The Role of Platelet-Activating Factor (PAF) and the Efficacy of ABT-491, a Highly Potent and Selective PAF Antagonist, in Experimental Allergic Rhinitis

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ABSTRACT
Platelet-activating factor (PAF) may be an important mediator of allergic rhinitis. In the present study we evaluated the effectiveness of a recently described PAF antagonist (ABT-491) in rat and guinea pig models of allergic rhinitis. PAF, when perfused through the nasal passages of anesthetized Brown Norway rats, provoked an acute increase, measured as dye leakage, in nasal vascular permeability evident within 15 min after exposure to PAF. ABT-491, given orally 1 hr before PAF challenge, inhibited the response in a dose-related manner (ED50 = 0.3 mg/kg). Intranasal perfusion with ovalbumin in rats sensitized to the antigen 18 to 21 days before challenge also induced an increase in vascular permeability. The antigen-induced leakage was inhibited a maximum of 74% (P ≤ .001) by pretreatment with ABT-491 (3 mg/kg p.o.). An antihistamine (mepyramine, 10 mg/kg i.p.), a serotonin antagonist (methysergide) and a 5-lipoxygenase inhibitor (A-79175) also exhibited efficacy in this model (56%, 87% and 65% inhibition, respectively). Near complete inhibition (93%, P ≤ .001) of the response was achieved by coadministration of ABT-491 and methysergide. In guinea pigs intranasal administration of PAF resulted in increased airway resistance that was inhibited in a dose-dependent manner by oral administration of ABT-491 (ED50 = 3 mg/kg). Antigen-induced nasal airway resistance, triggered by exposure of sensitized animals to aerosolized ovalbumin, was also inhibited by ABT-491 (maximum inhibition 64%, P ≤ .05; 10 mg/kg p.o.). The effectiveness of the antagonist was increased to 80% protection by coadministration with either an antihistamine or a 5-lipoxygenase inhibitor, agents which were separately insignificant in blocking the response to antigen. These results suggest a therapeutic utility for ABT-491, perhaps in combination with other anti-inflammatory agents, in the treatment of allergic rhinitis.

Allergic rhinitis is an inflammatory disease of the upper airway. Symptoms of the disease, which include congestion, rhinorrhea formation, sneezing, itching and loss of smell, are triggered by immunoglobulin E-mediated activation of mucosal mast cells, basophils and eosinophils. Activation of these inflammatory cells causes the release of numerous mediators including PAF, histamine, leukotrienes, cytokines and prostaglandins that can in turn recruit additional inflammatory cells, trigger the release of further inflammatory mediators and stimulateafferent nerves (Belser et al., 1996; Garrelds et al., 1996; Krause 1995; Meltzer 1995; Naclerio et al., 1991; Nonaka et al., 1996).

Of inflammatory mediators potentially involved in allergic rhinitis, PAF is of particular interest because it is perhaps the most potent mediator for inducing vascular leakage, a response that may contribute to rhinorrhea formation and congestion (Evans et al., 1988, 1989; Hwang et al., 1985). Because of its potent proinflammatory properties, PAF has been the subject of several studies that implicate it in bronchial asthma (Braquet et al., 1987; Summers and Albert, 1995). However, the role of PAF in allergic rhinitis is less well established. Several studies have been done with a few PAF receptor antagonists in animal models of the disease. CV-3988 blocked vascular permeability and nasal airway resistance increases induced by topical application of PAF, and WEB-2086 and SM-10661 attenuated antigen-induced increase in late-phase nasal airway resistance in guinea pigs (Misawa and Iwamura, 1990; Narita and Asakura, 1993). In the clinical setting, installation of PAF into the nose induces rhinorrhea, nasal neutrophil influx and nasal hyper-responsiveness to subsequent allergen challenge (Anderson and Pipkorn 1988; Leggieri et al., 1991; Miadonna et al., 1996). PAF, and its metabolite (lyso-PAF), have been detected in nasal fluids and plasma of patients with rhinitis (Labrakis-Lazana et al., 1988; Miadonna

ABBREVIATIONS: PAF, platelet-activating factor; OA, ovalbumin; BSA, bovine serum albumin.
et al., 1989; Shirasaki and Asakura, 1990). These results implicate PAF in allergic rhinitis and raise the possibility that PAF antagonists may be useful in the treatment of the disease.

