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Effect of FK3657, a non-peptide bradykinin B_2 receptor antagonist, on allergic airway disease models

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Abstract

Bradykinin has been suggested to be involved in allergic diseases. In this study, we tested the effect of FK3657 ((*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)-oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl]acrylamide), an orally active non-peptide bradykinin B_2 receptor antagonist, on allergic airway disease models in guinea pigs. FK3657 given orally inhibited bradykinin-induced or dextran sulfate (an activator of kinin–kallikrein cascade)-induced bronchoconstriction and plasma extravasation in the lower airways (trachea and main bronchi) and nasal mucosa of guinea pigs with ED_{50} of 0.04–0.23 mg/kg. In the antigen-induced dual asthmatic response model of guinea pigs, FK3657 significantly attenuated the late phase asthmatic response, but not the immediate asthmatic response. FK3657 also significantly inhibited the 2,4-tolylene diisocyanate (TDI)-induced plasma extravasation in nasal mucosa of TDI-sensitized guinea pigs. These results suggest that oral FK3657 may be useful for asthma or allergic rhinitis as a therapeutic drug.

Keywords: Bradykinin; Bradykinin B_2 receptor; Bradykinin receptor antagonist; Asthma; Rhinitis; FK3657

1. Introduction

Two types of kinin receptor, the bradykinin B_1 receptor and the bradykinin B_2 receptor, are known so far, and bradykinin exerts its effect via either type of bradykinin receptor. Bradykinin B_2 receptors are constitutively expressed and mediate most of the physiological actions of kinins, whereas bradykinin B_1 receptors are highly inducible upon inflammatory stimulation or tissue injury, suggesting that they are involved in inflammation and/or nociception (Bhoola et al., 1992; Calixto et al., 2000).

Bradykinin has a potent pro-inflammatory activity, suggested to be involved in allergic airway diseases. In allergic patients, kinins are generated in the nose or lung in response to antigen (Baumgarten et al., 1992; Christiansen et al., 1992; Majima et al., 1996; Proud et al., 1983). Nasal provocation by bradykinin, but not by a selective bradykinin B_1 receptor agonist, can induce nasal symptoms, nasal congestion, rhinorea and irritation, and increase vascular permeability (Churchill et al., 1991; Proud et al., 1988; Doyle et al., 1990). The bradykinin-induced or allergen-induced nasal response is suppressed by a bradykinin B_2 receptor antagonist (Austin et al., 1994; Proud et al., 1995). These results indicate that bradykinin B_2 receptors have a role in nasal allergy. Likewise, inhaled bradykinin, but not a selective bradykinin B_1 receptor agonist, causes bronchoconstriction in asthmatic patients, suggesting that bradykinin B_2 receptors may also be more important in asthma than bradykinin B_1 receptors (Polosa and Holgate, 1990). Thus, bradykinin B_2 receptor antagonists should have therapeutic potential as anti-allergic drugs.

In this study, we tested the effect of FK3657 ((*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)-oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl]acrylamide), an orally active non-peptide bradykinin B_2 receptor antagonist (Asano et al., 1997), on allergic disease models in guinea pigs.

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2. Materials and methods

2.1. Animals

Hartley male guinea pigs were purchased from Japan SLC (Shizuoka, Japan). All studies were performed in accordance with the guidelines of the Fujisawa Pharmaceutical Animal Experimental Committee.

2.2. Reagents

FK3657 (Fujisawa Pharmaceutical, Osaka, Japan) was dissolved with 0.1 mol/l HCl and given orally in a volume of 5 ml/kg.

Bradykinin (Sigma, St. Louis, MO, USA), sodium dextran sulfate (Nacarai Tesque, Kyoto, Japan), pancuronium bromide (Sankyo, Tokyo, Japan), captopril (Sigma), propranolol (Nacarai), chicken egg albumin (Seikagaku, Tokyo, Japan), Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA), 2,4-tolylene diisocyanate (TDI; Nacarai), Evans blue (Merck, Darmstad, Germany), and bovine serum albumin (Sigma) were purchased.

