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Effect of DP-1904, a thromboxane synthetase inhibitor, on antigen- and spasmogen-induced bronchoconstriction in rodents

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Abstract

The effect of DP-1904 [6-(1-imidazolylmethyl)-5, 6, 7, 8-tetra-hydronaphthalene-2-carboxylic acid hydrochloride], a selective thromboxane synthetase inhibitor, was examined on antigen- and spasmogen-induced bronchoconstriction in rodents. Oral administration of DP-1904 (1, 3, 10 mg/kg) as well as OKY-046 (sodium (*E*)-3[4-(1-imidazolylmethyl)-phenyl]-2-propanoate, 100 mg/kg), significantly inhibited immunoglobulin G-mediated bronchoconstriction in actively sensitized guinea pigs. Immunoglobulin E-mediated bronchoconstriction in actively sensitized rats was also inhibited by both DP-1904 (1, 10 mg/kg) and OKY-046 (100 mg/kg). DP-1904 (3–30 mg/kg) and OKY-046 (30 mg/kg) suppressed leukotriene D_4-induced bronchoconstriction in guinea pigs. In these models, the endogenous levels of thromboxanes significantly increased following the stimulus (antigen and leukotriene D_4). DP-1904 (10 mg/kg) inhibited the increase in thromboxane level in both plasma and bronchial alveolar lavage fluid. These actions of DP-1904 persisted for more than 12 h, indicating a long-lasting effect of DP-1904 on bronchoconstriction. The results showed that the biological activity of DP-1904 in our rodents models is more potent than that of OKY-046 (Ozagrel), which is available as an anti-asthma agent in Japan.

Keywords: DP-1904; OKY-046; Thromboxane synthetase inhibitor; Bronchoconstriction; (Guinea pig); (Rat)

1. Introduction

Several mediators are postulated to play pathophysiological roles in the onset and development of bronchial asthma. Thromboxane A_2, a product of arachidonic acid metabolites, which was discovered by Hamberg et al. (1975) and which is generated in inflammatory cells, such as mast cells, alveolar macrophages, platelets, neutrophils and eosinophils, causes platelet aggregation and acts as a potent constrictor of vascular and airway smooth muscle. It also causes plasma exudation in airways. Previous reports suggest that thromboxane A_2 may be involved in not only airway smooth muscle constriction but also in increased airway hyperresponsiveness. Some studies have also demonstrated that the level of thromboxane-derived products is increased in bronchial asthma (Shepherd et al., 1985; Wenzel et al., 1989; Sladek et al., 1990; Kumlin et al., 1992) and that administration of a thromboxane A_2 synthetase inhibitor prevents bronchial asthma and airway hyperresponsiveness (Makino et al., 1990; Taylor et al., 1991).

OKY-046, a thromboxane synthetase inhibitor, which first became available as an antiasthmatic agent in Japan, and AA-2414 ((±)-7-(3,5,6-trimethyl-1, 4-benzoquinon-2-yl)-7-phenylheptanoic acid), a thromboxane receptor antagonist, inhibit the antigen-induced asthmatic response and bronchial responsiveness (Iwamoto et al., 1988; Fujimura et al., 1986, 1990; Kurosawa, 1995) and alter airway cough sensitivity (Fujimura et al., 1995). In contrast, treatment for one week with a thromboxane synthetase inhibitor did not affect airway reactivity to methacholine in asthmatic individuals (Gardiner et al., 1993). These reports indicate that the role of thromboxane A_2 in the development or maintenance of hyperresponsiveness in asthma is still controversial.

DP-1904, a novel thromboxane synthetase inhibitor, causes a selective, potent and long-lasting inhibition of thromboxane synthesis and platelet aggregation (Irie et al.,

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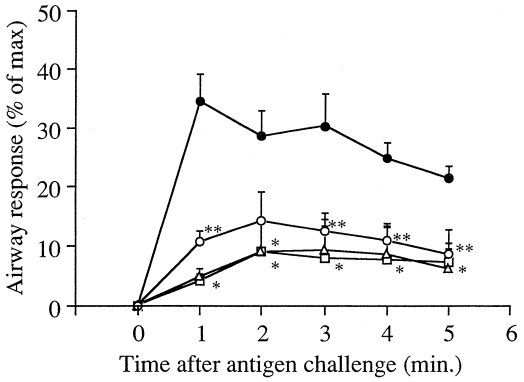

