Terfenadine Effect on the Bronchoconstriction, Dermal Response, and Leukopenia Induced by Platelet-Activating Factor*

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We have investigated the protective effect of oral terfenadine, a H<sub>1</sub> antagonist, on the dermal and pulmonary response, and changes of circulating WBCs to injected and inhaled platelet activating factor. Nine men with mild asthma participated in a double-blind, crossover study using terfenadine, 120 mg, or placebo. Three hours after administration of study drug, pulmonary function was measured, and a PAF challenge was performed. Skin test to histamine and PAF was performed prior to study drug, and 2.5 hours after drug. Circulating WBC count was determined prior to PAF inhalation and during the PAF challenge. There was a significant improvement in pulmonary function on terfenadine. Terfenadine significantly inhibited the wheal and flare response to histamine and the flare response to injected PAF. Terfenadine did not have an effect on the change in circulating WBC count or the change in pulmonary function to inhaled PAF. These results suggest a limited role for endogenous histamine for the effects of PAF.

(PAF = platelet activating factor)

Methods

Subjects

Nine male subjects with mild asthma were included in the study. The study consisted of two treatment days, placebo or terfenadine (120 mg). The sequence of the treatment days was randomly assigned and double blinded. A complete history and physical was performed for each subject. Subjects discontinued antihistamines two days prior to and during the one week protocol, but could take inhaled betagonists as needed, except for 8 h prior on study days. None of the subjects was receiving oral or inhaled corticosteroids, inhaled cromolyn sodium, oral theophylline, or long acting antihistamines during the study period. Mean percent predicted FEV<sub>1</sub> for placebo day was 71 ± 16 (range 43 to 95), and mean percent predicted FEV<sub>1</sub> for terfenadine day was 73 ± 16 (range 51 to 104). The mean percent FEV<sub>1</sub> values were not significantly different. The protocol was approved by the Institutional Review Board of Creighton University, and all subjects signed a consent form.

Experimental Protocol

We studied the effects of terfenadine or placebo on the effect of PAF-acether may play an important role in the pathogenesis of asthma. PAF has been reported to increase nonspecific bronchial hyperresponsiveness in humans. It has been previously shown that chlorpheniramine, a histamine antagonist, could inhibit the systemic and partially attenuate the airway response to inhaled PAF. We sought to further determine the potential role of antihistamines in the pathogenesis of PAF-mediated responses. To do so, we evaluated the effect of terfenadine or placebo on PAF-induced changes in pulmonary function, leukocyte changes, and on PAF skin tests.

Eight sites for intradermal injections were used over the flexor surface of both forearms, four sites on each forearm. Intradermal skin tests were done using PAF (0.02 ml of 200 μg/ml), Lyso-PAF (0.02 ml of 200 μg/ml), saline solution, and histamine (0.02 ml of 275 mg/ml). Maximum diameters were measured for wheal and flare at 90 degrees to each other. The area for the wheal and flare was determined using the product of the two diameters. The total WBC count was determined using an automated counter. The differential blood counts were obtained by reading 200 cells. C<sub>P</sub>-PAF was dissolved in 100 percent ethanol and diluted in phenol-buffered saline solution and 0.25 percent human serum albumin. The final concentration of ethanol in the administered C<sub>P</sub>-PAF was less than 2 percent. Lyso-PAF was prepared in a similar fashion.

Pulmonary function tests were performed using a pneumotach (Cybermedics CMV). Partial flows (pFEF<sub>max</sub>) were done on a 13.5 L spirometer. A timed inhalation method, from a nebulizer was employed for the PAF inhalation. The output of the nebulizer averaged .03 ml/actuation. In brief, the subjects slowly inhaled PAF starting from 25 percent of the inspiration capacity to 75 percent of inspiratory capacity, which results in a consistent inhalation pattern for all doses. This inhalation procedure also ensures a full inspiratory breath is not taken. Deep inspiratory efforts may result in bronchodilation, especially in asthmatics. Full vital capacity maneuvers were not performed during the PAF challenge, so as not to interfere with small changes in PAF-induced bronchoconstriction. At the end of the PAF challenge (218 minutes after drug, Fig 1), a full vital capacity measure was obtained, to compare to the FEV<sub>1</sub>, measured at 180 min following drug. The measurement of pFEF<sub>max</sub> has been previously outlined. In brief, it is an expiratory flow measurement (L/s) following a partial inspiratory maneuver, and measured at 60 to 80 percent from the peak of a baseline full inspiratory maneuver.

