Effects of PAF Antagonist, BN52021, on the PAF-, Methacholine-, and Allergen-Induced Bronchoconstriction in Asthmatic Children*  

Kue-Hsiung Hsieh, M.D.

Platelet-activating factor (PAF) is a inflammatory mediator capable of inducing protracted inflammation of the airways and bronchial hyperreactivity. Twenty-one asthmatic children were evenly divided into three groups and each group performed a double-blind, placebo-controlled and crossover study on the effect of aerosolized BN52021, a PAF antagonist, on the bronchoconstriction induced by PAF, methacholine, or specific allergen, respectively. One group of healthy children was included for comparison. Total WBC, neutrophils, and eosinophils were counted before and after PAF challenge. The results showed the following: (1) six of seven asthmatics and one of seven normal subjects gave a positive bronchial provocation with PAF; (2) in asthmatics, prior inhalation of BN52021 could inhibit the bronchoconstriction induced by PAF (6/6) and allergen (3/7), but not by methacholine; and (3) 5 min after inhalation of PAF, there was a marked decrease of peripheral blood eosinophils and neutrophils that could be inhibited by prior inhalation of BN52021 in normal subjects but not in asthmatics. These findings support the idea that PAF may be involved in the pathogenesis of bronchial asthma and PAF antagonist may have a role in the prevention and treatment of this disease. (Chest 1991; 99:877-82)

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\begin{align*}
\text{PAF} &= \text{platelet-activating factor; RAST = radioallergosorbent test; HBSS = Hanks' balanced salt solution; DMSO = dimethyl sulfoxide} \\
\end{align*}
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The leaves of Ginkgo biloba have been used in traditional Chinese medicine for a long time for respiratory diseases. Ginkgolide B (BN52021), the most active PAF antagonist isolated from the trees, has been shown to be a potent inhibitor of PAF-induced thrombocytopenia, bronchoconstriction, and pulmonary anaphylaxis in guinea pigs.

Inhalation may be the optimal route for the administration of antiasthmatic drugs to achieve the maximal therapeutic effect and to reduce the systemic side effects. The purpose of this trial was to examine the effect of aerosolized BN52021 on the immediate bronchoconstriction induced by inhalations of PAF, methacholine, and specific allergen in asthmatic children. The effect of BN52021 on the total counts of WBC, neutrophil, and eosinophil in the peripheral blood induced by PAF inhalation was also studied.

**Materials and Methods**

**Study Populations**

Twenty-one stable asthmatic children (16 boys and 5 girls, aged 8 to 15 years old), and 7 age-matched healthy children (5 boys and 2 girls, aged 8 to 15 years old) were studied. All the asthmatic children had elevated serum IgE levels, positive intradermal skin test (dilution of 10⁻⁴ g/ml, Torii, Japan), and positive radioallergosorbent test (RAST) (Pharmacia, Sweden) to the house dust mite, Dermatophagoides pteronyssinus, and eosinophilia (>400 cells/μm³). Asthmatic children were selected as stable if they had not had an asthmatic attack in the last month, they had not taken medication in the last two weeks, and their forced expiratory volume in 1 s (FEV₁) was at least 80% of the predicted normal. Healthy subjects had normal pulmonary function and had had no respiratory tract infection in the previous two weeks. Informed consents were...
Table 1—Characteristics of Study Populations

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>FEV1, yr</th>
<th>Serum IgE, IU/ml</th>
<th>Dp-IgE RAST, % Bound</th>
<th>Frequency of Attacks, /yr</th>
<th>Methacholine PD20, U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>11.8 ± 3.0</td>
<td>98.9 ± 8.2</td>
<td>68 ± 52</td>
<td>0.8 ± 0.3</td>
<td>180.4 ± 44.2</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF group</td>
<td>7</td>
<td>10.9 ± 2.7</td>
<td>91.6 ± 7.8</td>
<td>1012 ± 943</td>
<td>14.7 ± 6.8</td>
<td>9.2 ± 2.5</td>
</tr>
<tr>
<td>Methacholine</td>
<td>7</td>
<td>11.0 ± 3.2</td>
<td>92.5 ± 6.9</td>
<td>983 ± 614</td>
<td>16.8 ± 7.2</td>
<td>8.5 ± 3.0</td>
</tr>
<tr>
<td>group</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergen group</td>
<td>7</td>
<td>10.5 ± 3.0</td>
<td>92.0 ± 6.7</td>
<td>954 ± 708</td>
<td>16.3 ± 7.7</td>
<td>8.5 ± 2.8</td>
</tr>
</tbody>
</table>

*PAF = platelet-activating factor.

obtained from the parents before the children were enrolled into the study. The asthmatic children were divided randomly into three groups (seven patients in each group). The characteristics of each group, including the control group (healthy children), are listed in Table 1.

