinin and albumin as well as TAME-esterase activity in nasal lavages after antigen challenge (Fig. 3). It is worth remembering that kinin generation depends on both enzymes and a substrate,

which come from the peripheral blood. This study did not distinguish whether terfenadine blocked some of the enzymes released from the mast cells or whether it blocked only the vascular influx that was generated. However, terfenadine clearly blocked this vasoactive mediator, and that blocking would be a positive effect in patients with allergic rhinitis because intranasal administration of bradykinin causes nasal congestion and increased vascular permeability.

TERFENADINE VERSUS LORATADINE IN A NASAL CHALLENGE MODEL

We recently completed a study that compared 60 mg of terfenadine and 10 mg of loratadine, another H_1 antihistamine, given twice daily for 7 days before antigen challenge. This was also a three-way crossover, placebo-controlled study. Fourteen patients with allergic rhinitis were evaluated, and the study was done outside their allergy season.

Both terfenadine and loratadine significantly reduced the number of sneezes ($p < 0.01$) compared with placebo as subjects were challenged with increasing doses of antigen. The amount of histamine recovered from nasal lavages was also significantly lower among subjects treated with terfenadine compared with those who received placebo ($p < 0.05$). Although loratadine decreased the amount of histamine recovered compared with placebo, the reduction was not significant.

Both loratadine and terfenadine reduced the amount of PGD_2 recovered in nasal lavages compared with placebo, but neither agent induced a significant reduction. Measurements of tryptase produced similar results: both agents reduced this enzyme, which is located in the mast cell granule, but neither did so significantly.

Loratadine reduced the amount of TAME-esterase produced but not significantly. The reduction produced by terfenadine, however, was significant ($p < 0.01$).

Unlike cetirizine in the study described above, terfenadine had no effect on the generation of LTC_4 . Loratadine suppressed the generation of LTC_4 but not significantly when compared with placebo. The recovery of albumin in subjects given placebo dramatically demonstrates the vascular permeability induced by antigen challenge. Both loratadine and terfenadine significantly decreased the amount of albumin recovered compared with placebo ($p < 0.05$). Both agents also significantly reduced the amount of kinin generated compared with placebo ($p < 0.05$).

DISK METHOD FOR ANTIGEN CHALLENGE

Although the nasal lavage method used in the studies just described provides an excellent approach to studying the biochemistry of the allergic reaction, it

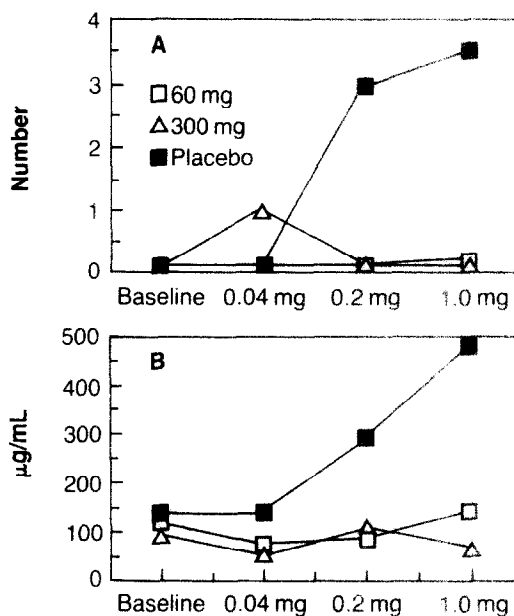


FIG. 2. A, Median sneezing response and B, level of albumin in recovered lavages after nasal challenge with histamine are both lower in 12 subjects treated after 1 week of pretreatment with either 60 or 300 mg of terfenadine twice daily compared with placebo. Baseline = response 20 minutes after last antigen challenge; 0.04, 0.2, and 1.0 mg = dose of histamine used for challenge. (From Naclerio RM, et al. Terfenadine, an H_1 antihistamine, inhibits histamine release in vivo in the human. *Am Rev Respir Dis* 1990;142:167-71.)

is not ideal. We premedicate patients with a decongestant to prevent the mucosal edema that follows a positive response to antigen challenge. In addition, this model cannot distinguish between the products of the two sides of the nose because they mix in the back of the nasopharynx. Therefore we are now developing a new challenge model that should allow us to determine both biochemical and physiologic responses to antigen.

The new technique involves placing a white filter paper disk on the nasal septum. Such disks can both deliver antigen to the nasal mucosa and collect secretions at fixed time intervals afterward. Thus antigen can be collected directly from the site of the challenge. Disks can also be placed on the contralateral side of the nose to assess reflex activity there.

