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Bronchoprotective effects of KF-19514 and cilostazol in guinea pigs in vivo

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Abstract

It has been shown that inhibitors of cyclic nucleotide phosphodiesterase III and IV have a bronchodilator effect. We compared the effects of a selective phosphodiesterase III inhibitor, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone (cilostazol) and a phosphodiesterase I/IV inhibitor, 5-phenyl-3-(3-pyridil) methyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (KF-19514) on antigen- and histamine-induced bronchoconstriction in guinea pigs in vivo. Intravenous administration of cilostazol and KF-19514 inhibited histamine- and antigen-induced bronchoconstriction in a dose-dependent manner. When assessing the resulting ED_{50} values, the activity of KF-19514 against antigen-induced bronchoconstriction was stronger than its antagonism of histamine-induced bronchoconstriction (0.004 vs. 0.056 mg/kg), while those of cilostazol were contrary (0.835 vs. 0.031 mg/kg). These results suggest that although both inhibitors of phosphodiesterase III and phosphodiesterase IV have a bronchodilator or bronchoprotective effect, phosphodiesterase IV inhibitors such as KF-19514 may be more useful for asthma treatment because KF-19514 had an anti-allergic effect in addition to the functional bronchodilator activity.

Keywords: Phosphodiesterase I/IV inhibitor; Phosphodiesterase III inhibitor; (Guinea pig); Anti-allergic effect; Bronchodilator effect

1. Introduction

It has been recognized that cyclic nucleotide phosphodiesterase regulates the metabolism of cyclic AMP and cyclic GMP in many kinds of cells and tissues. Phosphodiesterase inactivates cAMP or cGMP by catalyzing the hydrolysis of the 3′-phosphoester bond to form the corresponding inactive 5′-nucleotide (Beavo and Reifsnyder, 1990). Increase in either cAMP or cGMP is associated with relaxation of airway smooth muscle (Torphy and Hay, 1990; Giembycz and Raeburn, 1991), and cAMP acts as a second messenger in inflammatory cells to inhibit chemotaxis, cytotoxicity and degranulation (Bourne et al., 1974; Plaut et al., 1980; Kuehl et al., 1987).

Recently, six distinct phosphodiesterase isozymes have been characterized in human airway smooth muscle: (1) phosphodiesterase I alpha and I beta (two calmodulin-stimulated phosphodiesterases), (2) phosphodiesterase II (cyclic GMP-stimulated phosphodiesterase), (3) phosphodiesterase III (cyclic GMP-inhibited phosphodiesterase), (4) phosphodiesterase IV (cyclic AMP-specific phosphodiesterase), (5) phosphodiesterase V (cyclic GMP-specific phosphodiesterase) (Torphy et al., 1993). Phosphodiesterase isozymes differ in their substrate selectivity, sensitivity to isozyme-selective inhibitors and tissue distribution (Nicholson et al., 1991; Thompson, 1991; Weishaar et al., 1985). Theophylline is a non-selective inhibitor of phosphodiesterase and effective for treatment of asthma. It is commonly believed that theophylline acts by inhibiting the activity of phosphodiesterases and increasing cellular cyclic nucleotide content.

In in vivo animal experiments and in vitro animal and human experiments, phosphodiesterase III inhibitors such as siguazodan, CI-930 and Org 9935 have been shown to have a bronchodilator effect (Torphy et al., 1993; Howell et al., 1993; Boer et al., 1992) and phosphodiesterase IV inhibitors such as rolipram and RO-20-1724 to have an anti-inflammatory effect (Howell et al., 1993) as well as a bronchodilator effect (Torphy et al., 1993; Boer et al., 1992). Recently, it was reported that phosphodiesterase IV inhibitors inhibited eosinophil infiltration in guinea pig airways (Underwood et al., 1993, 1994; Lagente et al., 1995).