Fig. 1. Effect of DP-1904 on antigen-induced bronchoconstriction in actively sensitized guinea pigs. Vehicle (control: ● $n = 20$) or DP-1904 (1 mg/kg: ○ $n = 6$, 3 mg/kg: △ $n = 12$, 10 mg/kg: □ $n = 18$) was orally given 1 h prior to the antigen challenge. Each point represents the mean ± S.E.M. for the above indicated number of animals per group. $* P < 0.05$ vs. control. $* * P < 0.01$ vs. control.

1986; Kanao et al., 1989; Tanaka et al., 1989; Ohnishi et al., 1992). We previously reported that DP-1904 was effective in protecting against non-specific airway hyperresponsiveness in rats and inhibited the platelet-activating-factor- and immunoglobulin G-induced release of eosinophil cationic protein from human eosinophils (Takami et al., 1995; Agrawal et al., 1997). The present study was conducted to elucidate the effects of DP-1904 on antigen- and spasmogen-induced airway smooth muscle contraction in rodents.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (270–550 g, Japan SLC, Shizuoka, Japan) and Sprague–Dawley rats (Charles River Japan, Kanagawa, Japan) were used in this study. The animals were housed in mesh-bottom cages under controlled conditions at a temperature of 23 ± 2°C and a relative humidity of 55 ± 15% with a 12-h light/dark cycle. They were allowed access to standard laboratory chow (RC-4 for guinea pigs and F-2 for rats, Oriental Yeast, Tokyo, Japan) and tap water ad libitum. All animals were given at least 1 week to acclimatize to the environment before the experiments were performed.

2.2. Chemicals

DP-1904 (6-(1-imidazolylmethyl)-5, 6, 7, 8-tetra-hydronaphthalene-2-carboxylic acid hydrochloride) and OKY-046 (sodium (*E*)-3-[4-(1-imidazolylmethyl)-phenyl]-2-propanoate), which are thromboxane synthetase inhibitors, were synthesized in our laboratory. The following substances were purchased commercially: mepyramine maleate, indomethacin, C_{18}-platelet-activating-factor (PAF), arachidonic acid and bradykinin (Sigma, St. Louis, MO, USA), leukotriene D_4 (Wako, Kyoto, Japan), acetylcholine chloride (Daiichi, Tokyo, Japan), egg albumin (grade V, 5× crystallized and lyophilized; Seikagaku Kogyo, Tokyo, Japan), Freund's complete adjuvant (Difco Lab., Detroit, MI, USA), urethane (Kishida Chemicals, Tokyo, Japan), succinylcholine chloride (Tokyo Kasei, Tokyo, Japan), sodium pentobarbital (Nembutal; Abbott Lab., North Chicago, MO, USA), heparin (Mochida, Tokyo, Japan), $Al(OH)_3$ (S-100, Kyowa Hakkho Chemical, Takamatsu, Japan). The radioimmunoassay kits for thromboxane B_2 and 11-dehydro thromboxane B_2 (New England Nuclear Res., Boston, MA, USA) and for 6-keto prostaglandin $F_{1\alpha}$ (Amersham, UK) were also purchased. All other chemicals were routinely available and of analytical grade. Chemicals were dissolved in distilled water (oral administration) or in physiological saline solution (saline, intravenous injection).

2.3. Sensitization

The following method was derived from that described by Kobayashi et al. (1989). To produce immunoglobulin (Ig) G_1 antibody, guinea pigs were actively sensitized by intradermal administration (1 ml) into the foot pads of egg albumin (10 mg) emulsified with an equal volume of Freund's complete adjuvant on day 0. On day 13 to 15, the sensitized animals were challenged with the antigen.