ABT-491 (Fig. 1) is a recently described novel PAF antagonist that inhibits PAF binding to human PAF receptors with a $K_i$ of 0.6 nM. The antagonist is selective and its activity is correlated with functional antagonism of PAF-mediated cellular responses (calcium mobilization, priming of superoxide generation, aggregation and degranulation) (Albert et al., 1997). In vivo ABT-491 is effective in PAF-challenge models with oral potencies ($ED_{50}$) between 0.03 and 0.4 mg/kg in rat, mouse and guinea pig. Given its intrinsic potency and in vivo activity, ABT-491 may be useful in the treatment of allergic rhinitis. To further explore this possibility we evaluated the efficacy of ABT-491 in experimental models of rhinitis, focusing on acute-phase responses. The present report describes the effects of ABT-491 on PAF and antigen-induced changes in nasal vascular permeability in rats and in nasal airway resistance in guinea pigs. The efficacy of the PAF antagonist in these models is compared with the effects of other anti-permeability agents (histamine and serotonin antagonists and a leukotriene synthesis inhibitor).

**Materials and Methods**

**Materials.** ABT-491 (4-ethyl-N,N-dimethyl-3-[3-fluoro-4-[(2-methyl-1H-imidazo-[4,5-c]pyridin-1-yl)methylbenzoyl]-1H-indole-1-carboxamide hydrochloride) and A-79175 ((R)-(+)-N-[3-[5-(4-fluorophenoxy)-2-furanyl]-1-methyl-2-propynyl-N-hydroxyurea) were synthesized at Abbott Laboratories (Brooks et al., 1995; Summers et al., 1996). Other drugs and reagents were purchased from Sigma Chemical Company, St. Louis, MO.

**Laboratory animals.** Male Hartley guinea pigs (300–350 g) were purchased from Charles River Laboratories, Wilmington, MA. Brown Norway rats (275–325 g) were purchased from Harlan Sprague Dawley, Indianapolis, IN. Animals were maintained in a light/dark cycle and were given continuous access to food and water for at least 1 week after purchase. Before use, animals were fasted overnight. All studies were performed in accordance with protocols approved by the Abbott Laboratories Animal Use and Care Committee and met guidelines approved by the American Veterinary Medical Association.

**Statistical methods.** The Student’s $t$-test was used for statistical comparison of group means with appropriate control groups. Percent inhibition of the control response was computed for each treatment group and, in the case of dose-response data, was fitted with a straight line using the logarithm of the dose as the $x$-value. Linear regression of the data was used to estimate $ED_{50}$ values (dose yielding 50% inhibition) in appropriate assays.

**Active sensitization to ovalbumin.** Guinea pigs were sensitized to ovalbumin (chicken egg, grade III, 30 mg/ml) for 30 min by an Ultrasonic Nebulizer (DeVilbiss Aerosonic, Somerset, PA) in-line with the small animal respirator. Animals were pretreated by an i.v. dose of meclofenamic acid (20–24 mg/kg) and urethane (1.0 g/kg), and the trachea was cannulated with PE-240 tubing in posterior direction for spontaneous breathing. A second cannula was then inserted in the caudal direction (retrograde), and the buccal cavity (mouth) was sealed with adhesive to allow for the isolated ventilation through the nares. This cannula was connected to a small animal ventilator (Harvard Instruments, South Natick, MA) (volume set at 10 ml/kg b.wt.; 45 breaths/min) with an in-line pressure transducer (Millar model MPC 500) to monitor insufflation pressure. The pressure signal was recorded and analyzed (peak) by an MI$^2$ data processing workstation (Modular Instruments XYZ System, Southeastern, PA). The animal was maintained at 37°C by being placed supine on a heated table and monitored via a rectal temperature probe.