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6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)- quinolinone (cilostazol) is a selective orally active phosphodiesterase III inhibitor (Tani et al., 1992) and has been prescribed for treatment of obstructive arteriosclerosis (Yasunaga and Mase, 1985). Recently we (Fujimura et al., 1995) showed that a single oral administration of 200 mg cilostazol reduced bronchial responsiveness to methacholine in normal volunteers. 5-Phenyl-3-(3-pyridil) methyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (KF-19514) is a newly developed phosphodiesterase I/IV inhibitor (Manabe et al., 1995). It is unknown whether intravenous administration of cilostazol and KF-19514 have a bronchoprotective and/or anti-allergic effect in guinea pigs in vivo.

The present study was designed to compare the protective effects of KF-19514 and cilostazol on histamine-induced bronchoconstriction and antigen-induced leukotriene-mediated bronchoconstriction (Fujimura et al., 1985) in guinea pigs in vivo.

2. Materials and methods

2.1. Animals

Male, albino, Hartley strain guinea pigs weighing 380–430 g were obtained from Sankyou Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal Experiments in our university, they were kept in conventional animal housing facilities for one week before use. They were allowed to drink and feed ad libitum. This animal experiment was performed according to the Principles of Laboratory Animal Care formulated by the Institute of Animal Experiments in Kanazawa University.

2.2. Passive sensitization of guinea pigs

Guinea pig homocytotropic antiserum was made according to the method of Santives et al. (1976). Briefly, 500 μg of ovalbumin was emulsified in Freund's complete adjuvant and injected intradermally (i.d.) into each guinea pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled and kept frozen until use. The antibody titer of this serum was 1:12 800, 1:6400 and 1:512 as estimated by passive cutaneous anaphylaxis (PCA) at 4 h, 24 h and 7 days, respectively. Normal guinea pigs were passively sensitized with 1.0 ml antiserum/kg intraperitoneally (i.p.).

2.3. Preparation of animals

Nonsensitized or passively sensitized guinea pigs were anesthetized with sodium pentobarbital (75 mg/kg i.p.). They were placed in the supine position and the trachea was cannulated with a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm).

After surgery, the guinea pig was artificially ventilated by a small animal respiratory pump (Model 1680, Harvard Apparatus, South Natick, MA, USA) adjusted to a tidal volume of 10 ml/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; Pao) (cmH_2O) were measured by the modified method of Konzett and Rössler (1940) described by Jones et al. (1982) using a pressure transducer (Model TP-603T, Nihon Kogyo, Tokyo, Japan). Since the change in Pao following inhalation of leukotriene C_4 (LTC_4) represented the average of the changes in pulmonary resistance (R_L) and reciprocal dynamic lung compliance (1/Cdyn) (Fujimura, 1983), we have used Pao as an overall index of bronchial response to bronchoactive agents.

When all procedures were completed, the animals were over-inflated by two times of tidal volume for two breaths by clamping the outlet port of the respirator to standardize the volume history of the lung (Fujimura, 1983).

2.4. Histamine-induced bronchoconstriction

Ten minutes after the preparation of non-sensitized guinea pigs, when Pao had stabilized, KF-19514 or cilostazol was intravenously administered. Ten minutes later, ascending doses of histamine (25, 50, 100 and 200 μg/ml) were inhaled at 5 min intervals under continuous ventilation. The aerosol was generated during a 30 s period by an ultrasonic nebulizer developed for small animals at our institution. The amount of aerosol was 15.2 μl/min, and 46.4% of the aerosol was deposited in the lung as measured by the radio-aerosol technique (Minami et al., 1983).

2.4.1. (Study 1) Effects of KF-19514

KF-19514 in a dose of 0.001 ($n = 8$), 0.01 ($n = 8$) or 0.1 mg/kg ($n = 8$) or saline ($n = 8$) was intravenously administered 10 min before histamine inhalation.

2.4.2. (Study 2) Effects of cilostazol

Cilostazol in a dose of 0.01 ($n = 7$), 0.1 ($n = 7$) or 1.0 mg/kg ($n = 7$) or dimethyl sulfoxide (DMSO) ($n = 7$) was intravenously administered 10 min before histamine inhalation.