Rats were actively sensitized by intraperitoneal injection of 100 μg of egg albumin in 0.5 ml saline mixed with 100 mg of dried and re-suspended $Al(OH)_3$ on day 0. On day 25, the sensitized animals were challenged with the antigen. In this study, the animals which produced anti-egg albumin immunoglobulin E (IgE) antibody were used. The IgE titer in the serum from actively sensitized rats was

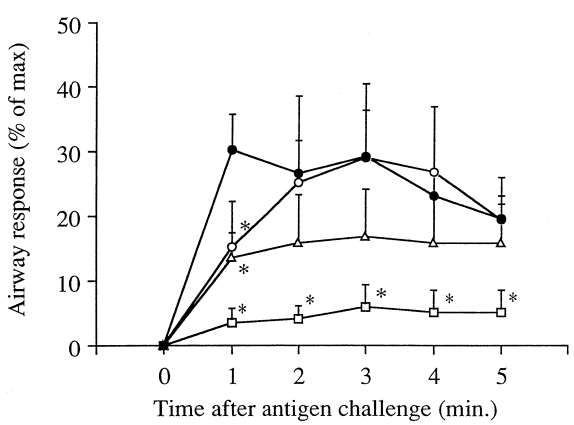

Fig. 2. Effect of OKY-046 on antigen-induced bronchoconstriction in actively sensitized guinea pigs. Vehicle (control: ● $n = 14$) or OKY-046 (10 mg/kg: ○ $n = 6$, 30 mg/kg: △ $n = 12$, 100 mg/kg: □ $n = 6$) was orally given 1 h prior to the antigen challenge. Each point represents the mean ± S.E.M. for the above indicated number of animals per group. $* P < 0.05$ vs. control.

Table 1
Effect of DP-1904, mepyramine and indomethacin on antigen-induced bronchoconstriction in actively sensitized guinea pigs

Compound	Dose (mg/kg, i.v.)	Peak response (% of maximum)	% Inhibition
Vehicle	–	52.2 ± 4.7	–
DP-1904	1.0	13.6 ± 2.6[a]	74 ± 4.9
Mepyramine	0.5	49.8 ± 4.8	4.5 ± 9
	5.0	43.8 ± 4.1	16.1 ± 7.8
Indomethacin	1.0	57.0 ± 4.5	−9.2 ± 8.6

DP-1904, mepyramine or indomethacin was given intravenously 2 min prior to the antigen challenge.
Values are the means ± S.E.M. of five experiments. Significantly different from vehicle control: [a] $P < 0.01$.

measured with the homologous passive cutaneous anaphylaxis assay, according to the method of Watanabe and Ovary (1977).

2.4. Measurement of antigen- and spasmogen-induced bronchoconstriction

Bronchoconstriction was measured according to Dahlback et al. (1984). Briefly, the animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The left jugular vein and right carotid artery were cannulated for intravenous dosing and for collecting the blood samples, respectively. Then, the trachea was cannulated and the animals were ventilated with a rodent ventilator (No. 7025, Ugo Basile, Italy) in a closed system. The inflation pressure was recorded through a side arm of the tracheal cannula connected to pressure transducer (TP-603T, Nihon Kohden, Tokyo). The respiratory rate was maintained at 60 strokes/min with a tracheal pressure of 10 cm H_2O. Spontaneous breathing was arrested with succinylcholine (1 or 5 mg/kg i.v.).

The animals were intravenously challenged with egg albumin (0.5 or 5 mg/kg) or the spasmogen; leukotriene D_4 (0.3 μg/kg), arachidonic acid (100–1000 μg/kg), PAF (0.01–0.1 μg/kg), bradykinin (0.5–5 μg/kg), histamine (5–50 μg/kg) or acetylcholine (5–50 μg/kg). The increase in inflation pressure evoked by the challenge was calculated as a percentage of the maximum intratracheal pressure obtained by clamping the trachea. Pulmonary responses were recorded for 3 or 5 min after the challenge.

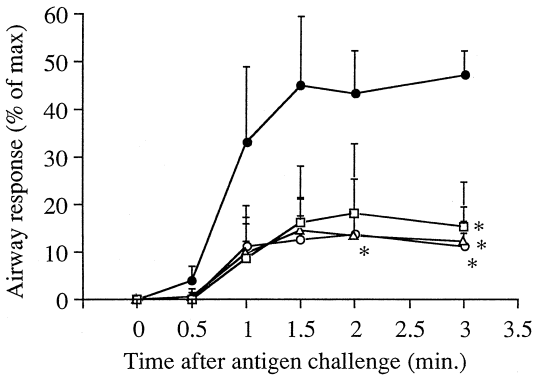

Fig. 3. Effect of DP-1904 and OKY-046 on IgE-mediated bronchoconstriction in actively sensitized rats. Vehicle (control: ●), DP-1904 (1 mg/kg: ○, 10 mg/kg: △) or OKY-046 (100 mg/kg: □) was orally given 1 h prior to the antigen challenge. Each point represents the mean ± S.E.M. for four animals in the control group or six animals in the other groups. * $P < 0.01$ vs. control.