On each study day, a baseline PFT was measured, blood drawn, and skin tests done (Fig 1). After the skin tests, terfenadine (120 mg) or placebo was administered. At intervals of 30 min, 1 h, and 2 h, postdrug PFTs were performed. At 2½ h postdrug, a second
Experimental Protocol

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
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<tbody>
<tr>
<td>0 min</td>
<td>baseline measurement</td>
</tr>
<tr>
<td>+ 30</td>
<td>Drug or Placebo</td>
</tr>
<tr>
<td>+ 60</td>
<td>PFT, Skin Tests</td>
</tr>
<tr>
<td>+ 90</td>
<td>PFT</td>
</tr>
<tr>
<td>+ 120</td>
<td>PFT</td>
</tr>
<tr>
<td>+ 150</td>
<td>Skin Tests</td>
</tr>
<tr>
<td>+ 180</td>
<td>CBC, PFT, PEF_{60-90}</td>
</tr>
<tr>
<td>+ 185</td>
<td>Saline</td>
</tr>
<tr>
<td>+ 190</td>
<td>PAF 200 ug/ml x 3 breaths</td>
</tr>
<tr>
<td>+ 193</td>
<td>PEF_{60-90} x 3, CBC</td>
</tr>
<tr>
<td>+ 195</td>
<td>PAF 200 ug/ml x 5 breaths</td>
</tr>
<tr>
<td>+ 198</td>
<td>PEF_{60-90} x 5, CBC</td>
</tr>
<tr>
<td>+ 200</td>
<td>PAF 200 ug/ml x 5 breaths</td>
</tr>
<tr>
<td>+ 203</td>
<td>PEF_{60-90} x 5, CBC</td>
</tr>
<tr>
<td>+ 210</td>
<td>PFT, CBC</td>
</tr>
</tbody>
</table>

Figure 1. Time sequence for experimental protocol.

Skin test (postdrug) was done. At 3 h postdrug, blood was drawn, and another PFT was performed. Following the 3 h pulmonary function test, baseline partial flow measurements were determined to establish a baseline (postdrug) PEF_{60-90}.

The subjects received saline solution and a second baseline pre-PAF PEF_{60-90} was determined (Fig 1). Following saline solution, there were three stages of PAF inhalations. The first stage was three breaths at 200 μg/ml PAF. Stage 2 and 3 were five breaths at 200 μg/ml PAF. At 2¼ min after each inhalation, blood was drawn, and PEF_{60-90} was measured. At 15 min poststage 3, blood was drawn, and a repeat PFT was performed.

Statistical significance used paired Student’s t-tests and ANOVA. A p value ≤0.05 was considered significant.

Figure 2. Baseline FEV1 (mean±SD) and postterfenadine and placebo FEV1 values. On terfenadine day, the 2 and 3.0 hour mean FEV1 values were significantly different than baseline (asterisk) and from placebo day (double asterisk). The post-PAF FEV1 values are shown.

Figure 3. The effect of PAF on the skin test flare and wheal (insert) are shown (mean±SD). The PAF flare was significantly inhibited on terfenadine day (asterisk).

Results

Pre-PAF Pulmonary Function

Baseline FEV1 values were not significantly different between placebo day and active drug day (Fig 2). There was not a significant difference in mean FEV1 values on placebo day. On terfenadine day, 2 h and 2.5 h mean FEV1 values were significantly higher than baseline, 30 min or 1 h. The terfenadine day showed significantly greater bronchodilation at 2 and 3 h postdrug, when compared to placebo day (Fig 2).

