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# Effect of combined leukotriene $D_4$ and thromboxane $A_2$ receptor antagonist on mediator-controlled resistance in guinea pigs

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**Abstract**

The effects of YM158 (3-[(4-*tert*-butylthiazol-2-yl)methoxy]-5′-[3-(4-chlorobenzenesulfonyl)propyl]-2′-(1*H*-tetrazol-5-ylmethoxy)benzanilide monosodium salt monohydrate), a new dual antagonist for leukotriene $D_4$ and thromboxane $A_2$ receptors, on antigen-induced increases in airway resistance were investigated in mediator-controlled novel asthmatic models using actively sensitized guinea pigs. While the predominant mediator was thromboxane $A_2$, complete inhibition of cyclooxygenase induced mediation by cysteinyl-leukotrienes. About 1-mg/kg indomethacin induced a state where both mediators participated equally. YM158 inhibited increases in resistance whether only one or both mediators were involved. When leukotriene $D_4$ and thromboxane $A_2$ equally participated, $ED_{50}$ values for 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate (pranlukast; 3.9 mg/kg) and 7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid (seratrodast; 2.1 mg/kg) were similar to that for YM158 (8.3 mg/kg), although those effects on the corresponding mediator-induced reaction were 10 times stronger than those of YM158. Additionally, the maximum inhibition of YM158 was stronger than those of either single receptor antagonist. In conclusion, YM158 has a potentially greater efficacy in wider types of experimental asthmatic models than single receptor antagonists. 

*Keywords:* Leukotriene; Thromboxane; YM158; Asthma

## 1. Introduction

Arachidonic acid metabolites of 5-lipoxygenase and cyclooxygenase action play important roles in the pathogenesis of bronchial asthma. Such metabolites, including the cysteinyl-leukotrienes leukotriene $C_4$, $D_4$, and $E_4$ (Samuelsson, 1983), are major constituents of the slow-reacting substance of anaphylaxis. This substance increases the vascular permeability (Peck et al., 1981; Rinkema et al., 1984) and contraction of airway smooth muscle (Dahlén et al., 1980; Ueno et al., 1982). It has been suggested that a blockade of the receptors of these substances might alleviate many signs and symptoms, or prevent the onset, of asthma. Some potent leukotriene $D_4$ receptor antagonists, such as 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate (pranlukast) (Obata et al., 1992; Nakagawa et al., 1992), zafirlukast (Krell et al., 1990) and montelukast (Jones et al., 1995), have already received marketing approval to treat bronchial asthma (Lazarus, 1998). Thromboxane $A_2$, another arachidonic acid metabolite, also has potent bronchoconstricting activity (Nagai et al., 1991; Francis et al., 1991), which influences airway hyperresponsiveness (Jones et al., 1992; Nagai et al., 1993; Fujimura et al., 1991). Thromboxane $A_2$ receptor antagonists, such as 7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid (seratrodast), and synthetase inhibitors have also received marketing approval as anti-asthmatic drugs in Japan (Samara et al., 1997; Kurashima et al., 1992). However, since leukotriene $D_4$ and thromboxane $A_2$ play different roles in the development and onset of asthma, a multi-pathway inhibitory agent might have more potent therapeutic effects in treating bronchial asthma.

Clinical trial results of leukotriene $D_4$ receptor antagonists (Barnes et al., 1997; Taniguchi et al., 1993; Adkins

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and Brogden, 1998) and thromboxane $A_2$ inhibitors (Samara et al., 1997; Kurashima et al., 1992) suggest that the predominant lipid mediator varies from patient to patient. Consequently, asthmatic patients can be roughly classified into leukotriene $D_4$-predominant, thromboxane $A_2$-predominant or equal participation types. We have already reported that the effects of leukotriene $D_4$ or thromboxane $A_2$ receptor antagonists on predominant lipid mediator-controlled asthmatic responses using passively sensitized guinea pigs (Arakida et al., 1999). In this study, a novel lipid mediator-controlled asthma model was developed, using actively sensitized guinea pigs whose asthmatic responses are modulated by the cyclooxygenase inhibitor indomethacin. This model was used to evaluate the effects of pranlukast, seratrodast and YM158 (Yokota et al., 1997; Arakida et al., 1998) on asthmatic response.