2.5. Collection of plasma and bronchoalveolar lavage fluid and measurement of prostanoid level

The plasma and bronchoalveolar lavage fluid were sampled from the same animals tested for antigen- and spasmogen-induced bronchoconstriction at 3 and 5 min after the challenge, respectively. Thromboxane B_2 and 11-dehydro thromboxane B_2 were quantified because thromboxane A_2 is unstable. Blood (1 ml) was withdrawn from the

Table 2
Effect of DP-1904 on TXs levels following antigen challenge in actively sensitized guinea pigs

Treatment	Plasma (pg/ml)		BALF (pg/ml)		Bronchoconstriction (% of maximum)
	TXB_2	11-dehydro TXB_2	TXB_2	11-dehydro TXB_2	
(a) 1 h prior to antigen challenge					
Normal	159 ± 11	71 ± 3	6 ± 1	6 ± 1	ND
Control	1775 ± 518	112 ± 13	83 ± 11	28 ± 5	50.9 ± 4.8
DP1904	169 ± 25[a]	58 ± 1[a]	14 ± 2[b]	9 ± 1[b]	8.4 ± 3.6[a]
(b) 12 h prior to antigen challenge					
Normal	123 ± 18	60 ± 6	4 ± 1	4 ± 1[a]	ND
Control	1111 ± 131	125 ± 13	77 ± 10	27 ± 4	54.5 ± 4.6
DP1904	329 ± 47[c]	67 ± 6[c]	14 ± 2[b]	5 ± 1[b]	24.3 ± 5.6[a]

DP-1904 (10 mg/kg) was administered orally 1 or 12 h prior to the antigen challenge.
Values are the means ± S.E.M. of six experiments.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.005$, significantly different from control.
ND means not done.

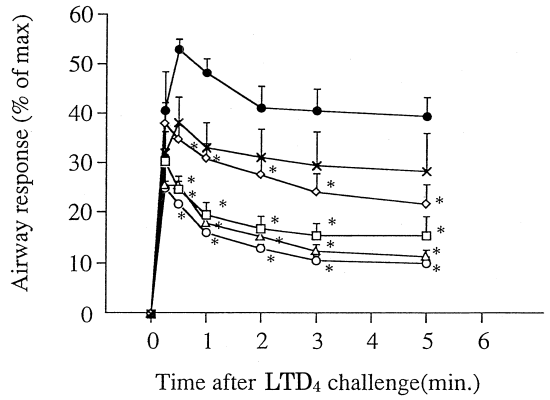

Fig. 4. Effect of DP-1904 and OKY-046 on LTD_4-induced bronchoconstriction in guinea pigs. Vehicle (control: ●), DP-1904 (3 mg/kg: □, 10 mg/kg: △, 30 mg/kg: ○) or OKY-046 (10 mg/kg: ×, 30 mg/kg: ◇) was orally given 1 h prior to the LTD_4 challenge. Each point represents the mean ± S.E.M. for five animals. * $P < 0.01$ vs. control.

carotid artery, using a 2.5-ml syringe containing 1 mM EDTA–2Na and 0.04 mM indomethacin. Bronchoalveolar lavage fluid was collected by perfusion of the airways with saline (5 ml) containing heparin (10 unit/ml), 1 mM EDTA–2Na and 0.04 mM indomethacin, using a 10-ml syringe. The fluid was gently sucked back into the syringe. Blood and bronchoalveolar lavage fluid were centrifuged at 1200 × *g* 4°C for 10 min. Thromboxane B_2, 11-dehydro thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$, a stable metabolite of prostaglandin I_2 were assayed by radioimmunoassay.