For PAF-challenged animals, 50 µg (in 50 µl of 0.1% BSA in phosphate-buffered saline) of PAF was instilled into each nostril just before a 30-min observation period. Nasal resistance (peak insufflation pressure) was measured at 1-min intervals throughout the observation period. Percent change at each interval was computed and used to calculate the area under the curve of percent change $vs.$ time by use of the trapezoidal rule. Five to eleven animals were used per treatment group. The combined control group contained 16 animals.

For antigen challenge, animals actively sensitized to ovalbumin were exposed to aerosolized ovalbumin (chicken egg, grade III, 30 mg/ml) for 30 min by an Ultrasonic Nebulizer (DeVilbiss Aerosonic, Somerset, PA) in-line with the small animal respirator. Animals were pretreated by an i.v. dose of meclofenamic acid (2 mg/kg) 10 min before antigen challenge. Each animal was ventilated at a rate of 45 breaths/min at a volume of 10 ml/kg of body weight. After the antigen challenge, nasal airway resistance was monitored during a 30-min period. At the end of the observation period, the nostrils were pinched closed to provide a means for normalizing each ovalbumin challenge to the maximum nasal resistance for each individual animal. Response to antigen was computed as percent maximum from nasal resistance measurements taken in duplicate 30 min after antigen challenge. Six or seven animals were used per treatment group. The combined control group contained 11 animals.

![Fig. 1. Structure of ABT-491.](image-url)
Animals receiving ABT-491 and A-79175 by the oral route were dosed 2 hr before challenge via a 5 French nasogastric tube with water and methylcellulose, respectively, as vehicle. Mepyramine, dissolved in water, was given as an i.p. injection 30 min before challenge.

Results

Nasal vascular permeability. Topical administration of PAF to the nasal passages of anesthetized Brown Norway rats provokes an acute inflammatory response that resulted in increased nasal vascular permeability. The response, measured as dye leakage, was evident within 15 min after perfusion with PAF (fig. 2A). After reaching a maximum 30 min after initiation of the infusion, the PAF response diminished to approximately half-maximal by the end of the observation period. The vascular leakage induced by PAF was inhibited by pretreatment with ABT-491 (fig. 2A). The inhibition, based on total dye release, was dose related (fig. 2B). An orally administered dose of 1 mg/kg of the antagonist, given 75 min before PAF challenge, inhibited the response by 75% and complete inhibition was achieved with a dose of 10 mg/kg. From these and the other results given in the figure 2B, the potency (ED$_{50}$) of ABT-491 for inhibiting the PAF-induced response was computed as 0.3 mg/kg.

A 5-lipoxygenase inhibitor A-79175 was also evaluated for efficacy in the PAF-challenge model. The inhibitor, when given at a dose (10 mg/kg) sufficient to block leukotriene biosynthesis (Brooks et al., 1995), had no significant effect on PAF-induced nasal vascular permeability (35.0 ± 3.6 vs. 28.9 ± 3.4 μg dye/2 hr).

Intranasal perfusion with OA in rats that had been sensitized to the antigen also induced an increase in nasal vascular permeability (fig. 3A). The response to OA, evident within 30 min after antigen infusion, was slower in development and had lesser magnitude than the PAF-induced response. In contrast to the PAF response, the antigen response did not peak but continued to increase throughout the perfusion period. As shown in figure 3A, oral pretreatment with 1 mg/kg ABT-491 inhibited the antigen-induced nasal vascular leakage. The maximum effective dose of ABT-491, 3 mg/kg, produced 74% inhibition of the antigen response (fig. 3B). Examination of this dose-response relationship yielded an ED$_{50}$ value of 0.5 mg/kg.

Other antipermeability agents were evaluated for efficacy in the antigen challenge model (table 1). Methysergide, a serotonin antagonist, inhibited the permeability response by 87%. A-79175, a 5-lipoxygenase inhibitor, exhibited a maximal inhibition of 77%. Mepyramine (antihistamine, 10 mg/kg i.p.) also significantly inhibited the response (56% inhibition). Combining the PAF antagonist (3 mg/kg) with the serotonin antagonist (10 mg/kg) resulted in 93% inhibition of the permeability response to OA. However, combined dosing of the PAF antagonist with the 5-lipoxygenase inhibitor resulted in inhibition (68%) no greater than that achieved with either agent alone.