## 2. Materials and methods

### *2.1. Animals*

All animal experiments were approved by the Animal Experimentation Ethics Committee of Yamanouchi Pharmaceutical. Male Hartley guinea pigs (Charles River Japan; Yokohama, Japan), weighing 400–730 g at the time of challenge, were used. The animals were given food and water ad libitum until the day before the experiment. Food was withheld overnight to eliminate the effect of food on absorption after oral administration of the test compounds.

### *2.2. Chemicals*

The following drugs and chemicals were used. YM158 (Arakida et al., 1998)], pranlukast (Obata et al., 1992) and seratrodast (Fukumoto et al., 1992) were synthesized by Yamanouchi Pharmaceutical (Tsukuba, Japan). Ovalbumin (grade VI), urethane, indomethacin, pyrilamine maleate, propranolol hydrochloride, and nordihydroguaiaretic acid were purchased from Sigma (St. Louis, MO, USA); gallamine triethioide (Flaxedil®) from Rhone-Poulenc Rorer (Paris, France); and methylcellulose (MC) from Shin-Etsu Chemical (Tokyo, Japan). Pyrilamine maleate, propranolol and ovalbumin were dissolved in 0.9% saline. Indomethacin was dissolved in 0.9% saline containing a few drops of 1 N NaOH solution. YM158, pranlukast and seratrodast were dissolved or suspended in a 0.5% aqueous MC solution for oral administration.

### *2.3. Sensitization and antigen-induced increase in airway resistance*

Animals were actively sensitized by three intraperitoneal injections of 5-μg ovalbumin containing 1 mg of Alum ($Al(OH)_3$) every 2 weeks. One week after the last sensitization, the animals were anesthetized with urethane (1.2 g/kg, i.p.). A tracheal cannula was inserted and attached to a constant volume respirator (Model 683; Harvard; South Natick, MA, USA). Animals were ventilated with a 10-ml volume of air/kg body weight at a rate