Skin Tests

PAF: PAF-induced flare on terfenadine day was significantly smaller (p<0.05) than responses on placebo day and compared to pre-terfenadine (Fig 3). PAF-induced wheal was not significantly affected by terfenadine or placebo (Fig 3).

Histamine: Histamine-induced wheal and flare were significantly smaller postterfenadine compared to before and after placebo and preterfenadine (Fig 4).

There was no response to intradermal skin tests to
Table 1—Effect of Inhaled PAF on Circulating WBC Count*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>PAF 1</th>
<th>PAF 2</th>
<th>PAF 3</th>
<th>15 min post-PAF 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>6,175 ± 578</td>
<td>5,137 ± 486</td>
<td>6,813 ± 60</td>
<td>7,200 ± 1092</td>
<td>8,757 ± 1109*</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>5,595 ± 455</td>
<td>4,833 ± 355</td>
<td>5,756 ± 753</td>
<td>7,900 ± 833†</td>
<td>8,267 ± 813†</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>3,219 ± 446</td>
<td>2,244 ± 342</td>
<td>3,870 ± 486</td>
<td>4,339 ± 896</td>
<td>5,813 ± 872*</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>2,714 ± 263</td>
<td>2,154 ± 375</td>
<td>3,228 ± 644</td>
<td>4,684 ± 644†</td>
<td>5,323 ± 713†</td>
</tr>
</tbody>
</table>

*WBC or neutrophil level was significantly higher than at baseline or PAF 1.
†WBC or Neutrophil level was significantly higher than at baseline, PAF 1 or PAF 2.
‡Neutrophil count was significantly lower than at baseline.

Lyso-PAF or saline solution (data not shown).

WBC Changes

There were no significant differences for baseline WBC or differential counts between placebo and terfenadine day. For total WBC on placebo day, post-PAF level (15 min after PAF 3) was significantly higher than baseline (p = 0.032), PAF dose 1 (p = 0.034), and PAF dose 2 (p = 0.043) (Table 1). Total WBC on terfenadine day was significantly higher at PAF dose 3 and post-PAF when compared to baseline, PAF dose 1, and PAF dose 2 (Table 1). The total WBC dropped after the first dose of PAF but did not reach statistical significance.

The change in total neutrophil count contributed predominantly to the change in total WBC (Table 1). The absolute neutrophil count showed similar results as total WBC except that PAF 1 on active drug day was also significantly lower than saline. Neutrophils for PAF 1 on placebo day also dropped, but the drop was not significant. When the neutrophil counts were expressed as percentage of baseline, however, the drop at PAF 1 was significant on both days (data not shown). Terfenadine did not inhibit the transient neutropenia seen with PAF.

There was no significant change in eosinophils, lymphocytes, basophils, or monocytes.

Post-PAF Pulmonary Function

The partial flow measurements (pFEF_{0-60}) on the active drug day were significantly higher than placebo day for saline (p = 0.05), PAF 1 (p = 0.034), and PAF 3 (p = 0.006) steps (Fig 5). When compared to percentage of pre-PAF saline, PAF 1 and PAF 3 were significantly lower than saline on active drug day (Fig 5, inset). The mean FEF_{15} value 15 min post-PAF inhalation (Fig 2) on active and placebo day revealed terfenadine did not protect against PAF-induced bronchoconstriction.

Discussion

In previous reports, histamine has been suggested to have a role in PAF-acether induced bronchoconstriction and dermal response. To further investigate the possible role of histamine in PAF-acether induced reactions and to investigate the potential of pharmacologic modulation of PAF responses, we utilized an oral antihistamine, terfenadine, at a dose known to cause bronchodilation in asthmatic subjects.