2.6. Drug treatment

For oral administration, DP-1904 (1–30 mg/kg) or OKY-046 (10–100 mg/kg) was given 1 h prior to the challenge. For intravenous administration, DP-1904 (1 mg/kg), mepyramine (0.5, 5 mg/kg) or indomethacin (1 mg/kg) was given 2 min prior to the challenge. To evaluate the duration of the effects, DP-1904 (10 mg/kg, p.o.) was given 1 or 12 h prior to the challenge. As a control, the vehicle for each test drug was used.

2.7. Statistical analysis

The results are expressed as the means ± S.E.M. for the indicated number of animals. The correlation coefficient between thromboxane level and bronchoconstriction was calculated by linear regression analysis. The dose causing 75% inhibition (ED_{75} value) was determined by linear regression analysis of plots for drug vs. % inhibition of bronchoconstriction for drug-treated compared with vehicle-treated animals. Statistical comparisons ($P < 0.05$) between groups were made by using Student's unpaired t test or Williams' multiple comparison test.

3. Results

3.1. Effect of DP-1904, OKY-046 and other compounds on antigen-induced bronchoconstriction in guinea pigs

In the guinea pig model, antigen challenge caused bronchoconstriction, with the peak response being reached within 1 min, whereafter it diminished gradually over the next 30 min. When administered orally, DP-1904 significantly reduced the antigen-induced bronchoconstriction at doses of 1 mg/kg to 10 mg/kg, and the ID_{75} value of DP-1904 was 1.91 mg/kg (Fig. 1). OKY-046 also significantly attenuated the bronchoconstriction at the dose of 100 mg/kg, and the ID_{75} value was 59.1 mg/kg. At the lower doses of 10 and 30 mg/kg, OKY-046 inhibited only the early phase of the constriction (Fig. 2). Intravenous injection of DP-1904 (1 mg/kg) also inhibited the antigen-induced bronchoconstriction (by 74% reduction), while mepyramine showed only a small effect (5–16%

Table 3
Effect of DP-1904 on prostanoid levels following LTD_4-induced bronchoconstriction in guinea pigs

Treatment	TXB_2 (pg/ml)		6-keto $PGF_{1\alpha}$ (pg/ml)	Bronchoconstriction (% maximum)
	Plasma	BALF	Plasma	
(a) 1 h prior to LTD_4 challenge				
Normal	306 ± 20	ND	2117 ± 201	
Control	1369 ± 210	124 ± 39	3631 ± 735	48 ± 6
DP1904	398 ± 61[a]	14 ± 1[a]	6511 ± 936[b]	20 ± 3[b]
(b) 12 h prior to LTD_4 challenge				
Normal	230 ± 18	ND	1459 ± 303	
Control	1432 ± 192	102 ± 19	2587 ± 328	51 ± 4
DP1904	329 ± 47[b]	18 ± 2[a]	3237 ± 351	25 ± 7[a]

DP-1904 (10 mg/kg) was administered orally 1 or 12 h prior to LTD_4 challenge.
Values are the means ± S.E.M. of six experiments.
ND means not done.
[a] $P < 0.05$.
[b] $P < 0.01$, significantly different from control.

Table 4
Effect of DP-1904 and OKY-046 on spasmogen-induced bronchoconstriction in guinea pigs

Spasmogen (μg/kg, i.v.)	% Inhibition		
	DP-1904 (mg/kg, p.o.)		OKY-146 (mg/kg, p.o.)
	3	30	30
Aracidonic acid (100–1000)	63	ND	28
PAF (0.01–0.1)	76	ND	40
Bradykinin (0.5–5)	82	ND	31
Acetylcholine (5–39)	ND	No effect	No effect
Histamine (5–50)	ND	No effect	No effect

DP-1904 or OKY-046 was administered orally 1 h prior to the spasmogen.
Values are the mean % inhibition ± S.E.M. of five experiments.
ND means not done.

reduction) and indomethacin had no effect (Table 1). When orally administered at 1 h and even at 12 h before antigen challenge, DP-1904 (10 mg/kg) significantly reduced the bronchoconstriction and significantly suppressed the increase in the endogenous levels of thromboxanes (thromboxane B_2 and 11-dehydro thromboxane B_2) in both the plasma and bronchoalveolar lavage fluid. This shows that DP-1904 had a long-lasting effect in this antigen-induced model (Table 2). When OKY-046 (50 mg/kg) was administered orally at 12 h before antigen challenge, it no longer affected the bronchoconstriction (data not shown). There was a significant correlation between the bronchoconstriction and thromboxanes levels in the plasma ($r = 0.71$, $n = 24$, $P < 0.01$) and bronchoalveolar lavage fluid ($r = 0.76$, $n = 24$, $P < 0.01$).