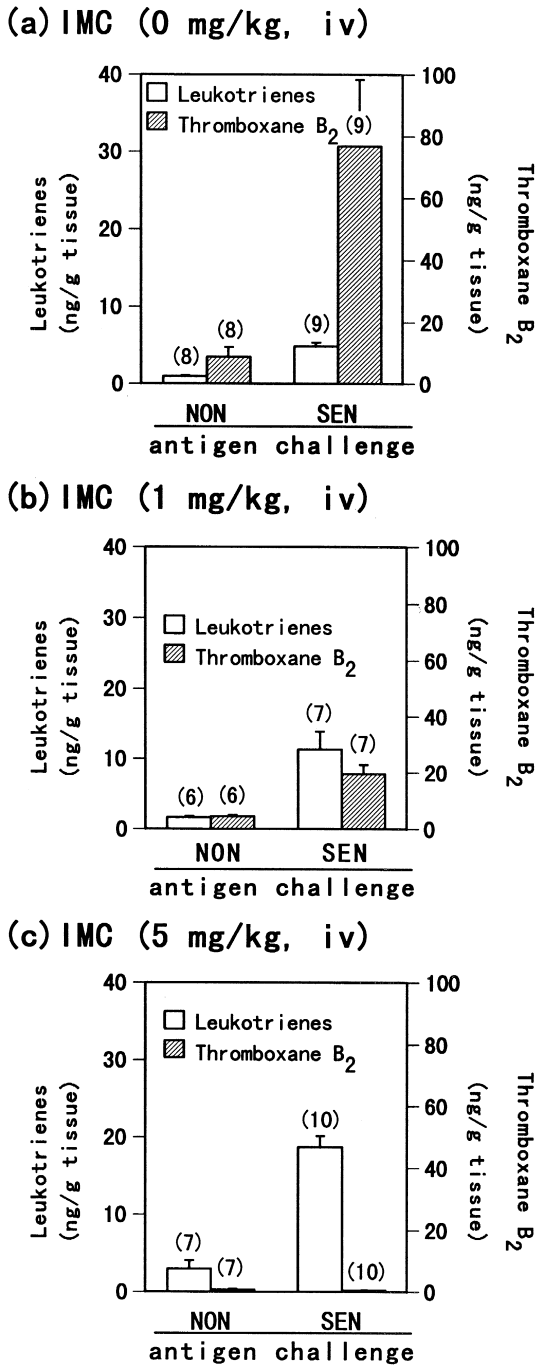


Fig. 1. Effects of indomethacin on lipid mediator content in lung tissue isolated from sensitized and non-sensitized guinea pigs. Lipid mediators were measured by ELISA after extraction of lipophilic components. Animals were challenged by the antigen after the intravenous injection of (a) 0, (b) 1 or (c) 5 mg/kg indomethacin (IMC). Values are the means ± S.E.M. of the indicated number of guinea pigs. SEN means sensitized, and NON means non-sensitized animals.

of 60 strokes/min. Airway resistance was measured with a respiratory function measuring apparatus (Model 6; Buxco Electronics; Sharon, CT, USA) connected to the tracheal cannula. The animals were pretreated with gallamine (1 mg/kg, i.v.), pyrilamine (2 mg/kg, i.v.) and propranolol (0.3 mg/kg, i.v.) at 10, 2 and 2 min prior to antigen challenge. After intravenous injection of ovalbumin, airway resistance was measured continuously for 15 min, and the values obtained at each minute were used to evaluate changes in airway resistance. The effects of compounds were evaluated using the percentage change in airway resistance from the pre-challenge value. The area under the time–response curve (AUC) for the 15 min of observation after antigen challenge was used as an index. The study compound was orally administered 1 h before antigen challenge.

### *2.4. Adjustment of lipid mediator contents and antigen amount*

The increase in pulmonary cysteinyl-leukotrienes and thromboxane $B_2$ induced by antigen challenge was examined after treatment with one of two doses of indomethacin. Briefly, an asthmatic reaction was induced in animals that had been intravenously administered 0, 1 or 5 mg/kg of indomethacin 3-min before antigen challenge. To evoke the same increase in airway resistances in all sensitized control groups, 0.6 mg/kg ovalbumin was given to animals, which received 0 or 1 mg/kg indomethacin, and 1 mg/kg ovalbumin was given to animals, which received 5 mg/kg indomethacin. The lungs were resected immediately after measuring airway functions, using the following method: The animal was sacrificed by