2.5. Antigen-induced bronchoconstriction

Passively sensitized guinea pigs were given diphenhydramine hydrochloride (60 mg/kg, i.p.) to block the action of histamine before surgery (Fujimura, 1983). Fifteen minutes after the preparation of the animals, when Pao had stabilized, KF-19514 or cilostazol was intravenously injected. Ten minutes after the treatment, the animals were challenged with nebulized ovalbumin dissolved in saline (1.0 mg/ml) without interrupting the constant ventilation in passively sensitized animals. The ovalbumin aerosol was generated for 30 s with an ultrasonic nebulizer.

2.5.1. (Study 3) Effect of KF-19514

A selective phosphodiesterase I/IV inhibitor, KF-19514, in a dose of 0.001 ($n = 7$), 0.01 ($n = 7$) or 0.1 mg/kg ($n = 7$) or saline ($n = 7$) was intravenously administered 10 min before ovalbumin challenge. In addition, saline was inhaled for 30 s 10 min after intravenous administration of saline in sensitized animals ($n = 7$).

2.5.2. (Study 4) Effect of cilostazol

A selective phosphodiesterase III inhibitor, cilostazol, in a dose of 0.01 ($n = 6$), 0.1 ($n = 6$) or 1.0 mg/kg ($n = 6$) or DMSO ($n = 6$) was intravenously administered 10 min before ovalbumin challenge. Furthermore, saline was inhaled for 30 s 10 min after intravenous administration of DMSO in sensitized animals ($n = 7$).

2.6. Statistical analysis

All data were shown as mean ± standard error of the mean (S.E.M.). Statistical differences were determined by analysis of variance (ANOVA) among 4 groups and unpaired *t*-test between 2 groups. Differences of time-course curves for percentage increase in Pao from the baseline value after ovalbumin provocation were analyzed among animals treated with KF-19514 (0.001, 0.01 and 0.1 mg/kg) and vehicle (saline) or cilostazol (0.01, 0.1 and 1.0 mg/kg) and vehicle (DMSO) with 2-factor repeated ANOVA. Dose–response curves for histamine-induced bronchoconstriction were also analyzed by 2-factor repeated ANOVA. A P value of 0.05 or less was considered to be significant. ED_{50} values of KF-19514 and cilostazol to inhibit histamine- and antigen-induced bronchoconstriction were calculated from a least-squares linear regression analysis.

2.7. Chemicals

The following chemicals were used: ovalbumin (Sigma, St. Louis, MO, USA), diphenhydramine hydrochloride (Sigma), sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA), dimethyl sulfoxide (DMSO) (Wako Pure Chemical, Osaka, Japan), histamine (Wako Pure Chemical), KF-19514 (5-phenyl-3-(3-pyridil) methyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one, Kyouwa Hakkou Kogyo, Shizuoka, Japan), cilostazol (6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone), Otsuka Pharmaceutical, Tokyo, Japan).

3. Results

3.1. Histamine-induced bronchoconstriction

3.1.1. Effects of KF-19514 (Study 1) (Fig. 1)

The mean value (±S.E.M.) of baseline Pao immediately before histamine inhalation was 10.0 ± 0.4, 9.6 ± 0.2, 9.4 ± 0.4 and 9.4 ± 0.2 cmH_2O with treatment with saline and 0.001, 0.01 and 0.1 mg/kg of KF-19514, respectively. There were no significant differences among them. Fig. 1 shows a preventive effect of intravenous administration of KF-19514 on bronchoconstriction induced by inhalation of histamine. The dose–response curve of the histamine-induced increase in Pao was significantly ($P < 0.01$ by 2-factor repeated ANOVA) and dose-dependently inhibited by KF-19514. The percent increase in Pao after inhalation of 100 μg/ml histamine was 173.5 ± 23.1, 145.6 ± 18.4, 114.9 ± 19.7 and 78.2 ± 15.7% with saline, 0.001, 0.01 and 0.1 mg/kg of KF-19514, respectively. The value was significantly lower with 0.01 ($P < 0.05$) and 0.1 mg/kg ($P < 0.02$) of KF-19514 than with saline. The percent increase in Pao induced by 200 μg/ml of histamine was also significantly lower with 0.01 ($P < 0.01$) and 0.1 mg/kg ($P < 0.01$) of KF-19514 compared with saline.