Nasal airway resistance. PAF (50 μg) instilled into each guinea pig nostril produced a steady increase in nasal resistance as compared with instillation of the 0.1% BSA vehicle alone (fig. 4A). The response was approximately 40% of the maximal nasal resistance achievable, as determined by a total occlusion obtained by pinching closed both nostrils. Pretreatment with ABT-491 (1 mg/kg), as a 2-hr oral pretreatment, significantly inhibited the increase in nasal resistance caused by PAF (fig. 4A). The inhibition, based on area under the curve, was dose-dependent. As shown in figure 4B, the 0.3 mg/kg dose of ABT-491 was not significantly different from the control PAF responses, whereas all remaining doses of ABT-491 (1.0, 3.0 and 10 mg/kg) were significantly inhibited by 63%, 65% and 88%, respectively. From these results, the ED$_{50}$ of ABT-491 was found to be 1.0 mg/kg. ABT-491 (1 mg/kg) also reduced the amount of rhinorrhea qualitatively observed after a PAF challenge but had no effect on histamine-induced nasal resistance (not shown). In additional studies the 5-lipoxygenase inhibitor (A-79175) was evaluated for activity against the PAF-induced response. When tested at a dose of 10 mg/kg A-79175 resulted in only weak (25%) nonsignificant inhibition of the AUC of the PAF-induced response (38.5 ± 5.1 vs. 50.1 ± 7.7).

In guinea pigs actively sensitized to ovalbumin, a 30-min aerosol challenge with antigen produced an increase in nasal resistance that remained essentially constant (55–60% of maximum) throughout the observation period (fig. 5A). ABT-491, given as a 2-hr oral pretreatment, inhibited the antigen-induced increase in nasal resistance. The maximum effective dose of ABT-491 (10 mg/kg) achieved 64% inhibition, based on nasal airway resistance 30 min after antigen challenge.
The 5-lipoxygenase inhibitor (A-79175) and an antihistamine (mepyramine) were also evaluated for efficacy in the guinea pig antigen challenge model (table 2). When administered alone, A-79175 (10 mg/kg p.o.) and mepyramine (10 mg/kg i.p.) attenuated the antigen response, although the inhibition achieved (43% and 29%, respectively) was not statistically significant. A combination of 10 mg/kg ABT-491 with either A-79175 or with mepyramine produced an additive effect (80% and 82% inhibition, respectively, P = .05) in the inhibitory activity for each compound.

**Discussion**

Increased nasal vascular permeability to macromolecules may be one of the underlying causes of rhinorrhea, a symptom of rhinitis (Garreld et al., 1996; Horvath et al., 1991; Krause 1995). Both PAF and antigen cause vascular leakage when applied topically to the nasal cavities of rats. The response to topical administration of PAF observed in the current study agrees well with previous studies demonstrating PAF-induced vascular leakage in the trachea, bronchi and upper airways in rats and guinea pigs (Evans et al., 1989; Kyriakopoulos et al., 1995; Misawa and Iwamura 1990). Similar nasal responses to antigen have also been reported (Misawa and Iwamura 1990; Takahashi et al., 1990). In the present study ABT-491 inhibited both PAF and antigen-induced nasal vascular permeability with approximately the same potency (ED$_{50}$ values of 0.3 and 0.5 mg/kg, respectively). The equivalence in potency suggests that the protective effect of ABT-491 for the antigen response is likely the result of the compound’s ability to compete with PAF for binding to the PAF receptor (Albert et al., 1997) and further supports the role of PAF in antigen response. These observations, coupled with those demonstrating that PAF is a potent mucous secretagogue (Hotchkiss et al., 1993), lend support to the concept that PAF plays a role in the rhinorrhea associated with allergic rhinitis. The present study is also the first to demonstrate oral activity of a PAF receptor antagonist for blocking antigen-induced nasal vascular permeability in a model of allergic rhinitis.