When the disk is removed from the nasal cavity, it is placed in a small Eppendorf. If the Eppendorf and the disk are weighed before secretions are collected, the amount of secretion collected over a fixed period can then be determined by noting the change in weight. We are now refining this method to assess the amount of mediators recovered by eluting secretions from the disks. To date we have been able to recover histamine, PGD_2 , and kinin.

We have studied terfenadine with this system. We

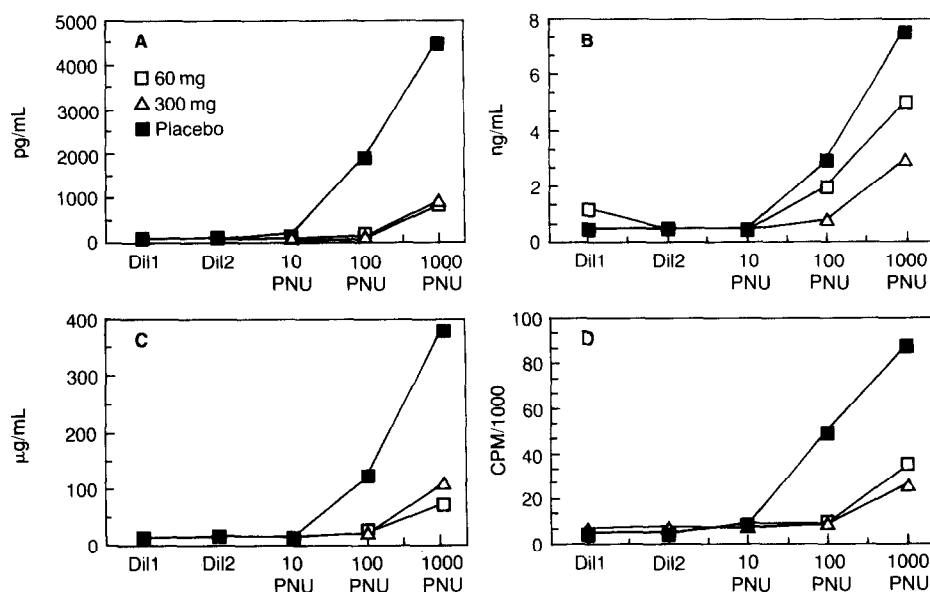


FIG. 3. Median levels of **A**, kinin, **B**, histamine, **C**, albumin, and **D**, TAME-esterase activity in recovered nasal lavages from 12 subjects after antigen challenge after 1 week of pretreatment with 60 or 300 mg of terfenadine twice daily. *Dil*, Diluent for antigen extract. (From Naclerio R, et al. Terfenadine, an H_1 antihistamine, inhibits histamine release in vivo in the human. *Am Rev Respir Dis* 1990;142:167-71.)

first wanted to make sure that we were measuring only H_1 antihistaminic effects and not anticholinergic effects. Methacholine, a cholinergic agonist, increases the amount of secretion. A single 60 mg dose of terfenadine administered 2 hours before methacholine challenge had no effect on the weight of secretions collected. This finding demonstrates that terfenadine has no anticholinergic effect and does not affect secretory volume.

Increasing doses of histamine in this disk model elicit a dose-related response on the ipsilateral side of the nose as well as an increased response on the contralateral side, as measured by the weight of secretions collected. The administration of 60 mg of terfenadine 2 hours before histamine challenge partially reduced both the ipsilateral and the contralateral responses. These results indicate that the secretory response is H_1 mediated, in part.

To study the effects of terfenadine on nasal antigen challenge with the use of this disk placement method, we evaluated eight patients with seasonal allergic rhinitis to ragweed. The study was double blind and was done outside the subjects' allergy seasons. A single oral dose of 60 mg of terfenadine or placebo was administered 2 hours before antigen challenge. We used this single dose of terfenadine rather than the 2-week dosing schedule that had been used in some earlier studies because we wanted to decrease any

potential effect of terfenadine on mediator release from mast cells, which happens when a steady-state blood level is achieved.

Antigen was put on 5 mm diameter, white filter paper disks, which were placed on the anterior part of the nasal septum for 1 minute. Prewashed collection disks were placed on the ipsilateral and contralateral sides of the nose for 30 seconds. The secretions were eluted in 300 µl of saline solution for 24 hours at 4° C. The amounts of mediators in the secretions were determined by radioenzyme assay. As usual, we counted the number of sneezes after antigen challenge. To assess any changes in airflow through the nasal cavity, we also measured nasal airway resistance on both sides of the nose by anterior rhinomanometry.