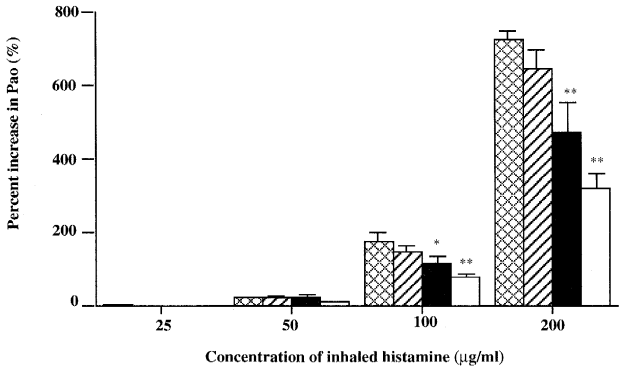

Fig. 1. Protective effect of intravenous administration of KF-19514 on bronchoconstriction induced by inhalation of histamine in guinea-pigs. The groups of treatment with saline ($n = 8$) (cross-hatched bars), treatment with 0.001 mg/kg of KF-19514 ($n = 8$) (hatched bars), treatment with 0.01 mg/kg of KF-19514 ($n = 8$) (solid bars) and treatment with 0.1 mg/kg of KF-19514 ($n = 8$) (open bars) are shown. Results are presented as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared with saline treatment group by the Mann-Whitney's *U*-test. Pao = pressure at the airway opening.

3.1.2. Effects of cilostazol (Study 2) (Fig. 2)

The mean value (±S.E.M.) of baseline Pao immediately before histamine inhalation was 9.7 ± 0.5, 9.6 ± 0.4, 9.4 ± 0.4 and 10.6 ± 0.4 cmH_2O with treatment with DMSO and 0.01, 0.1 and 1.0 mg/kg of cilostazol, respectively. There were no significant differences among them. As shown in Fig. 2, the dose–response curve of the histamine-induced increase in Pao was significantly ($P < 0.01$ by 2-factor repeated ANOVA) inhibited by cilostazol in a dose-dependent manner. The percent increase in Pao after inhalation of histamine (100 μg/ml) was 267.7 ± 56.5, 194.7 ± 61.8, 105.8 ± 21.3 and 77.5 ± 24.0% with DMSO, and 0.01, 0.1 and 1.0 mg/kg of cilostazol. These values were significantly ($P < 0.02$ and $P < 0.01$, respectively) lower with 0.1 and 1.0 mg/kg of cilostazol than with DMSO in a dose-dependent manner. The percent increase in Pao caused by 200 μg/ml histamine was also

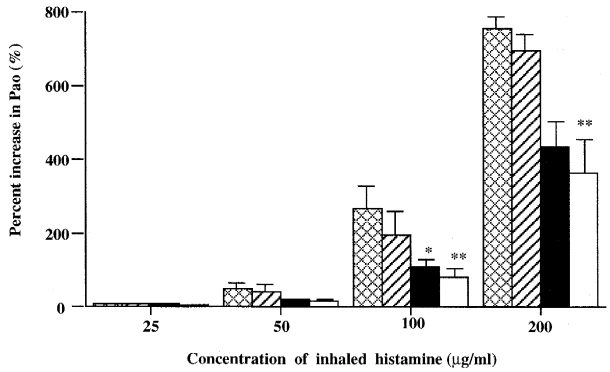

Fig. 2. Protective effect of intravenous administration of cilostazol on bronchoconstriction induced by inhalation of histamine in guinea pigs. Treatment with DMSO ($n = 7$) (cross-hatched bars), treatment with 0.01 mg/kg of cilostazol ($n = 7$) (hatched bars), treatment with 0.1 mg/kg of cilostazol ($n = 7$) (solid bars) and treatment with 1.0 mg/kg of cilostazol ($n = 7$) (open bars) are shown. Results are presented as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared with DMSO treatment group by the Mann-Whitney's *U*-test. Pao = pressure at the airway opening.

significantly ($P < 0.01$) lower with 0.1 mg/kg of cilostazol compared with DMSO.