Although the maximum inhibition resulting from administration of ABT-491 was substantial (74%), the PAF antagonist did not provide complete protection against the antigen-induced permeability response. This less-than-complete inhibition suggests that other mediators in addition to PAF are involved in the permeability response. Histamine, a product of activated mast cells, is well established as a mediator of vascular permeability in the lower airways (Evans et al., 1988, 1989). In the present study an antihistamine blocked a significant portion (56%) of antigen-induced nasal vascular permeability, thus supporting the role of histamine in the upper airway response to antigen. This result is in good accord with results from previous studies in rats and guinea pigs (Mizuno et al., 1991; Takahashi et al., 1990). Because antihistamines are commonly used therapy in allergic rhinitis (Krause 1995), the rat and guinea pig models appear to be clinically predictive, at least as far as the role of histamine is concerned.

In addition to PAF and histamine, serotonin and leukotrienes are also involved in nasal vascular leakage induced by antigen in Brown Norway rats, as is evidenced by the effectiveness of a serotonin antagonist and a 5-lipoxygenase inhibitor in the antigen challenge model. The number of mediators potentially involved in the permeability response...
to antigen raises the possibility of combination therapy. In the current study nearly complete inhibition (93%) was achieved with a combination of the PAF antagonist and the serotonin antagonist. However, in contrast to the nasal resistance response to antigen discussed below, combined dosing of the PAF receptor antagonist and the 5-lipoxygenase inhibitor did not increase inhibition of the permeability response above that was achieved with either agent alone. This suggests that PAF and leukotrienes may share a common pathway in inducing vascular leakage. One possibility suggested previously by others is that the PAF-induced response is mediated in part by the generation of leukotrienes (Misawa and Iwamura 1990; Seeds et al., 1995). In the present study the 5-lipoxygenase inhibitor A-79175, administered at a dose effective against antigen challenge, had no effect on the PAF-induced response. It thus appears that in sensitized rat model vascular leakage induced by PAF in the nasal cavity is independent of leukotriene biosynthesis.

Nasal congestion, in addition to rhinorrhea, is another symptom of the acute allergic response associated with allergic rhinitis (Krause 1995). Increased nasal airway resistance that mimics the nasal blockage seen in allergic rhinitis is induced experimentally by antigen administration to sensitized animals (Mizuno et al., 1991; Narita and Asakura 1993). In the present study we observed a response to PAF administered topically to the nasal passages of guinea pigs comparable in magnitude (40% of maximum) with that induced by inhalation of aerosolized antigen (55–60% maximum). These results, which are similar to those obtained with PAF administered via inhalation (Misawa and Iwamura 1990), indicate a role for PAF in the acute upper airway response to antigen.

The effectiveness of the PAF receptor antagonist ABT-491

![Fig. 4. PAF-induced increase in nasal airway resistance of guinea pigs and inhibition with ABT-491. (A) PAF (50 μg) was instilled into the nostril of guinea pigs given drug vehicle (squares) or 1 mg/kg ABT-491 (triangles) orally 2 hr before challenge. Negative controls are indicated by circles. Nasal airway resistance was monitored at 1-min intervals and is expressed as percent change. Values are the mean ± S.E.M. (B) Area under the curve (AUC) for the percent change vs. time plots were computed for groups receiving the indicated dose of ABT-491 and are presented as the mean ± S.E.M. Percent inhibition of the PAF response is shown above each bar. Statistical comparison vs. antigen control: *P ≤ .05; ***P ≤ .005.](image)