Oral terfenadine has been previously shown to attenuate pulmonary symptoms during seasonal antigen exposure and cold air, ultrasonically nebulized distilled water, exercise, and histamine, but not methacholine challenges. Terfenadine has also been shown to be potentially beneficial in asthma, in single dose studies, by inducing bronchodilation at clinically relevant doses, even without loading doses. We, as others, have reported significant improvement in FEV₁ 2 to 3 h after a single dose of 120 and 240 mg of terfenadine. In this report, the 120 mg resulted in clinically relevant bronchodilation (Fig 2). The mean percentage of improvement in FEV₁ in the reported subjects receiving terfenadine, as compared to baseline, is comparable to the bronchodilation seen with oral short-acting theophylline or caffeine preparations. Two recent reports suggest terfenadine, in addition to H₁ receptor antagonism, actually inhibits the release of histamine. It is possible that terfenadine actually inhibits bronchoconstriction, rather than being a bronchodilator. Further studies of this possibility are needed.

Despite the significant improvement in FEV₁, ter-
fenadine did not protect against PAF-induced bronchoconstriction, when measured either by FEV₁ or by changes in pFEF₆₅₋₉₀. Smith et al demonstrated an attenuation of pulmonary function changes after PAF inhalation using oral chlorpheniramine. In their report, chlorpheniramine did not alter baseline pulmonary function. In the same report, two of seven subjects receiving chlorpheniramine did not reach a PC₉₀S Gaw by the largest PAF dose (1,000 μg/ml), and PC₉₀S Gaw values were extrapolated from dose-response curves. There is a difference in the total doses of inhaled PAF in these two studies. It is possible with higher doses of inhaled PAF that lung mast cells are stimulated to release histamine. In our study, terfenadine altered baseline function but did not prevent the drop in FEV₁ or pFEF₆₅₋₉₀ percent changes from pre-PAF inhalation values. It has been shown, using radioactively-labeled antagonists of PAF-acether, that specific binding sites for [³H]PAF exist in human and guinea pig tissue. It is conceivable, therefore, that PAF-induced bronchoconstriction could be directly mediated by smooth muscle PAF receptors.

It has been clearly shown that inhaled PAF can induce a significant, although transient, reduction in total WBC and neutrophil counts, with a subsequent rebound above baseline. The mechanism is presumably through the induction of neutrophil margination in the pulmonary vascular bed, possibly through PAF receptor activation of pulmonary vascular endothelial or smooth muscle cells. The observation that terfenadine did not affect the reduction in WBC or neutrophil counts would add support to the hypothesis.

A PAF injection into the dermis has been shown to induce both an early wheal and flare reaction and a later cellular infiltrative response. It has been previously reported the local administration of chlorpheniramine did not affect the wheal response to injected PAF but did significantly attenuate the flare response. With oral terfenadine, there was a significant reduction in the flare response to injected PAF, but no change in the wheal response. As expected, terfenadine significantly affected the wheal and flare to injected histamine.

In a report by Chung et al, ketotifen, an antihistamine with additional antiallergy properties, attenuated the intradermal PAF (200 ng) induced wheal and flare but not the immediate bronchoconstriction to inhaled PAF. The authors concluded that the dermal response to PAF was histamine-mediated, but the pulmonary response was not. Ketotifen does have recognized nonantihistamine antiallergic effects, possibly accounting for the differences in dermal response compared to terfenadine.

Histamine-induced wheal is mediated by H₁ and H₄ receptor increases in vascular permeability. Flare response is mediated by histamine-mediated axon reflexes. Terfenadine inhibited the flare, but not wheal, response to injected PAF. The effect of PAF on wheal formation is possibly through PAF receptors in vascular tissue, resulting in direct plasma extravasation and is not inhibited by H₁ receptor antagonists.

The fact terfenadine attenuated the flare response to PAF would suggest it could be caused by PAF-receptor mediated histamine release from mast cells, or that PAF could stimulate nerve endings, resulting in release of substance P (or other neuropeptides) which may then act upon mast cells to release histamine. Substance P has been demonstrated to cause histamine release from human cutaneous mast cells. A direct inhibition of axon reflexes by terfenadine cannot be excluded, however.

A terfenadine dose sufficient to clinically improve pulmonary function did not affect PAF inhalation, blood cell changes, and wheal response to PAF. Terfenadine attenuated the histamine wheal and flare and the PAF flare. The results would suggest a limited role of endogenous histamine in PAF-induced responses.

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