3.2. Antigen-induced bronchoconstriction

3.2.1. Effects of KF-19514 (Study 3) (Fig. 3)

The Pao value before saline challenge with treatment with saline was 11.4 ± 0.4 cmH_2O, and the Pao values before ovalbumin challenge were 13.1 ± 0.7, 10.4 ± 0.2, 11.9 ± 0.9 and 13.3 ± 1.1 cmH_2O with treatment with saline and 0.1, 0.01 and 0.001 mg/kg of KF-19514, respectively. There were no significant differences among them. Time-courses of percent increase in Pao from the baseline value after saline and ovalbumin challenge in the 5 groups are shown in Fig. 3. These curves showed that antigen-induced bronchoconstriction was significantly ($P < 0.01$ by 2-factor repeated ANOVA) inhibited by KF-19514 in a dose-dependent manner. The peak values after ovalbumin challenge were 235.7 ± 23.3, 159.6 ± 23.5, 111.9 ± 14.0 and 77.5 ± 6.4% with saline, 0.001, 0.01 and 0.1 mg/kg of KF-19514 and the values were significantly ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively) lower with 0.001, 0.01 and 0.1 mg/kg of KF-19514 than with saline. As shown in Fig. 3, 0.001, 0.01 and 0.1 mg/kg of KF-19514 significantly inhibited the increases in Pao after ovalbumin challenge each time.

3.2.2. Effects of cilostazol (Study 4) (Fig. 4)

The Pao value before saline challenge with DMSO treatment was 11.5 ± 0.3 cmH_2O, and the values before ovalbumin challenge were 11.0 ± 0.4, 10.6 ± 0.5, 10.4 ± 0.3 and 10.0 ± 0.6 cmH_2O with treatment with DMSO and 0.01, 0.1 and 1.0 mg/kg of cilostazol, respectively, and there were no significant differences among them. Time-courses of percent increase in Pao from the baseline value after ovalbumin challenge in the 5 groups are shown in Fig. 4. The time-course curve was significantly ($P < 0.01$ by 2-factor repeated ANOVA) inhibited by cilostazol in a dose-dependent manner. The peak values after ovalbumin challenge were 252.5 ± 10.3, 223.3 ± 13.6, 189.1 ± 18.1 and 139.4 ± 19.1% with DMSO, 0.01, 0.1 and 1.0 mg/kg of cilostazol and the value was significantly ($P < 0.01$) lower with 1.0 mg/kg of cilostazol than with DMSO.

To evaluate the influence of DMSO on the antigen-induced bronchoconstriction time-courses of percent increase in Pao from the baseline value after challenge with ovalbumin were compared between animals treated with DMSO and saline (control in Study 3). There was no significant difference between these.

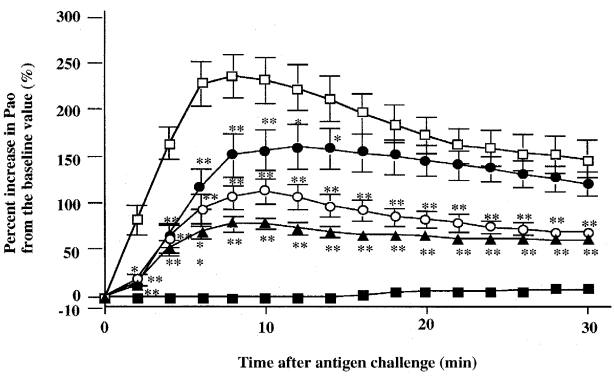

Fig. 3. Time-course of percent increase in pressure at the airway opening (Pao) from pre-ovalbumin provocation value after ovalbumin provocation in passively sensitized guinea pigs treated intravenously with saline ($n = 7$) (open squares), 0.001 mg/kg of KF-19514 ($n = 7$) (solid circles), 0.01 mg/kg of KF-19514 ($n = 7$) (open circles) and 0.1 mg/kg of KF-19514 ($n = 7$) (solid triangles) 10 min before the ovalbumin challenge. The value of saline provocation in passively sensitized guinea pigs intravenously treated with saline is also shown as solid squares ($n = 7$). Results are presented as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared with saline treatment group by the Mann-Whitney's *U*-test.