2.3. Bradykinin-induced bronchoconstriction

Bronchoconstriction by exogenous bradykinin (i.v. injection of bradykinin) or endogenously generated bradykinin (i.v. injection of dextran sulfate) was induced as described previously (Inamura et al., 1997). Briefly, the animals were anesthetized with sodium pentobarbital and the trachea, jugular vein, and esophagus were cannulated for mechanical ventilation (10 ml/kg, 60 strokes/min), drug administration, and FK3657 administration. Pulmonary insufflation pressure was monitored by a pressure transducer connected to the side arm of the tracheal cannula. Spontaneous breathing was suppressed by i.v. injection of pancuronium bromide (0.1 mg/head), and then propranolol (10 mg/kg, s.c.) was injected. After 10 min, bradykinin (4.2 μg/kg, i.v.) or dextran sulfate (2 mg/kg, i.v.) was injected. FK3657 or the vehicle was administered 30 min before bradykinin or dextran sulfate injection via the esophageal cannula.

Bronchoconstriction was expressed as a percentage of the maximal increase of the pulmonary insufflation pressure achieved by clamping off the trachea (percentage increase).

2.4. Bradykinin-induced plasma extravasation

The animals were injected with a mixture of Evans blue (20 mg/kg), captopril (1 mg/kg), and bradykinin (2.1 μg/kg), or sodium dextran sulfate (0.32 mg/kg) in a volume of 2 ml/kg of saline into the cephalic vein. Ten minutes later, the animals were killed and the pulmonary artery was perfused with saline (50–100 ml). The trachea and main bronchi were dissected, weighed, and stored at −30 °C until use. For the nasal study, the animals were anesthetized with pentobarbital and injected with a mixture of Evans blue (40 mg/kg), captopril (1 mg/kg), and sodium dextran sulfate (0.56 mg/kg) into the cephalic vein. Fifteen minutes later, the animals were killed and perfused with saline systemically. The nasal mucosa was removed, weighed, and stored at −30 °C until use. For the determination of background plasma extravasation in both lower airway and nose, saline was injected in place of bradykinin.

FK3657 or the vehicle was given p.o. 30 min before the injection of bradykinin or dextran sulfate.

Evans blue was extracted from the tissues by incubating them in formamide at 37 °C overnight, and quantified spectrophotometrically by measuring the absorbance at 620 nm wavelength. Tissue Evans blue dye content was expressed as nanogram dye per milligram wet weight tissue.

2.5. Antigen-induced dual asthmatic response

The animals were immunized by i.p. injection of 1 ml/head of emulsion prepared by vigorous mixing an equal volume of egg albumin solution (20 mg/ml in saline) and Freund's complete adjuvant. After 23 days, immunized conscious animals were challenged with nebulized egg albumin solution (5% in saline) with an ultrasonic nebulizer (NE-U10B, Omron) for 3 min in a two-chambered, whole body plethysmograph (model P, Buxco electronics, Sharon, CT, USA). The animals received 10 mg/kg of mepyramine (pyriramine maleate, Sigma) intraperitoneally to protect from anaphylactic death 30 min before the challenge. FK3657 (1.8 mg/kg) or the vehicle was given 30 min before and 3 h after the antigen challenge. Since FK3657 at 1 mg/kg was not effective in a preliminary experiment, we selected the 1.8-mg/kg dose to determine the minimal effective dose.

Specific airway resistance of conscious animals in the chamber was measured using a non-invasive respiratory analyzer (Model 1 PMUA + SAR, Buxco) based on the method described earlier (Pennock et al., 1979). After the baseline value was obtained, the animals were challenged with antigen. Then, specific airway resistance was measured at 2 min, 2, 4, 5, 6, 7, 8, and 24 h after the antigen challenge. In a preliminary experiment, we observed no significant diurnal change from baseline in specific airway resistance in saline-challenged animals (data not shown).

