Studies on Anti-allergic Action of AH 21-132, a Novel Isozyme-Selective Phosphodiesterase Inhibitor in Airways

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ABSTRACT—The effects of AH 21-132, a type III and IV phosphodiesterase (PDE) inhibitor, on allergic reactions in the airway were studied by comparing them with the effects of rolipram, a type IV PDE inhibitor, and aminophylline, a non-selective PDE inhibitor. The following results were obtained: 1) AH 21-132, rolipram and aminophylline inhibited the antigen-induced contraction of isolated guinea pig tracheal muscle in vitro. 2) AH 21-132 and aminophylline inhibited antigen-induced histamine release from human lung tissue fragments. 3) Antigen-induced accumulation of inflammatory cells including eosinophils and macrophages in mice bronchoalveolar lavage fluid was clearly inhibited by AH 21-132 and rolipram, but not by aminophylline. 4) AH 21-132, rolipram and aminophylline inhibited immediate phase bronchoconstriction induced by either an intravenous or an aerosol challenge of antigen in guinea pigs. 5) AH 21-132 and rolipram inhibited the aeroantigen challenge-induced late phase increase in the airway resistance in guinea pigs, but aminophylline did not. These results suggest that AH 21-132 has an anti-allergic effect in the airway and that these actions may be beneficial for the treatment of allergic bronchial asthma.

Keywords: Phosphodiesterase inhibitor (type III/IV), Aminophylline, Bronchodilator, AH 21-132, Bronchial asthma

Theophylline is an effective anti-asthma drug because it has bronchodilation, pulmonary anti-allergic and anti-inflammatory activity (1–3). The molecular mechanism of these actions, however, has been debated for many years. Several mechanisms for the anti-asthmatic effect of theophylline have been proposed, including adenosine-receptor antagonism (4, 5). Despite the complexity of theophylline’s multiple actions, many studies have suggested that its bronchodilatory effect and some other effects may involve the inhibition of one or more forms of phosphodiesterase (PDE) (6–9).

(±)-cis-6-(p-Acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methyl-benzo-[c][1,6]naphtyridine (AH 21-132) is a benzonaphtyridine derivative structurally similar to papaverine, a non-selective PDE inhibitor. AH 21-132 differs from papaverine in its structural moiety, physicochemical nature and biochemical activities. The most important difference is its selective inhibition of specific PDE isozymes. AH 21-132 selectively inhibits types III and IV isozymes derived from a homogenate of guinea pig cardiac ventricles and bovine trachealis (10–13). Regarding the pharmacological profile of AH 21-132, it is an effective relaxant of both human and guinea pig airway smooth muscle preparations and also actively prevents spasmogen-induced contraction of airway smooth muscles (10–13). In the guinea pig in vivo, AH 21-132 inhibits platelet activating factor (PAF)-induced airway hyperreactivity and eosinophilia. Although previous studies have established that AH 21-132 is effective against the bronchoconstriction and airway inflammation caused by chemical substances, relatively little is known about its effect on allergic reactions. The purpose of the present study is to investigate the effect of AH 21-132 on the allergic reaction in the airway.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs weighing 300–400 g and 7-week-old male Balb/c mice were used. These animals were purchased from Japan SLC, Inc. (Hamamatsu).

Drugs

(±)-cis-6-(p-Acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methyl-benzo-[c][1,6]naphtyridine bis hydrogen maleinate (AH 21-132) and aminophyll-
line were kindly donated by Japan Sandz, Tokyo. 4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-2-pyridinone (rolipram) was kindly donated by Dr. S. Tanaka (Nissan Chemical, Ltd., Tokyo). AH 21-132 and aminophylline were dissolved in saline, and rolipram was suspended in saline.

**Antigen-induced contraction of trachea isolated from sensitized guinea pigs**

Guinea pigs were immunized twice with ovalbumin (OA) at doses of 5 mg on day 0 (i.p.) and 10 mg on day 4 (s.c.). One month after the first immunization, the guinea pigs were stunned and exsanguinated. The trachea was excised, trimmed of excess tissue and cut longitudinally in pigs were stunned and exsanguinated. The trachea was excised guinea pigs

Antigen-induced immediate asthmatic response (IAR) in guinea pigs

The procedure has been described previously (15). In brief, guinea pigs were passively sensitized by an i.v. injection of anti-benzylpenicilloxy bovine gamma globulin (BPO-BGG) guinea pig serum (0.5 ml/kg). Forty-eight hours after the sensitization, the trachea of the guinea pig was cannulated under anesthesia with pentobarbital sodium (50 mg/kg, i.p.). The cannula was connected to both a transducer (MFP-1100, TV-142, TV-241, TP-602T; Nihon Kohden) and a respirometer (RM-25, RPM-6018; Nihon Kohden). The antigen (BPO-BSA, 40 μg/kg) was then injected i.v. into the guinea pigs. Alterations in respiration were measured by monitoring changes in the respiratory rate and volume and changes in the ratio of expiratory time to inspiratory time. Drugs were given p.o. 1 hr before the antigen challenge.