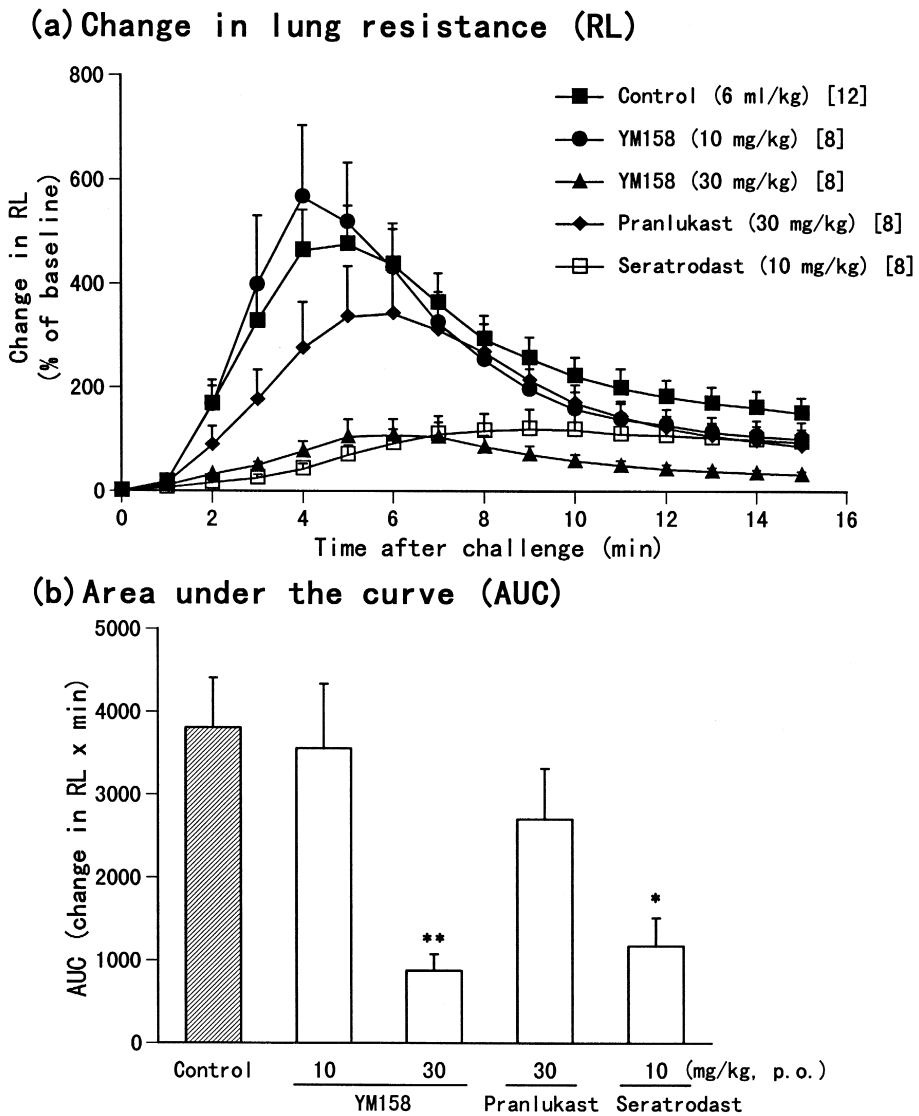


Fig. 2. Effects of YM158, pranlukast and seratrodast on increased airway resistance (RL) induced by intravenously injected ovalbumin in actively sensitized guinea pigs not given indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. sensitized control using Dunnett's multiple range test.

exsaguination, the chest cavity was opened to allow perfusion with buffer A (0.9% saline containing 10 μM of indomethacin and 10 μM of nordihydroguaiaretic acid) from the pulmonary artery to the left atrium, and then the lung parenchyma was removed and homogenized in 5 ml of buffer A.

### 2.5. Determination of leukotriene $C_4/D_4/E_4$ and thromboxane $B_2$ levels in lungs

The isolated and homogenized lung tissue was added to ethanol, stored at 4°C for 30 min, and centrifuged at $2000 \times g$, 4°C for 15 min. The supernatant was retained and dried under reduced pressure. The residue was dissolved in 5 ml of $H_2O$ and adjusted to pH 3–4 by addition of a 1-M citric acid solution.

This sample was applied to an octadecylsilyl silica cartridge (SEP-PAK $C_{18}$; Waters Associates; Milford, MA, USA). To obtain the thromboxane $B_2$ fraction, a stable metabolite of thromboxane $A_2$, the cartridge was washed with a 15% aqueous ethanol solution followed by petroleum ether. The lipophilic components were then eluted with 4.5 ml of ethyl acetate. To obtain leukotriene $C_4/D_4/E_4$ fraction, the cartridge was washed with distilled water and 15% aqueous ethanol solution, and eluted with 2.0 ml of ethanol. These eluted fractions were dried using a rotary evaporator under reduced pressure, and then these samples were dissolved in ELISA buffer. Leukotriene $C_4/D_4/E_4$