After the measurement of specific airway resistance at 24 h, the animals were killed with an excess of sodium pentobarbital, and bronchoalveolar lavage was conducted with 10 ml/kg of saline three times. The bronchoalveolar lavage fluid was centrifuged, the cells were resuspended in saline, and the total cell number was counted. In a separate experiment, the cells of bronchoalveolar lavage fluid in egg albumin- or saline-challenged animals were

collected at 24 h after challenge, stained with Hemacolor® (Merck), and differentially counted to specify the cell type increased by egg albumin challenge.

2.6. TDI-induced nasal plasma extravasation

The animals were sensitized with TDI as described previously (Sugawara et al., 1993) with some minor modification. TDI (2 μl) was instilled into both nostrils of the guinea pig at days 0, 2, 4, 7, 14, 21, 28, and 35. We confirmed TDI-specific immunoglobulin (Ig) production in our sensitization protocol by the homologous passive cutaneous anaphylaxis method. Blood samples were collected from anesthetized animals via the saphenous vein on days 14, 21, 28, and 35, and the serum was separated. For TDI-specific immunoglobulin E (IgE) detection, the serum diluted two-fold with saline was injected intradermally (0.1 ml/site). One week later, 0.5 ml of a mixture of TDI-conjugated bovine serum albumin (0.23 mg/ml) and Evans blue dye (20 mg/ml) in saline was injected intravenously. TDI-conjugated bovine serum albumin was prepared as described (Tse and Pesce, 1979). Thirty minutes later, the diameter of the blue spot at the injected site was measured. A blue spot with >5 mm diameter was judged as a positive reaction. For the detection of TDI-specific immunoglobulin G (IgG), the serum was pre-incubated at 56 °C for 2 h to inactivate IgE, diluted 16-fold, and injected intradermally (0.1 ml/site). The next day, the animals were challenged with TDI-conjugated bovine serum albumin and the reaction was judged as described above.

On day 36 or 37, a nasal response was induced by instillation of 5 μl of 2% TDI-ethyl acetate solution into both nostrils of the sensitized animals after Evans blue injection (40 mg/kg, i.v.). Ten minutes later, the nasal mucosa was removed and weighed, and Evans blue was extracted and quantified as described for the bradykinin-induced plasma extravasation study. For the determination of background plasma extravasation, only ethyl acetate was inoculated. FK3657 or the vehicle was administered 45 min before TDI challenge.

2.7. Statistics

The data were expressed as means ± S.E.M. For the dual asthmatic response study, the significance of differences in specific airway resistance between the vehicle group and the FK3657 group was assessed with the Wilcoxon rank sum test at each time point. The late asthmatic response was also quantified by calculating the area under the curve of specific airway resistance values plotted against time (from 4 to 8 h) above baseline. The difference between the vehicle group and the FK3657 group was assessed with the Aspin–Welch test (*t*-test). For other studies, Students-*t*-test or Aspin–Welch test (*t*-test) was also used to assess the significance of differences between two groups, and Dunnett's multiple comparison test was used to assess the significance of differences between the vehicle group and FK3657-treated groups. $P<0.05$ was always considered significant.

ED_{50} values were calculated by the least square regression method. In plasma extravasation studies, the background value was subtracted from the values for all other groups, and the ED_{50} value was then calculated.

3. Results

3.1. Effect of FK3657 on bradykinin-induced airway responses

We first tested the effect of FK3657 given orally on lower airway responses and nasal response induced by bradykinin or dextran sulfate, which activate the kinin–