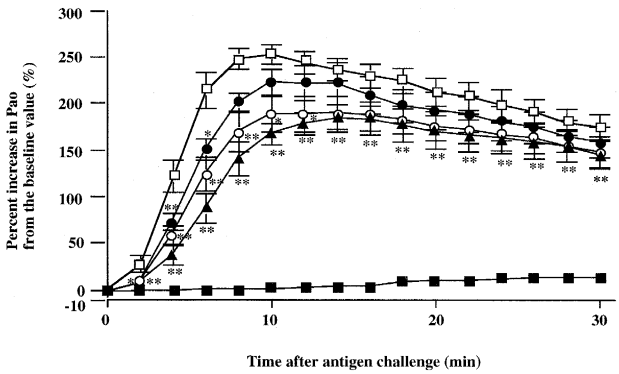

Fig. 4. Time-course of percent increase in pressure at the airway opening (Pao) from pre-ovalbumin provocation value after ovalbumin provocation in passively sensitized guinea pigs treated intravenously with DMSO ($n = 6$) (open squares), 0.01 mg/kg of cilostazol ($n = 6$) (solid circles), 0.1 mg/kg of cilostazol ($n = 6$) (open circles) and 1 mg/kg of cilostazol ($n = 6$) (solid triangles) 10 min before the ovalbumin challenge. The value of saline provocation in passively sensitized guinea pigs intravenously treated with DMSO is also shown as solid squares ($n = 6$). Results are presented as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared with DMSO treatment group by the Mann-Whitney's *U*-test.

3.3. ED_{50} values for inhibitory effect of KF-19514 and cilostazol on histamine- and antigen-induced bronchoconstriction

The peak percent increase in Pao after inhalation of ovalbumin and 100 μg/ml histamine was 267.7 ± 56.5 and 252.5 ± 10.3%, respectively, in control animals in cilostazol studies (Study 2 and Study 4), and these were not different. However, the values were 173.5 ± 23.1 and 235.7 ± 23.3% in control animals in the KF-19514 studies (Study 1 and Study 3), and the former was lower than the latter. So the ED_{50} value of KF-19514 against 200 μg/ml histamine-induced increase in Pao was also calculated for reference.

The resulting intravenous ED_{50} values of treatment with KF-19514 were 0.056 and 0.004 mg/kg in 100 μg/ml histamine- (Study 1) and antigen-induced bronchoconstriction (Study 3), respectively. The ED_{50} value of KF-19514 against 200 μg/ml histamine-induced bronchoconstriction was 0.053 mg/kg. The resulting ED_{50} values of treatment with cilostazol were 0.031 and 0.835 mg/kg in histamine- (Study 2) and antigen-induced bronchoconstriction (Study 4), respectively.

4. Discussion

In the present study intravenous administration of cilostazol and KF-19514 inhibited both histamine- and antigen-induced bronchoconstriction, which is mainly mediated by leukotrienes in guinea-pigs treated with antihistamines (Fujimura, 1983; Fujimura et al., 1985), in a dose-dependent manner. When assessing the resulting ED_{50} value, the activity of KF-19514 against antigen-induced bronchoconstriction was greater than its antagonism of histamine-induced bronchoconstriction (0.004 vs. 0.056 mg/kg), while the inhibitory effect of cilostazol on antigen-induced bronchoconstriction was rather weak compared with that on histamine-induced bronchoconstriction (0.835 vs. 0.031 mg/kg). The inhibitory effects of KF-19514 and cilostazol against histamine-induced bronchoconstriction are relatively weak compared with that of a β_2-agonist, salbutamol, with an ED_{50} of 0.001 mg/kg previously shown by us (Mizuhashi, 1994).

The Pao measurement used in this study does not clearly distinguish between airway edema and airway smooth muscle contraction. Since Pao increased immediately after antigen and histamine inhalation and the bronchodilator procaterol almost completely inhibited the increases in Pao (Fujimura et al., 1991), it is likely that the increase in Pao is indicative of bronchoconstriction in our experimental system.