Experimental protocol

Each patient group was challenged with only one of the following stimulants: PAF, methacholine, and mite allergen. The control group performed PAF challenge.

The effects of aerosol BN52021 on PAF, methacholine, and allergen challenges were studied using a double-blind, placebo-controlled, crossover method. For methacholine and allergen studies, each subject performed bronchial provocation twice, with a washout interval of seven days. After baseline pulmonary function tests (which should be greater than 80 percent of the predicted normal), subjects were instructed to inhale five puffs of either BN52021 or placebo (diluent of BN52021) and pulmonary function tests were repeated 10 minutes later. If there was no significant decrease of FEV1, (≥10 percent), then the FEV1 of this time was taken as “baseline” and bronchial provocation with assigned stimulant was then performed. To obtain a dose response of BN52021 on the one hand and to avoid tachyphylaxis to PAF on the other hand, PAF challenge was done for four consecutive days, pretreated each day blindly with either placebo or one of the three concentrations of BN52021 (see below), respectively.

Solutions of methacholine chloride (J.T. Baker Chemical, Phillipsburg, NJ) were prepared freshly each time. The concentrations of methacholine were 0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10, and 25 mg/ml. One inhalation unit was defined as one inhalation of methacholine of 1 mg/ml. Solutions of PAF (Sigma, St. Louis, MO) were prepared just before use. The concentrations of PAF were 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 mg/ml. One inhalation unit was defined as one inhalation of 1 mg/ml of BN52021 (a gift from Dr. Pierre Braquet, IHB, Le Plessis-Robinson, France) dissolved, according to the manufacturer’s instruction, in dimethylsulfoxide (DMSO), (Merck, Darmstadt, West Germany) as stock solution (5 × 10−4 M) and was diluted in hot HBSS (37°C) to three concentrations of 450, 150, and 50 mg/ml (1.6 × 10−4 M, 1.3 × 10−6 M). BN52021 remained soluble in HBSS when cooled to room temperature before use. HBSS containing DMSO of 10-fold dilution (weight/volume) was used as placebo. As both BN52021 and DMSO were highly diluted, no evident bitter taste or odor could be detected. Moreover, only those who showed less than 10 percent decrease in FEV1 after inhaling placebo or drug would participate in the study.

Pulmonary function was measured (using Microspiro Hi-298, Chest Corporation, Tokyo, Japan). Bronchial provocations were performed according to the standard method of Chai et al.14 A nebulizer (DeVilbiss model 646) was connected to a dosimeter consisting of an input solenoid valve delivering 20 psi of compressed air that generated aerosols of particle size of 2 to 5 μm, and a timer was set at 0.5 s for each inspiratory breath. The timing of the opening of the valve was manually triggered by the patient through the hand-held reset switch and the thermistor breath sensing unit. Each breath of testing agent was delivered from functional residual capacity to inspiratory capacity. The bronchial provocation tests were stopped if a greater than 20 percent decrease of FEV1 of baseline was reached, or frank asthmatic attack occurred or no significant drop in FEV1 was observed even after inhaling the highest dose of stimulants. The total WBC, neutrophil, and eosinophil counts were checked before challenge and at 5 and 10 minutes after the completion of PAF challenges. Symptoms such as throat irritation, cough, flushing, dyspnea, and tachycardia were recorded during the test and within the 24 h after challenge.

Statistics

Data were expressed as mean ± SD and were analyzed by paired Student’s t test. A p value of <0.05 was considered significant.

RESULTS

As shown in Table 1, the four studied groups were comparable regarding the age and baseline FEV1. The asthmatic patients had a much smaller methacholine PD20 than did controls, but no difference was found among the three asthmatic groups.

The protective effect, as measured by changes in FEV1, of aerosol BN52021 on PAF-induced bronchoconstriction in normal individuals and asthmatic children is shown in Figure 1. BN52021 itself had no effect on the baseline FEV1. Although six of the seven asthmatics showed a greater than 20 percent decrease in FEV1 after inhaling the highest dose of PAF (1,000 mg/L), only one of the seven normal subjects showed a greater than 20 percent decrease in FEV1. Prior inhalation of BN52021 was able to inhibit the bronchoprotective effect of PAF in both normal subjects (Fig 1A) and asthmatics (Fig 1B). BN52021 at the concentration of 50 ng/ml had only a little effect on PAF-induced bronchospasm; at a concentration of 150 ng/ml, BN52021 could inhibit PAF-induced bronchospasm in four of the six responders and at a concentration of 450 ng/ml, BN52021 was capable of preventing PAF-induced bronchospasm in all six responders. However, as the dose-response study of BN52021 was done successively, a cumulative effect of BN52021 should be considered. After the dose-dependent nature of inhibitory effect of BN52021 on PAF-induced bronchospasm was established, BN52021 at a concen-
either challenge group activating allergen tration were aited subjects seven (location formed blind, protective effect of placebos) were omitted for clarity.