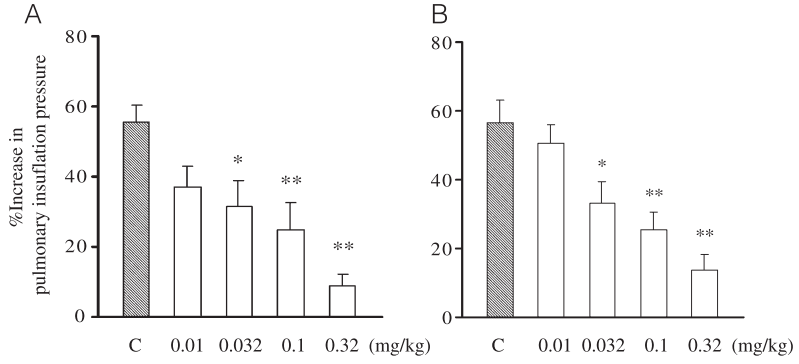

Fig. 1. Effect of FK3657 on bradykinin (A)- or dextran sulfate (B)-induced bronchoconstriction. FK3657 at the doses indicated or the vehicle (C group) was given p.o. After 30 min, bronchoconstriction was induced by bradykinin (4.2 μg/kg) or dextran sulfate (2 mg/kg) injection (i.v.). Data are means ± S.E.M. for seven or eight animals. *$P<0.05$, **$P<0.01$ compared with C group (Dunnett's multiple comparison test).

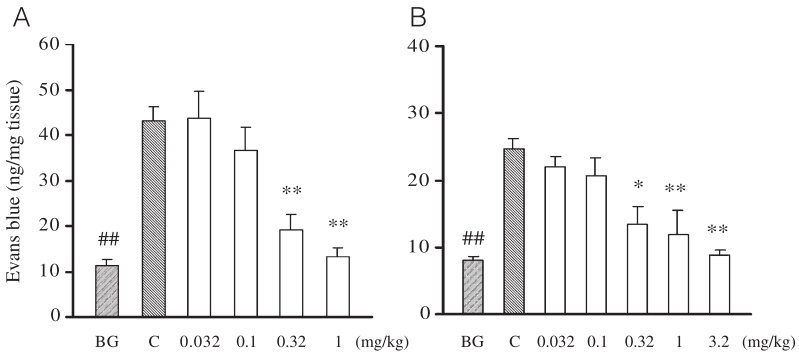

Fig. 2. Effect of FK3657 on bradykinin (A)- or dextran sulfate (B)-induced plasma extravasation in the trachea and main bronchi. FK3657 at the doses indicated or the vehicle (C group) was given p.o. After 30 min, bradykinin (2.1 μg/kg) or dextran sulfate (0.32 mg/kg) was injected (i.v.). The animals in the BG group were injected with a saline solution of Evans blue and captopril without bradykinin or dextran sulfate. Data are means ± S.E.M. for four or five animals. $^{*}P<0.05$, $^{**}P<0.01$ compared with C group (Dunnett's multiple comparison test). $^{\#\#}P<0.01$ compared with C group (*t*-test).

kallikrein cascade to generate bradykinin (Kluft, 1978; Morimoto et al., 1996).

FK3657 dose-dependently inhibited the bronchoconstriction induced by either bradykinin or dextran sulfate (Fig. 1). ED_{50} was 0.04 or 0.07 mg/kg for bradykinin- or dextran sulfate-induced response, respectively. FK3657 also dose-dependently inhibited the plasma extravasation induced by either exogenous or endogenous bradykinin (Fig. 2). ED_{50} was 0.20 or 0.23 mg/kg, respectively.

For dextran sulfate-induced plasma extravasation in nasal mucosa, FK3657 dose-dependently inhibited the response with ED_{50} value of 0.21 mg/kg (Fig. 3).

3.2. Effect of FK3657 on antigen-induced dual asthmatic response model

In egg albumin-sensitized guinea pigs, the antigen challenge induced a dual phase increase of specific airway resistance, immediate asthmatic response, and late asthmatic response, which appeared within a few minutes and 4–8 h after the antigen challenge, respectively (Fig. 4).

FK3657 did not show any significant effect on the immediate response. However, FK3657 significantly attenuated the late response at 1.8 mg/kg (Fig. 4) at 6 and 8 h after antigen challenge. The area under the curve above baseline between 4 and 8 h is shown in Fig. 5. The area under the curve was significantly reduced by 52% in the FK3657 group.