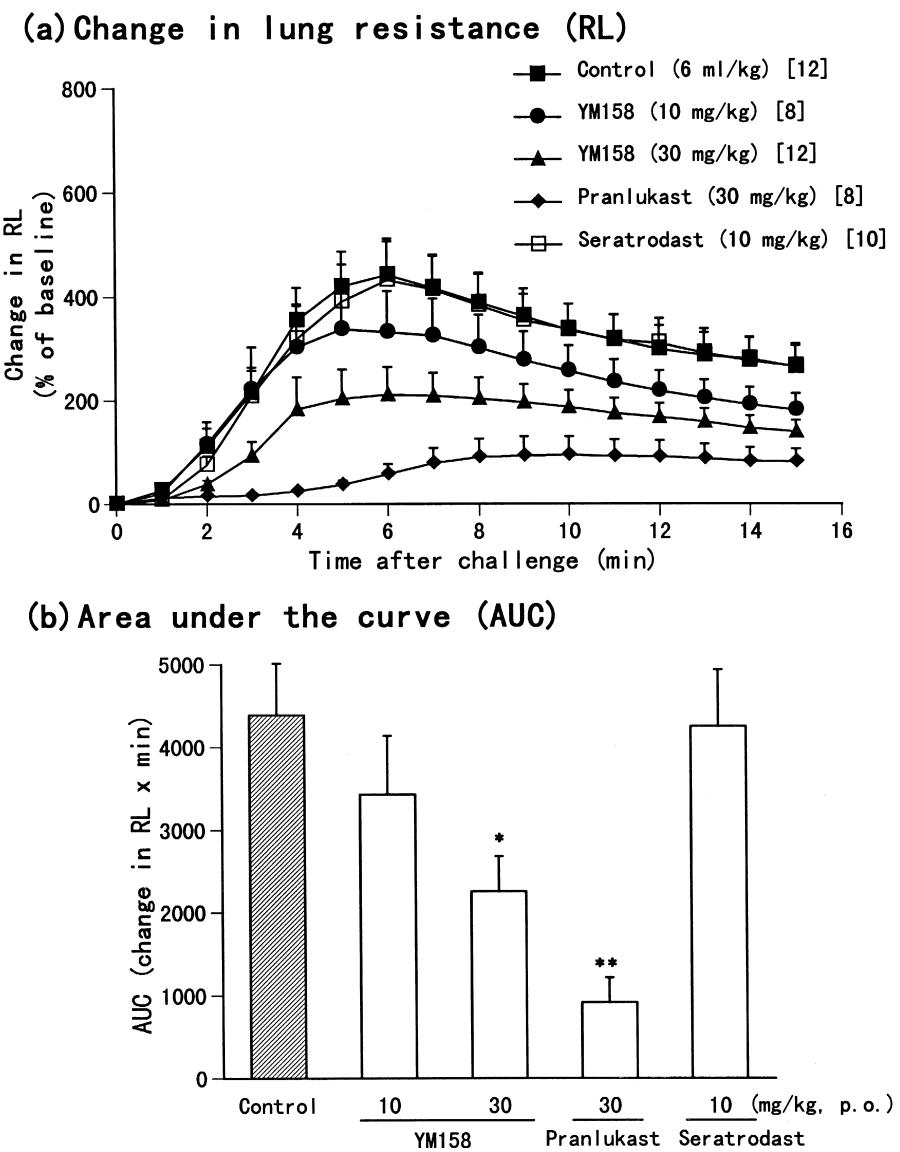


Fig. 3. Effects of YM158, pranlukast and seratrodast on increased airway resistance (RL) induced by intravenously injected ovalbumin in actively sensitized guinea pigs pretreated with 5 mg/kg indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. sensitized control using Dunnett's multiple range test.

and thromboxane $B_2$ levels in isolated guinea pig lung tissue extracts were measured using an ELISA kit (Amersham International, Buckinghamshire, England).

The lipid recovery rate in each experiment was measured by adding a standard radioactive lipid to samples isolated from normal guinea pigs. Lipid amounts were normalized to amount of radioactivity recovered.