3.2. Effect of DP-1904 and OKY-046 on antigen-induced bronchoconstriction in rats

In the rat model, antigen challenge caused bronchoconstriction, with the peak response being reached within 1.5 min, whereafter the response remained stable throughout the observation period. When administered orally, DP-1904 (1 and 10 mg/kg) significantly inhibited the bronchoconstriction. OKY-046 also significantly attenuated the bronchoconstriction at the dose of 100 mg/kg, but the effect was less than that of DP-1904 (Fig. 3). The plasma thromboxane B_2 level was significantly elevated after the antigen challenge. The increase in the plasma level of thromboxane B_2 was completely inhibited by DP-1904 (data not shown).

3.3. Effect of DP-1904 and OKY-046 on leukotriene D_4- and other spasmogen-induced bronchoconstriction in guinea pigs

Oral administration of DP-1904 (3–30 mg/kg) significantly attenuated leukotriene D_4-induced bronchoconstriction. At 30 mg/kg, OKY-046 significantly reduced this bronchoconstriction (Fig. 4). Oral treatment with DP-1904 (10 mg/kg) significantly reduced the bronchoconstriction and the increase in endogenous levels of leukotriene D_4-induced thromboxane B_2 in both plasma and bronchoalveolar lavage fluid at 1 h and even at 12 h after the challenge, as observed in the antigen-induced guinea pig model, indicating again the long-lasting biological activity of DP-1904. In addition, DP-1904 significantly increased the level of plasma prostaglandin F_{1_α} after the leukotriene D_4 challenge (Table 3).

Bronchoconstriction was also induced by intravenous injection of arachidonic acid, PAF, bradykinin, histamine or acetylcholine. Oral administration of DP-1904 (3 mg/kg) as well as OKY-046 (30 mg/kg) effectively counteracted the bronchoconstriction induced by arachidonic acid, PAF and bradykinin. DP-1904 and OKY-046 had no significant effect on the bronchoconstriction provoked by histamine and acetylcholine (Table 4).

4. Discussion

It has been suggested that the arachidonate metabolites, leukotrienes and thromboxane, play an important role in the pathogenesis of asthma. The biological activities of these mediators, which include bronchoconstriction, increase in microvascular permeability, formation of mucosal edema, are more potent than those of histamine and acetylcholine on a molar basis. Furthermore, recent studies have demonstrated the presence of these mediators in plasma, bronchoalveolar lavage fluid, and urine in asthmatic patients after allergen challenge. Therefore, the regulation of these mediators may provide a novel target for the treatment of bronchial asthma. A large number of peptide leukotriene antagonists, thromboxane synthetase inhibitors, and thromboxane antagonists have been actively developed by the pharmaceutical industry. Among them, a thromboxane synthetase inhibitor, OKY-046, first became available as an antiasthmatic agents in Japan. In the present study, we investigated the effect of DP-1904, a thromboxane synthetase inhibitor, on antigen- and spasmogen-induced bronchoconstriction in rodents.

In actively sensitized guinea pigs, allergen challenge caused marked bronchoconstriction and the liberation of