In this model, the cell number in the bronchoalveolar lavage fluid was increased by the antigen challenge (antigen challenge group, $(2.2 \pm 0.40) \times 10^7$ cells/animal ($n=8$) vs. saline challenge group, $(0.44 \pm 0.073) \times 10^7$ cells/animal ($n=6$); $P<0.01$). The increase in cell number was attributed to the increase of neutrophils (from 0.09×10^6 to

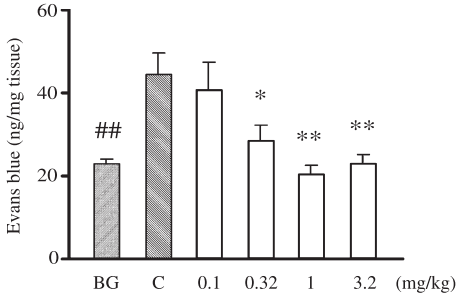

Fig. 3. Effect of FK3657 dextran sulfate-induced plasma extravasation in the nasal mucosa. FK3657 at the doses indicated or the vehicle (C group) was given p.o. After 45 min, dextran sulfate (0.56 mg/kg) was injected (i.v.). The animals in the BG group were injected with a saline solution of Evans blue and captopril without bradykinin. Data are means ± S.E.M. for four or five animals. $^{*}P<0.05$, $^{**}P<0.01$ compared with C group (Dunnett's multiple comparison test). $^{\#\#}P<0.01$ compared with C group (*t*-test).

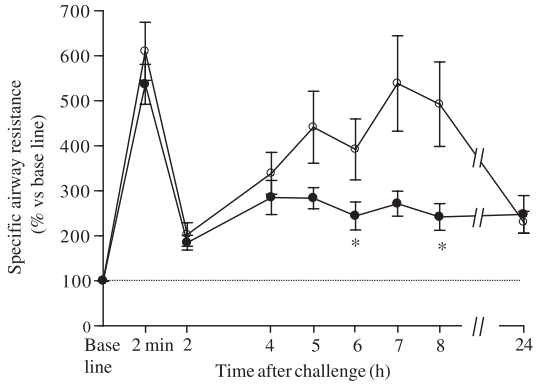

Fig. 4. Effect of FK3657 on antigen-induced dual asthmatic response. The animals were given FK3657 (1.8 mg/kg, closed circle) or the vehicle (open circle) at 30 min before and 3 h after the antigen challenge. Changes in specific airway resistance are presented as percentages of the baseline value for each animal. Data are means ± S.E.M. for 20–23 animals, since 2/22 in the FK3657 group and 3/23 animals in the vehicle group died between 4 and 8 h. $^{*}P<0.05$ compared with the vehicle group (Wilcoxon rank sum test).

9.1×10^6), macrophages (from 2.6×10^6 to 7.0×10^6), eosinophils (from 0.21×10^6 to 1.7×10^6), and lymphocytes (from 0.96×10^6 to 3.6×10^6), indicating that considerable airway inflammation occurred on antigen challenge. The cell number in the bronchoalveolar lavage fluid in the FK3657 group ($(2.7 \pm 0.50) \times 10^7$ cells/animal) was no different from that in the vehicle group ($(2.4 \pm 0.26) \times 10^7$ cells/animal) 24 h after the antigen challenge, suggesting that FK3657 had no inhibitory effect on antigen-induced inflammatory cell infiltration in this model.