![Fig. 5. Antigen-induced nasal airway resistance of guinea pigs and inhibition with ABT-491. (A) Guinea pigs given drug vehicle (squares) or 10 mg/kg ABT-491 (triangles) orally 2 hr before challenge were exposed to aerosolized antigen for 30 min. Negative controls are indicated by circles. After antigen challenge, nasal airway resistance was monitored at 1-min intervals and is expressed as percent maximum. Values are the mean ± S.E.M. (B) Dose-response relationship. Nasal airway resistance of guinea pigs given vehicle or ABT-491 was measured 30 min after exposure to antigen. Values, expressed as percent maximum, are the mean ± S.E.M. Percent inhibition of the antigen response is shown above each bar. Statistical comparison vs. antigen control: * P ≤ .05.](image)

**TABLE 2**

Effect of anti-PAF, histamine and leukotriene agents on antigen-induced nasal airway resistance in guinea pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nasal Airway Resistance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>17 ± 4</td>
<td>100</td>
</tr>
<tr>
<td>Antigen control</td>
<td>57 ± 9</td>
<td>0</td>
</tr>
<tr>
<td>ABT-491</td>
<td>31 ± 12</td>
<td>64</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>45 ± 9</td>
<td>29</td>
</tr>
<tr>
<td>A-79175</td>
<td>40 ± 11</td>
<td>43</td>
</tr>
<tr>
<td>ABT-491 and mepyramine</td>
<td>24 ± 4**</td>
<td>82</td>
</tr>
<tr>
<td>ABT-491 and A-79175</td>
<td>25 ± 5*</td>
<td>80</td>
</tr>
</tbody>
</table>

*a Each drug, alone or in combination, was given orally or, in the case of mepyramine, intraperitoneally, at a dose of 10 mg/kg.

*b Nasal airway resistance was measured 30 min after exposure to antigen and is expressed as mean percent maximum ± S.E.M.

Statistical comparison vs. antigen control: * P ≤ .05; ** P ≤ .01.

The effectiveness of the PAF receptor antagonist ABT-491
in inhibiting both the PAF and the antigen-induced increases in nasal airway resistance (oral ED_{50} values of 1.0 and 5.5 mg/kg, respectively) further supports the role of PAF in the response of the upper airways to antigen. CV-3988, a first-generation PAF receptor antagonist, given intravenously (10 mg/kg) has previously been shown to inhibit antigen-induced nasal airway resistance (Misawa and Iwamura 1990). The present study extends these results by demonstrating the oral activity of a potent PAF receptor antagonist for inhibiting the acute phase airway response to antigen.

As for antigen-induced vascular leakage, other mediators in addition to PAF have been implicated in the airway response. Histamine and leukotrienes are capable of inducing nasal airway resistance, and leukotrienes may be particularly important in the late-phase response to antigen (Howarth et al., 1993; Knapp 1990; Mizuno et al., 1991; Narita and Asakura 1993). In the present study the 5-lipoxygenase inhibitor A-79175 and the antihistamine mepyramine, agents which were effective in inhibiting antigen-induced vascular leakage, had only weak and not statistically significant effects (43% and 29% inhibition, respectively) on antigen-induced nasal airway resistance. The lack of effect of the antihistamine agrees well with results from previous studies in guinea pigs and with the clinical observation that H_{1}-antagonists do not alleviate nasal congestion (Krause 1995; Misawa and Iwamura 1990). The marginal effect of the 5-lipoxygenase inhibitor suggests that leukotrienes, which are important mediators of nasal vascular permeability, may play less of a direct role in the acute nasal airway resistance response to antigen. Despite the lack of efficacy when given as monotherapies, both the antihistamine and the 5-lipoxygenase inhibitor, when given in combination with the PAF receptor antagonist, increased the extent of inhibition of the airway response to antigen, compared with treatment with the PAF receptor antagonist alone. These results illustrate the complexity of interaction among inflammatory mediators and the potential utility of combination therapies.

In summary, the present study demonstrates the potent oral activity of ABT-491 in animal models of allergic rhinitis. The PAF receptor antagonist, alone and in combination with other anti-inflammatory drugs, was effective at inhibiting antigen-induced increases in nasal vascular leakage and nasal airway resistance, responses that mimic primary symptoms of allergic rhinitis. These results support the potential clinical utility of an orally active PAF antagonist for the treatment of this disease.

References
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