thromboxane B_2 and 11-dehydro thromboxane B_2 into both plasma and bronchoalveolar lavage fluid. The results obtained here are consistent with those of Nambu et al. (1990), although they did not determine the 11-dehydro thromboxane B_2 levels. Oral administration of DP-1904 significantly inhibited the antigen-induced bronchoconstriction. When compared with the inhibitory effect of OKY-046, DP-1904 was approximately 30-fold more potent than OKY-046. This was supported by the results of in vitro and ex vivo studies in which DP-1904 was more potent in inhibiting thromboxane synthesis than OKY-046 (Kanao et al., 1989) and had a better bioavailability than OKY-046 in humans (Tanaka et al., 1989, 1990). The inhibitory effects of DP-1904 (10 mg/kg) on bronchoconstriction and thromboxane synthesis persisted for more than 12 h, despite its short half-time in plasma (about 0.5 h). A possible explanation for the discrepancy between the short half-life and the long-lasting action of DP-1904 on thromboxane B_2 synthesis could be its high affinity and specificity for thromboxane synthetase in platelets and airway tissues (Tanaka et al., 1989). We also examined the duration of effect of OKY-046 in the model, but OKY-046 did not significantly inhibit bronchoconstriction when administered orally 8 h before antigen challenge. Interestingly, the magnitude of bronchoconstriction correlated to the reduction in thromboxane levels in plasma and bronchoalveolar lavage fluid, indicating that thromboxane A_2 acts as an important mediator in this model. A maximally effective dose of mepyramine, a histamine H1 receptor antagonist, showed only a partial effect, excluding a dominant role of histamine, which is consistent with the results described by Kobayashi et al. (1989). Furthermore, indomethacin, at a dose suppressing the increase in thromboxane B_2 in plasma (data not shown), was not effective in this model. Indomethacin causes shunting of arachidonic acid to the 5-lipoxygenase pathway, resulting in the enhanced production of peptide-leukotrienes and in the diminished production of relaxing prostanoids such as prostaglandin I_2 and prostaglandin E_2 (Hitchcock, 1980; Undem et al., 1987). The shunting effect on arachidonic metabolism caused by indomethacin accounts for the failure of indomethacin to reduce the bronchoconstriction.

In the actively sensitized rat model in which IgE antibody is involved, it has been reported that 1-benzyl imidazole, methysergide and phenidone prevent allergen-induced broncoconstriction (Dahlback et al., 1984), suggesting that thromboxanes, 5-HT and peptide-leukotrienes participate in the development of bronchoconstriction in this model. DP-1904 and OKY-046 effectively relieved the IgE-mediated bronchoconstriction in the model. However, DP-1904 was active in lower doses than those of OKY-046.

DP-1904 and OKY-046 also reduced leukotriene D_4-induced bronchoconstriction. This result supports the suggestion that leukotriene D_4-induced bronchoconstriction is mediated in part through endogenous thromboxane A_2 (Weishman et al., 1982; Mong et al., 1986). It has been reported that OKY-046 inhibits thromboxane levels and increases plasma 6-keto prostaglandin $F_{1\alpha}$ levels after antigen challenge in guinea pigs (Nambu et al., 1990). Therefore, we examined the effect DP-1904 on prostanoid levels in leukotriene D_4-induced bronchoconstriction. We found that DP-1904 significantly inhibited endogenous thromboxanes formation in plasma, and there was a significant increase in 6-keto prostaglandin $F_{1\alpha}$ levels after leukotriene D_4 challenge. This phenomenon can be explained by shunting mechanisms in arachidonic acid metabolism (Dworski et al., 1992; Trochtenberg et al., 1992). Further, the inhibitory effect of DP-1904 on thromboxanes synthesis persisted for more than 12 h, indicating the long-lasting ability of DP-1904 to decrease leukotriene D_4-induced bonchoconstriction.

DP-1904 and OKY-046 also inhibited the bronchoconstriction induced by arachidonic acid-, PAF- and bradykinin, suggesting that these constrictions might be mediated in part via thromboxanes synthesis, as shown in a previous report (Rossoni et al., 1980; Lefort et al., 1984). DP-1904 inhibits human platelets thromboxane A_2 synthetase ($IC_{50} = 1.9$ nM), but is 5×10^6 times less active in inhibiting sheep seminal vesicle cyclooxygenase, bovine aorta prostaglandin I_2 synthetase and guinea pig 5-lypoxgenase (unpublished data) and does not interact directly with the PAF receptor (Agrawal et al., 1993). Thus, a direct effect of DP-1904 on smooth muscle can be excluded.

In conclusion, the current results show that DP-1904 is a potent and long-acting inhibitor of antigen- and spasmogen-induced bronchoconstriction in rodent models, and that these biological activities of DP-1904 are more potent than those of OYKY-046 (Ozagrel) on a molar basis.

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