An antigen dose of 333 PNUs produced significantly more sneezes in the placebo group compared with those who had been treated with 60 mg of terfenadine 2 hours before challenge ($p < 0.05$).

On the ipsilateral side, airway resistance as measured by anterior rhinomanometry remained constant through two baseline measures and one control challenge with diluent (saline solution) and then increased on challenge with 333 PNUs of antigen. On the contralateral side no increase occurred in airway resistance. These results were the same regardless of whether patients received terfenadine or placebo before antigen challenge. These findings are consistent

with experience from clinical practice, that is, that H_1 antihistamines have no significant effect on congestion in patients with allergic rhinitis.

The weight of secretions collected increased only marginally from two baseline measures to control challenge measurements at 30 seconds and 2 minutes, regardless of whether subjects had received terfenadine or placebo. The secretion weight increased significantly 30 seconds after allergen challenge compared with the control diluent challenge ($p < 0.05$). The secretion weight remained at approximately the same level 2 minutes after challenge. Significantly more secretion was collected from placebo group subjects than from terfenadine-treated subjects at 30 seconds ($p < 0.05$), but the difference between the two groups at 2 minutes was not significant.

The findings were the same on the contralateral side: secretion weight increased significantly 30 seconds after antigen challenge compared with the control diluent challenge ($p < 0.05$). Again, the difference between the terfenadine group and the placebo group was significant at 30 seconds ($p < 0.05$) but not at 2 minutes.

As our control study with methacholine demonstrated, it is not an anticholinergic action of terfenadine that is decreasing the secretory weight. It is important to note that H_1 histamine is not totally responsible for producing secretions on the contralateral side. Although it has a dramatic effect on sneezing, H_1 histamine has a much less marked effect on contralateral secretions. Some other mechanism must be involved in this reflex activity because the contralateral response to histamine was entirely abolished in the control experiment.

The amount of histamine collected from secretions increased dramatically 30 seconds after antigen challenge in both the terfenadine and the placebo groups. Although more histamine was recovered from the placebo group than from the terfenadine group, the difference was not significant at 30 seconds. At 2 minutes, as the amount of histamine recovered began to decline, the level in the placebo group was significantly higher than in the terfenadine group ($p < 0.05$). Histamine levels continued to decline and could not be measured about 10 minutes after challenge.

No histamine was released on the contralateral side. As just noted, secretions increased on that side. Thus it is clear that increased secretions alone do not account for increased levels of histamine.

It was reasonable to assume that the 333 PNU dose of antigen that we used was too high to permit this H_1 antihistamine to have a significant effect on re-

ducing histamine levels after antigen challenge. Therefore we decided to reduce the dose of antigen to 33.3 PNUs.

This reduced dosage of antigen was still enough to cause an increase in sneezing as the 10-fold higher dose used in the first challenge. Now, however, terfenadine almost completely abolished the sneezing response compared with placebo ($p < 0.05$).

Pretreatment with 60 mg of terfenadine 2 hours before challenge with 33.3 PNUs of antigen significantly decreased the secretion weight 30 seconds after challenge on the ipsilateral side compared with placebo ($p < 0.05$). However, on the contralateral side the decrease in secretion weight compared with placebo was not significant. A comparison of these results with those after the 333 PNU antigen challenge indicates that terfenadine has a somewhat greater effect in reducing secretions at the lower antigen dose.

When the dose of antigen was lowered to 33.3 PNUs, pretreatment with terfenadine suppressed antigen-induced histamine more than placebo at 30 seconds and 2 minutes but not significantly so. It is possible that this trend would have become significant if more than eight subjects had been studied. As in the challenge with 333 PNUs of antigen, no histamine was produced on the contralateral side.

The results of this study support the conclusion that sneezing is a reflex-mediated response through H_1 histamine receptors. Nasal airway resistance changes are not mediated through H_1 receptors; either other receptors or other mediators are involved.

Nasal secretions after allergen challenge are to a degree caused by released histamine, but other mediators also contribute to these events. These other mediators are especially important in initiating nasosnasal reflex secretions. If it were solely histamine that caused reflex activity on the contralateral side, one would expect that an H_1 antihistamine would block that response almost completely. However, that did not happen in this model.

Terfenadine tends to decrease histamine release after allergen challenge in this model, as it did in our lavage model.² We anticipate that the newer disk model will lead to a better understanding of the physiology and timing of mediator release from nasal mast cells.

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