KF19514 is a new, potent and highly selective inhibitor of phosphodiesterase I/IV isoenzymes (Manabe et al., 1995). IC_{50} values of KF19514 for phosphodiesterase I, phosphodiesterase II, phosphodiesterase III, phosphodiesterase IV and phosphodiesterase V in vitro are 0.28, > 10, > 10, 0.41 and > 10 μmol, respectively (personal communication by O. Manabe, Kyowa Fermentation, Shizuoka, Japan). It has been shown that KF19514 relaxes guinea-pig isolated tracheal smooth muscle contraction caused by histamine with an IC_{50} value of 0.5 μmol and inhibits peptide leukotriene release from eosinophils with an IC_{50} value of 0.098 μmol (Manabe et al., 1995). Both oral and intravenous administration of phosphodiesterase IV inhibitors such as rolipram and RO-20-1724 have been shown to have anti-inflammatory effects (Howell et al., 1993; Lagente et al., 1994, 1995; Underwood et al., 1993) as well as bronchodilator effects (Torphy et al., 1993; Boer et al., 1992). Howell et al. (1993) have reported that rolipram has pulmonary anti-allergic effects, including inhibition of antigen-induced full and leukotriene-dependent bronchoconstriction and prevention of antigen-induced airway hyperreactivity, with minimal relaxant activity of airway smooth muscle. Teixeira et al. (1994) have reported that rolipram effectively inhibits allergic- and mediator-induced eosinophil accumulation but not edema formation or neutrophil accumulation. On the other hand, the phosphodiesterase I/V inhibitors such as zaprinast (Howell et al., 1993; Boer et al., 1992) and the phosphodiesterase I inhibitors such as vinpocetin (personal communication by O. Manabe) have no effect in the regulation of airway smooth muscle tone. From these findings, it is considered that the inhibitory effect of KF19514 on antigen- and histamine-induced bronchoconstriction results from its antagonism of phosphodiesterase IV other than phosphodiesterase I. When the resulting ED_{50} values for the inhibitory effect of KF-19514 on histamine- and antigen-induced bronchoconstriction were compared, the ED_{50} value against antigen-induced bronchoconstriction was lower than that against histamine-induced bronchoconstriction, suggesting that KF19514 may have anti-allergic activity such as inhibition of antigen-induced mediator release in addition to the nonspecific functional bronchoprotective or bronchodilator effect, by inhibiting phosphodiesterase IV but not by inhibiting phosphodiesterase I. The anti-allergic activity of KF19514 resulting from the inhibition of phosphodiesterase IV may be due to the stabilization of mast cells, in which phosphodiesterase IV appears to be the predominant phosphodiesterase isozyme (Torphy et al., 1992). Other mechanisms in addition to the suppression of mast cell degranulation may also be proposed because it has been shown that rolipram reduces antigen-induced airway eosinophil influx when administered after antigen exposure: after mast cell degranulation has already occurred (Sturm et al., 1990).

Cilostazol is well known as a potent and highly selective inhibitor of phosphodiesterase III isozyme (Tani et al., 1992). This agent causes reduction of platelet aggregation and dilatation of blood vessel (Yasunaga and Mase, 1985). It has been prescribed for treatment of obstructive arteriosclerosis in Japan and Korea from 1988 and 1991, respec-

tively. It has been shown that cilostazol selectively inhibits phosphodiesterase III from human platelets and human umbilical cord vein endothelial cells in vitro with IC_{50} values of 0.19 and 0.71 μmol while cilostazol requires a 13–300-times higher concentration to inhibit the other phosphodiesterase subtypes (Tani et al., 1992). The IC_{50} values of cilostazol for phosphodiesterase I, phosphodiesterase II, phosphodiesterase III, phosphodiesterase IV and phosphodiesterase V in vitro have been shown to be $>$ 100, 100, 0.2–0.5, 50 and 8–15 μmol, respectively.