**Antigen-induced increase in respiratory resistance (Rrs) in conscious guinea pigs**

Guinea pigs were sensitized twice with OA, i.p. (0.5 mg on day 0 and 1 mg on day 2). On days 22, 24, 27, 31, 36 and 38, they were exposed for 1 min to an aerosol of OA solution (0.1, 0.2, 0.4, 0.5, 1.0 and 1.0%, respectively) generated by a nebulizer. Drugs were given orally 1 hr before every inhalation. The changes of Rrs by the OA inhalations were measured on days 22 and 38. The effect of drugs on the Rrs changes was evaluated for 30 min from immediately after the end of the inhalation on day 22 and for 270 min from 90 min after that on day 38. To expose the animals to the aerosol and measure the Rrs change on days 22 and 38, the animals were placed in a transparent chamber (20 × 20 × 20 cm) and inhaled aerosol spray via a snout-covering plastic face mask connected to a Devilbiss Pulmo Aide nebulizer (Somerset, PA, USA) (spray volume, 0.5 ml/min; mass particle diameter distribution, 0.5–5.0 μm; compressed air, 12 L/minute). On days 24, 27, 31 and 36, the animals were exposed to OA using another nebulizer (Ultrasonic nebulizer TUR-3200, Nihon Kohden). Rrs was measured by the method described by Mead and modified by Yamauchi et al. (16). Briefly, the guinea
The guinea pig was positioned in a body-plethysmograph chamber with the head outside of the chamber. The respiratory airflow from the snout-covering face mask and the oscillating pressure in the body-chamber with a 30 Hz sine wave pressure at 10 cmH2O were recorded with a differential pressure transducer, and these signals were displayed simultaneously on an X-Y oscilloscope and recorded on a polygraph. Rrs was calculated using the following formula: Rrs = pressure/flow (H2O pressure/ml/min). The results are expressed as the percent change of the Rrs value in the individual animals.

Statistical analyses

Data are expressed as the mean ± standard error of the mean (S.E.M.). Statistical analyses were performed by Student's paired two-tailed t-test and Dunnett's multiple range test.

RESULTS

Antigen-induced contraction of trachea isolated from sensitized guinea pig

Figure 1 shows the effect of AH 21-132, rolipram and aminophylline on the antigen-induced contraction of sensitized guinea pig trachea. AH 21-132 at a concentration between 10^{-6} and 10^{-5} M inhibited antigen induced contraction of guinea pig tracheal smooth muscle. Rolipram and aminophylline at a concentration between 10^{-5} and 10^{-4} M also inhibited the reaction.

<table>
<thead>
<tr>
<th>Table 1. Effect of AH 21-132, rolipram and aminophylline on histamine release from passively sensitized human lung fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Spontaneous</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>AH 21-132</td>
</tr>
<tr>
<td>10^{-5}</td>
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<td>10^{-4}</td>
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<td>10^{-5}</td>
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<tr>
<td>Aminophylline</td>
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<td>10^{-4}</td>
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<td>Rolipram 10^{-6}</td>
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<tr>
<td>Aminophylline 5 x 10^{-5}</td>
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<tr>
<td>10^{-4}</td>
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<tr>
<td>Aminophylline 5 x 10^{-5}</td>
</tr>
</tbody>
</table>

The examined agents were added to the reaction mixture 5 min before the antigen challenge. The results are shown as the mean ± S.E.M. of 5 sets of each experiment. *P<0.05, **P<0.01, statistically significant difference from the control response (t-test).
Histamine release from human lung fragment

Table 1 shows the effect of AH 21-132, rolipram and aminophylline on antigen-induced histamine release from human lung fragment. AH 21-132 inhibited the histamine release at concentrations of $10^{-5}$ and $10^{-4}$ M. Aminophylline at a concentration of $10^{-4}$ M also inhibited the release, but rolipram did not inhibit it at any concentration.