3.3. Effect of FK3657 on TDI-induced nasal response

Repeated application of TDI to the guinea pig nostril sensitizes the animal causing the formation of TDI-specific IgE and inducing allergic symptoms and an inflammatory response accompanied by mast cell degranulation upon TDI challenge (Sugawara et al., 1993; Tanaka et al., 1988). We confirmed TDI-specific Ig production in our sensitization protocol by the homologous passive cutaneous anaphylaxis method. TDI-specific IgG production was detected from day 21 in some animals (8/9 animals), and on day 28, TDI-specific IgG was detected in all animals (9/9 animals). TDI-specific IgE was also detected from day 28 in some animals (4/7 animals), and on day 35, we could detect IgE production in 5/7 animals. These results indicate that simple TDI inoculation into guinea pig nostrils induced a TDI-specific immune response, consistent with results of previous studies (Sugawara et al., 1993; Tanaka et al., 1988).

In TDI-sensitized guinea pigs, challenge with TDI on day 36 or 37 induced vigorous plasma extravasation in the nasal mucosa (Fig. 6). FK3657 partially but significantly suppressed the TDI-induced nasal plasma extravasation (Fig. 6). The inhibitory effect reached a plateau at 1 mg/kg or more with a maximal percentage inhibition of 45%.

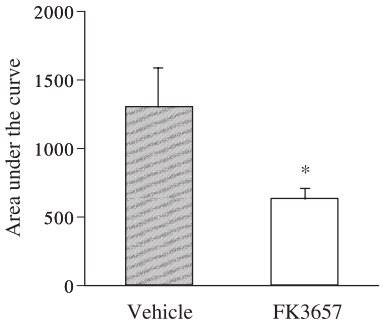

Fig. 5. Effect of FK3657 (1.8 mg/kg) on antigen-induced late asthmatic response. The area under the curve of the specific airway resistance values plotted against time (from 4 to 8 h) above baseline was calculated for each animal. Data are means ± S.E.M. for 20 animals in each group, since 2/22 in FK3657 group and 3/23 animals in vehicle group died between 4 and 8 h. *$P < 0.05$ compared with the vehicle group (*t*-test).

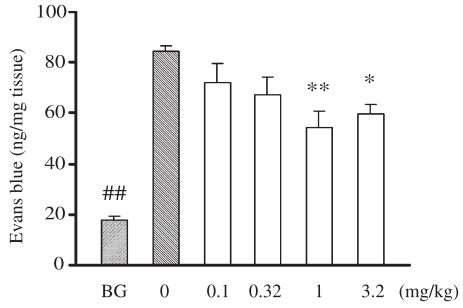

Fig. 6. Effect of FK3657 on TDI-induced plasma extravasation in nasal mucosa. FK3657 at the doses indicated or the vehicle (C group) was given p.o. After 45 min, 2% TDI in ethyl acetate was inoculated. The animals in the BG group were inoculated with ethyl acetate without TDI. Data are means ± S.E.M. for eight animals. *$P < 0.05$, **$P < 0.01$ compared with C group (Dunnett's multiple comparison test). $^{\#\#}P < 0.01$ compared with C group (*t*-test).

4. Discussion

FK3657 given orally inhibited bradykinin- and dextran sulfate-induced plasma extravasation in the lower airways (trachea and main bronchi) as well as bronchoconstriction. It also inhibited dextran sulfate-induced plasma extravasation in the nasal mucosa. These results indicate that FK3657 given orally inhibits bradykinin-induced airway responses potently, which is consistent with previous reports (Asano et al., 1997; Inamura et al., 1997; Watanabe et al., 1999).

Bronchial asthma is a chronic inflammatory disease of the airways. In some asthmatic patients, antigen challenge elicits dual phase asthmatic responses, an immediate asthmatic response and a late asthmatic response. The late asthmatic response appears several hours after the antigen challenge and shows some pathophysiological features of chronic bronchial asthma, that is, infiltration of inflammatory cells into airways and a sustained decrease of respiratory function (O'Byrne et al., 1987). The effect on the late phase asthmatic response may reflect the therapeutic potential better, and animal models of late asthmatic response have been used (Hutson et al., 1988). In our antigen-induced asthma model of guinea pigs, inhalation of antigen induced the characteristic dual asthmatic responses. Oral FK3657 significantly attenuated the antigen-induced late phase asthmatic response. The suppressive effect was partial, but seemed to be maximal for FK3657, since the effect of FK3657 at 3.2 mg/kg was not greater than that at 1.8 mg/kg in our preliminary study (data not shown). These results indicate that bradykinin B_2 receptors are at least partly involved in the late response. In a sheep model, another bradykinin receptor antagonist also suppressed the late phase asthmatic response and,