In this study, the intravenous administration of cilostazol inhibited histamine- and antigen-induced bronchoconstriction in a dose-dependent manner. When assessing the resulting intravenous ED_{50} values of cilostazol in histamine- and antigen-induced bronchoconstriction, the former value (ED_{50} = 0.031 mg/kg) was lower than the latter value (ED_{50} = 0.835 mg/kg). There may be at least two explanations for this difference of activity against the two spasmogens. First, in addition to the histamine H_1 receptor-mediated bronchoconstriction, histamine has been shown to act at the histamine H_2 receptors in several tissues, including inflammatory cells, to stimulate adenyl cyclase activity and raise cAMP concentrations (Lichtenstein and Gillespie, 1973). The histamine H_2 receptor-mediated increases in cAMP may be amplified by cilostazol to accentuate its bronchodilator activity. Second, the ability of phosphodiesterase inhibitors to induce bronchorelaxation is dependent upon the type of contractile agent used to induce tone (Torphy et al., 1988). Thus, it is conceivable that histamine-induced contractions are intrinsically more sensitive to phosphodiesterase III inhibitors than antigen-induced contractions. Howell et al. (1993) reported that CI-930 inhibited antigen-induced full bronchoconstriction, but as opposed to rolipram inhibited both histamine-induced bronchoconstriction and antigen-induced full bronchoconstriction to a similar degree. In their study, they demonstrated that the inhibition of antigen-induced full or leukotriene-dependent bronchoconstriction by CI-930 is attributed to airway smooth muscle relaxation rather than to pulmonary anti-allergic activity. These results suggest that a selective phosphodiesterase III inhibitor, cilostazol, has airway smooth muscle relaxation without apparent anti-allergic effects.

It has been recognized that theophylline is effective for the treatment of asthma and the proposed mechanisms of action are, (1) inhibition of phosphodiesterases, (2) adenosine receptor antagonism, (3) mobilization of intracellular Ca^{2+}, (4) release of catecholamines, (5) antagonism of prostaglandins and others (Persson et al., 1986). Although the mechanism of action of this agent remains unclear, phosphodiesterase inhibition may play an important role in the anti-asthmatic action. Howell et al. (1993) suggested that the pulmonary anti-allergic activity of this agent results in part from inhibition of the phosphodiesterase IV isozyme and the bronchodilator activity results in part from inhibition of the phosphodiesterase III isozyme. The IC_{50} value of theophylline as an inhibitor of cAMP phosphodiesterase is 30 μg/ml, not vastly different from the effect on blood levels in humans (10–20 μg/ml). Kuehl et al. (1987) expected that synergism with endogenous inflammatory prostaglandins may decrease this value further in vivo. They have suggested that the efficacy of theophylline in asthma is not based on the direct effect on bronchial smooth muscle, but is primarily resulting from suppressing mediator release from relevant white cells by inhibition of cAMP phosphodiesterase.

Although theophylline is useful in the treatment of asthma, the value is limited by a narrow therapeutic index and a wide range of gastrointestinal, central nervous system and cardiovascular adverse effects (Weishaar et al., 1985; Persson et al., 1986; Jenne, 1987). These adverse effects are generally ascribed to its lack of selectivity. To reduce these adverse effects, it may be reasonable to use selective phosphodiesterase isoenzyme inhibitors.

In conclusion, our results indicate that the phosphodiesterase I/IV inhibitor KF-19514 has a pulmonary anti-allergic effect with a functional bronchoprotective effect and this pulmonary anti-allergic effect is probably due to inhibition of the release of chemical mediators (leukotrienes and possibly other mediators). On the other hand, the selective phosphodiesterase III inhibitor cilostazol has a functional bronchoprotective effect without apparent anti-allergic effects. KF-19514 and cilostazol, especially the former, may be useful for the treatment of asthma and further studies should be carried out to determine the clinical application. In addition, as Underwood et al. (1994) have suggested that the combined inhibition of both phosphodiesterase III and IV by dual phosphodiesterase III/IV inhibitors such as zardaverine acts in an additive or synergistic manner to inhibit bronchospasm in guinea pigs, the usefulness of combination of phosphodiesterase III and IV inhibitors in lower doses should be evaluated to avoid these side effects.

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