The specificity of the inhibitory effect of BN52021 on PAF-induced bronchospasm was studied by using methacholine challenge. Figure 2 shows that there was no difference in methacholine PD20 units between placebo and BN52021 pretreated groups (40.8 ± 20.2 U vs 41.2 ± 20.6 U).

To study the possible role of PAF in the pathogenesis of allergic bronchial asthma, a double-blind, placebo-controlled, crossover allergen challenge study was conducted. Patients were instructed to inhale either BN52021 or placebo and then perform the allergen challenge. Two tests were done seven days apart. Figure 3A shows that inhalation of mite allergen extract-induced bronchospasm in all of seven mite-sensitive asthmatic children. Importantly, the allergen-induced bronchoconstriction could be inhibited by a prior inhalation of BN52021 in three of seven patients (Fig 3B), and the threshold of allergen sensitivity was increased in each patient after inhalation of BN52021.

Table 2 shows the effect of aerosol BN52021 on the PAF-induced changes of peripheral WBC counts. Total numbers of WBC, neutrophil, and eosinophil counts were decreased markedly 5 minutes after PAF inhalation in both normal subjects and asthmatic children, but they returned to nearly normal value at 10 minutes. The decrease of eosinophil and neutrophil counts after PAF inhalation was greater in asthmatics than in normal subjects. Prior inhalation of BN52021 could inhibit PAF-induced decrease in eosinophil and neutrophil counts in normal, but not in asthmatic children.

**Discussion**

In human and in animal models, PAF is a potent bronchoconstrictor no matter whether given intravenously or by aerosol. We observed similar
results in this study. The magnitude of decrease of FEV₁ and the frequency of response to PAF inhalation were much greater in asthmatics than in normal controls (Fig 1). In addition, BN52021 was less efficient in inhibiting the PAF-induced bronchoconstriction in patients than in normal subjects, especially at low concentration. Rubin et al.¹⁵ also reported a greater decrease of FEV₁ at 1,000 mg/L of PAF in asthmatics (10 percent) than in normal subjects (5 percent), and the asthmatic responders were approximately four times more sensitive to PAF than the normal responders. The hyperresponsiveness of asthmatic airways to PAF may be due to the differences in the function and/or the number of PAF receptors or the activity of PAF acetylhidrolase.³⁰

The mechanisms by which PAF induces bronchoconstriction are still unclear. In addition to a possible direct contractile effect,²¹ several lines of evidence indicate that PAF-induced bronchoconstriction may be a platelet-dependent inflammatory process: (1) the PAF-induced bronchoconstriction could be abrogated by prior depletion of circulating platelets by using an anti-platelet antiserum,³⁶,³² and (2) histamine secretion from circulating platelets had been shown to be involved in PAF-induced bronchospasm in rabbits.³³,³⁴ However, it should be kept in mind that all of these experiments were done in animals rather than in humans.

Recently, PAF has been postulated to play a central role in the pathogenesis of bronchial asthma because PAF is unique, among allergic mediators, by having the capacity to induce protracted inflammation of the airway wall. The role of PAF in bronchial asthma may be furthered by the results of the present experiments with BN52021 in allergic asthmatics and normals.

Table 2 — The Effects of Aerosolized BN52021 on the Platelet-Activating Factor (PAF)-Induced Decrease of WBC, Neutrophils, and Eosinophils, (Mean ± SD) in the Peripheral Blood in Normal and Asthmatic Children

<table>
<thead>
<tr>
<th>Experimental Protocol</th>
<th>Time after Experiment, min</th>
<th>Total WBC</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normals</td>
<td>Asthmatics</td>
<td>Normals</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>7,560±456</td>
<td>11,280±576</td>
<td>4,210±246*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>218±48†</td>
<td>758±149*</td>
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</tr>
<tr>
<td>BN52021</td>
<td>0</td>
<td>7,880±510</td>
<td>12,680±634</td>
<td>4,060±343</td>
</tr>
<tr>
<td></td>
<td></td>
<td>246±53</td>
<td>821±205†</td>
<td></td>
</tr>
<tr>
<td>BN52021</td>
<td>5</td>
<td>4,540±201</td>
<td>7,550±394</td>
<td>1,940±192*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3,480±263</td>
<td>4,890±100</td>
<td>137±39†</td>
</tr>
<tr>
<td>BN52021</td>
<td>10</td>
<td>6,640±360</td>
<td>8,070±416</td>
<td>3,840±256</td>
</tr>
<tr>
<td></td>
<td></td>
<td>236±57</td>
<td>495±93‡</td>
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</tr>
<tr>
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<td>12,680±634</td>
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<tr>
<td></td>
<td></td>
<td>236±57</td>
<td>495±93‡</td>
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</tbody>
</table>