Airway inflammation in mice

Repeated inhalations of antigen to actively sensitized mice resulted in an increase in the number of monocytes and eosinophils in BALF. Table 2 shows the effect of the examined agents on the increase of inflammatory cells in BALF. AH 21-132 and rolipram at a dose of 3 mg/kg inhibited the increase of total leukocytes, monocytes and eosinophils, in terms of cell number. On the other hand, aminophylline at doses of 1 and 3 mg/kg did not affect the total number of leukocytes, monocytes and eosinophils.

### Table 2. Effect of AH 21-132, rolipram and aminophylline on antigen-induced inflammatory cell accumulation in BALF in mice

<table>
<thead>
<tr>
<th>mg/kg</th>
<th>total ($\times 10^5$ cells/BALF)</th>
<th>monocytes</th>
<th>eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.60±0.22</td>
<td>2.90±0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>6.03±0.62</td>
<td>3.82±0.28</td>
<td>1.94±0.54</td>
</tr>
<tr>
<td>AH 21-132</td>
<td>1.40±0.46</td>
<td>3.89±0.22</td>
<td>0.89±0.30</td>
</tr>
<tr>
<td>Rolipram</td>
<td>3.19±0.46</td>
<td>2.82±0.28</td>
<td>0.26±0.07</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>3.79±0.33</td>
<td>3.35±0.29</td>
<td>0.40±0.13</td>
</tr>
<tr>
<td>3</td>
<td>3.24±0.29</td>
<td>2.56±0.14</td>
<td>0.35±0.12</td>
</tr>
<tr>
<td>5</td>
<td>5.20±0.58</td>
<td>3.30±0.42</td>
<td>1.52±0.28</td>
</tr>
<tr>
<td>3</td>
<td>6.20±1.76</td>
<td>3.96±0.63</td>
<td>2.20±0.31</td>
</tr>
</tbody>
</table>

Mice were immunized with OA i.p. on days 0 and 12. Starting on day 22, the mice were exposed to OA-aerosol 3 times every 4th day. The examined agents were given i.p. for 10 consecutive days from day 21 to the day of the final inhalation. The results are shown as the mean±S.E.M. of 7 mice. *P<0.05, **P<0.01, statistically significant difference from the control (Dunnett’s multiple range test). a): P<0.05, statistically significant difference from the normal mice (t-test).

**Fig. 2.** Effects of AH 21-132, rolipram and aminophylline on antigen-induced immediate asthmatic response in guinea pigs. Guinea pigs were challenged by an i.v. injection of BPO-BSA 48 hr after passive sensitization with anti-BPO-BGG guinea pig serum. The examined agents were given p.o. 1 hr before the antigen challenge. The results are shown as the mean±S.E.M. of 4 to 6 guinea pigs. The number of respirations (rate), tidal volume (volume) and ratio between Ex/In before antigen-challenge were 90.8±5.62 times/minute, 442.9±75.24 ml/minute and 1.5±0.32, respectively. Ex/In: Ratio of expiration time to inspiration time. *P<0.05, statistically significant difference from the time-matched control response (t-test). ○: Control, AH 21-132 and aminophylline (○: 10 mg/kg, □: 30 mg/kg), rolipram (□: 3 mg/kg, △: 10 mg/kg).
Antigen-induced IAR in guinea pigs

Figure 2 shows the effect of AH 21-132, rolipram and aminophylline on antigen-induced IAR in guinea pigs. When BPO-BSA was injected i.v. into guinea pigs sensitized passively, the respiratory rate and volume decreased and the ratio of expiration time to inspiration time increased in comparison with the pre-challenge level. AH 21-132 and aminophylline at a dose of 30 mg/kg inhibited the responses. Rolipram at a dose of 10 mg/kg shows clear and almost complete inhibition of the responses.

Antigen-induced increase in Rrs in conscious guinea pigs

An aerosolized OA-induced increase in Rrs on day 22 was observed at 1–10 min after the antigen exposure (immediate phase response), in the guinea pigs, but an increase in Rrs at 2–6 hr later (late phase response) was not observed. Figure 3 shows the effects of the examined agents on the immediate airway response. AH 21-132, rolipram and aminophylline administered orally at doses of 10 and 30 mg/kg significantly reduced the increase of Rrs in the immediate phase response on day 22.

On day 38, the challenge with aerosolized 1.0% OA provoked biphasic airway responses observed at 1 min and at
2–6 hr after the antigen-challenge (immediate and late phase responses). The peak increase in Rrs in the late phase response was observed at 4 hr after the antigen-challenge. Figure 4 shows the effect of the examined agents on the late phase response on day 38. The increase in Rrs was clearly inhibited by AH 21-132 and rolipram at doses of 10 and 30 mg/kg. Aminophylline showed an inhibition at 10 mg/kg and showed a tendency to inhibit the late phase response at 30 mg/kg. The inhibitory potencies of AH 21-132 and rolipram were more potent than that of aminophylline.