concomitantly, inflammatory cell infiltration (Abraham, 1992). We could not obtain any evidence of the inhibitory effect of FK3657 on antigen-induced inflammatory cell accumulation. It is more likely that FK3657 might block the action of bradykinin on smooth muscle or blood vessels to attenuate the late phase bronchoconstriction and/or edema in our model.

FK3657 partially but significantly suppressed TDI-induced immediate nasal plasma extravasation in TDI-sensitized guinea pigs, contrasting with the effect on the immediate asthmatic response. It has been shown that repeated sensitization of guinea pigs with TDI induces TDI-specific IgE production, allergic symptoms, and an inflammatory response accompanied by mast cell degranulation (Sugawara et al., 1993; Tanaka et al., 1988), indicating that type I allergic responses are involved in the model. We also confirmed the production of TDI-specific IgG and IgE in animals sensitized with our sensitization protocol. Thus, our results indicate that bradykinin B_2 receptors are partly involved in the immediate response in the nose. These results were quite consistent with a previous report by Ricciardolo et al. (1994) that also showed partial inhibition of antigen-induced plasma extravasation by a bradykinin B_2 receptor antagonist, icatibant, in guinea pig nose.

The reason why the effect of FK3657 on the immediate response in the nose is different from that in lower airways in our models is unclear. It is unlikely that it simply results from tissue difference, since in some guinea pig models, a bradykinin B_2 receptor antagonist suppressed the antigen-induced immediate bronchial response (Farmer et al., 1992; Featherstone et al., 1996; Ikemura et al., 1998). The effect of the bradykinin B_2 receptor antagonist appeared to be variable depending on the models used. The clue may be in the process of bradykinin formation. It has been reported that allergy-related mediators such as histamine and leukotriene C_4 affect the bradykinin generation (Jin et al., 1992; Proud, 1998). Tachykinins also interact with the kinin system in allergic inflammation (Bertrand and Geppetti, 1996). Thus, it is possible that the formation of such mediators is so variable among allergic models that the formation of bradykinin might consequently be variable. Since histamine H_1 receptor antagonists are often used to prevent anaphylactic shock in guinea pig allergy models including our asthma model, this kind of experimental procedure may also affect the contribution of bradykinin to the subsequent antigen-induced response. In any case, our results together with those of others indicate that bradykinin B_2 receptors are involved in the allergic response in airways, once bradykinin is generated.

Only one bradykinin B_2 receptor antagonist, icatibant, has so far been evaluated for efficacy in clinical trials with allergic diseases. Icatibant suppressed the mite antigen-induced nasal response in chronic allergic patients and grass pollen-induced nasal inflammation and hyperreactivity in seasonal nasal allergy patients (Austin et al., 1994; Turner et al., 2001). On the other hand, it failed to show a beneficial effect for seasonal nasal allergy and showed only limited efficacy for asthma (Akbary and Bender, 1993; Akbary et al., 1996). Since icatibant was administered locally in all these studies, the short duration of action possibly due to mucociliary clearance may partly explain the reason for these discouraging results (Proud, 1998). In addition, a rather high dose of bradykinin B_2 receptor antagonist may be required to block the action of bradykinin in allergic responses, since the effective doses of FK3657 in our allergy models (1–1.8 mg/kg) were substantially higher than the ED_{50} (0.04–0.23 mg/kg) obtained from experiments with bradykinin-induced airway responses. Potent and orally active bradykinin B_2 receptor antagonists such as FK3657 may overcome these problems and their therapeutic value in allergic diseases should be evaluated.

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