*p<0.0001.  †p<0.001.  ‡p<0.003.  §p<0.02.  ||p=0.001.  **p<0.0001.
Type that is evident in asthma, i.e., infiltration of the airways by mononuclear cells, neutrophils, and eosinophils.\textsuperscript{7,25,26} This unique inflammatory process, which could be induced by PAF inhalation in both animals\textsuperscript{9-11} and humans,\textsuperscript{14,15} may account for the induction of persistent bronchial hyperreactivity, the hallmark of bronchial asthma. Additional lines of evidence include the detection of PAF in the sputum of asthmatic subjects\textsuperscript{12} and the demonstration that PAF may be produced by alveolar macrophages obtained from asthmatics.\textsuperscript{27} The recent availability of specific PAF receptor antagonists has made it possible to clarify the exact role of PAF in asthma. It has been shown that oral administration of BN52063, a ginkgolides mixture, was able to inhibit both the PAF- and antigen-induced acute and late-onset cutaneous responses in healthy and atopic subjects.\textsuperscript{28-30} More importantly, this same preparation could also suppress the immediate response to inhaled allergen challenge in asthmatics via oral route.\textsuperscript{31} These studies provide evidence that PAF may be involved in the pathogenesis of bronchial asthma and the specific PAF antagonists may have a therapeutic role in the prevention and treatment of this disease.

As only the oral route of administration had been used in previous studies to examine the effect of PAF antagonists on PAF- and allergen-induced bronchoconstriction, we used an aerosol form in this study, hoping to achieve the maximal effect with fewer side effects. Our results showed that inhalation of five puffs of BN52021 at a concentration of 450 ng/ml was able to inhibit completely the immediate bronchoconstriction induced by PAF inhalation in both normal and asthmatic individuals and the allergen-induced immediate bronchoconstriction in some asthmatics. Moreover, no obvious adverse effects were observed. In previous studies, however, more than 100 mg of oral BN52063 was needed to suppress the PAF- and allergen-induced wheal and flare skin reaction and bronchoconstriction.\textsuperscript{30-31} The specificity of BN52021 was further confirmed by the failure of BN52021 to block methacholine-induced bronchospasm.

Tachyphylaxis had been shown to occur rapidly after repeated inhalation of PAF,\textsuperscript{14,15,32,33} and this phenomenon may interfere with the interpretation of the efficacy of BN52021 in blocking PAF-induced bronchospasm. In this study, subjects were instructed to inhale twofold increasing doses of PAF every 15 minutes during PAF provocation challenge, but no clear-cut tachyphylaxis was found. The reasons for the discrepancy between this series and other studies are unknown; however, Cuss et al\textsuperscript{14} found that not all tested individuals showed consistent tachyphylaxis when Vp30 was measured, and Rubin et al\textsuperscript{15} also demonstrated a steady decrease of SCaw with increasing PAF. Furthermore, BN52021 was shown to block the PAF-induced bronchoconstriction in a dose-dependent manner (Fig 1). Therefore, tachyphylaxis cannot be used as the major factor to explain the effectiveness of BN52021 in blocking the bronchoconstrictive effect of PAF. Thus, this is the first study demonstrating the efficacy of an aerosolized form of PAF antagonist in suppressing the immediate bronchoconstrictive response to PAF challenge in normal subjects and asthmatic children and bronchospasm induced by allergen challenge in asthmatic children. These findings may support the possible role of specific PAF antagonist in the attenuation and treatment of bronchial asthma.

Several investigators reported that PAF inhalation was capable of recruiting eosinophil into the guinea pig lung tissues,\textsuperscript{3,4,6} causing neutrophilia in bronchial lavage fluid in dogs,\textsuperscript{32} and decreasing neutrophils in peripheral blood in man,\textsuperscript{33} which correlated with the induction of bronchial hyperreactivity. Thus, the findings obtained in this study that aerosol BN52021 was able to inhibit the PAF-induced decrease of WBC, neutrophil, and particularly eosinophil in the peripheral blood may explain partly the effectiveness of BN52021.

In conclusion, BN52021 is able to suppress PAF- and allergen-induced immediate bronchospasm; however, studies including more cases and with a longer period of observation are needed before a solid conclusion can be made. More importantly, although no clinically evident adverse effects have been found on those tested patients nearly one year after the study and DMSO had been shown to have no cytotoxic effect on K562 cell line,\textsuperscript{34} evaluation of the safety of long-term inhalation of BN52021 dissolved in DMSO by using animal models is absolutely required before advocacy of its clinical use.

\textbf{REFERENCES}

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