**DISCUSSION**

Small et al. (17) reported that theophylline, unlike AH 21-132, antagonized adenosine in depressing the twitches elicited by electrical transmural stimulation in longitudinal smooth muscle of guinea pig ileum. Bewley and Chapman (18) and Boyle et al. (19) reported that removing the airway epithelial cells potentiated the relaxing activity of
AH 21-132, but not that of aminophylline and theophylline. In the present results, AH 21-132 exerted inhibitory effects on all experimental allergic reactions in the airway. Aminophylline showed inhibitory effects on acute allergic bronchoconstriction and histamine release, but not on the antigen-induced accumulation of inflammatory cells in BALF in mice or antigen-induced late phase increase in Rrs in guinea pigs. Rolipram had anti-allergic action in the airway, but did not suppress antigen-induced histamine release. These evidence suggest that AH 21-132 has a pharmacological profile different from that of methylxanthines.

Recently, many investigators have suggested that bronchial asthma should be considered as an inflammatory disease, especially when eosinophilia and airway edema are present (20, 21). Antigen challenge-induced acute phase reactions in the skin and airway appears within 60 min and then is often followed by an intense inflammatory reaction termed the late phase reaction. The late phase reaction usually appears 3 to 48 hr after the antigen challenge and is of great interest due to its similarity to the clinical manifestation of chronic allergic diseases. In addition to the late phase reaction, eosinophilia is another undesired symptom of bronchial asthma. Eosinophil derived harmful substances such as eosinophil cationic protein, eosinophil peroxidase and others play an important role in desquamation of airway epithelial cells which results in airway hyperreactivity. The late phase allergic reaction and airway eosinophilia are, therefore, key reactions in the development of the chronic disease state in asthma. AH 21-132 shows clear inhibition of both the late phase reaction and airway eosinophilia, suggesting a therapeutic value of this agent for allergic chronic airway diseases such as bronchial asthma.

There are a few reports indicating that the anti-spasmodogenic effect of AH 21-132 on airway smooth muscle is due to the inhibition of cAMP PDE (type III and IV). In these experiments, acetylcholine and histamine were used as a spasmogen. In the present study, AH 21-132 clearly inhibited antigen-induced acute bronchoconstriction in vitro and in vivo. Whereas histamine is one of the most important mediators of the allergic reaction, many reports have indicated that there are different contractile mechanisms between antigen- and histamine-induced contractions. Therefore, further experiments will be necessary to elucidate the inhibitory mechanism of AH 21-132 on antigen-induced acute bronchoconstriction, especially the participation of the inhibition of PDE isozymes. In the present study, AH 21-132 showed more potent inhibition on antigen-induced bronchoconstriction than rolipram, whereas these two agents have been reported to have similar inhibitory activity against the type IV PDE isozyme. This may mean the inhibition of type III PDE may participate in the AH 21-132 induced inhibition of allergic bronchoconstriction. Further experiments will focus on the relationship between the inhibition of type III PDE and inhibition of allergic bronchoconstriction by AH 21-132.

Rolipram inhibited the accumulation of inflammatory cells and antigen-induced broncho-constriction, probably due to its potent and selective inhibition of type IV PDE isozymes. These pharmacological profiles seem to indicate the usefulness of rolipram as a remedy for bronchial asthma. Rolipram is, however, not suitable for the treatment of asthma because of its suppression of the central nervous system. Theophylline is useful in the treatment of asthma, but its value is limited by a narrow therapeutic index and wide range of gastrointestinal, central nervous system and cardiovascular side effects. In our recent experiments, whereas significant abnormal behavioral alterations including head twitches and foreleg shaking were observed after the administration of rolipram (10 mg/kg) or aminophylline (10 mg/kg) to rats, AH 21-132 showed no significant alteration of behavior in rats. These unpublished data of ours suggest that AH 21-132 shows less effect on the central nervous system than rolipram and aminophylline. Further studies on the effectiveness and side effects of some other agents will be necessary to find the best selective anti-asthmatic agent with PDE inhibition. One of the most important requirements for the development of such a drug (PDE inhibitors) is that it combines the efficacy of theophylline with fewer side effects.

In conclusion, the present results show that AH 21-132 suppresses experimental allergic reactions in the airway effectively, while the relationship between the anti-allergic action and the inhibition of PDE is still unclear. Further experiments will be necessary to explain the molecular mechanism of the anti-allergic action of AH 21-132.

REFERENCES