### 2.6. Statistical analysis

Experimental results are expressed as the mean ± S.E.M. For evaluation of each compound's effectiveness on airway resistance in each group, multiple comparison of AUC values during the 15 min after antigen challenge were analyzed by Dunnett's multiple range test. *P*-values of less than 0.05 were defined as significant. $ED_{50}$ values were estimated by regression analysis (linear regression using the maximum likelihood method). These analyses were performed using statistical analysis system (SAS).

## 3. Results

### 3.1. Lipid mediator content in antigen-challenged guinea pig lung tissue

The predominance of either lipid mediator, leukotriene $C_4/D_4/E_4$ or thromboxane $B_2$ (a stable metabolite, which

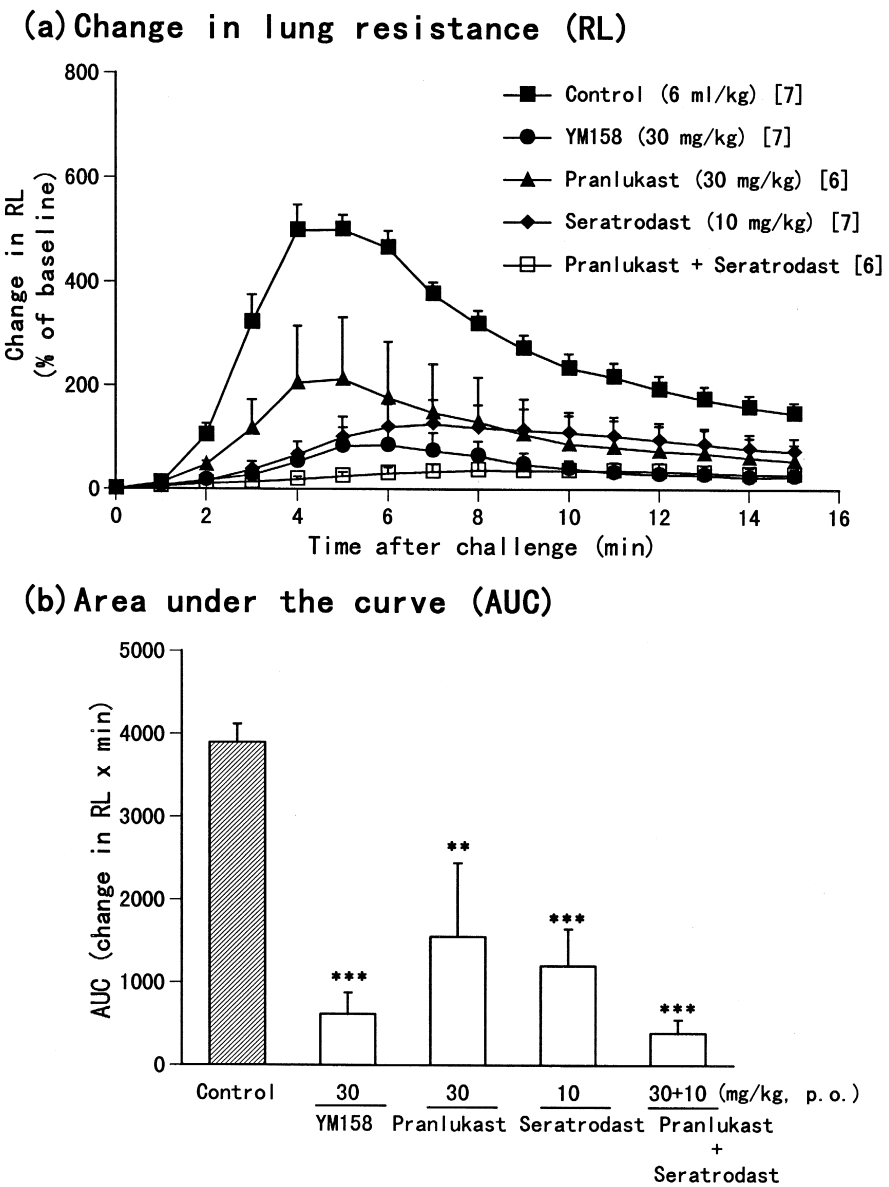


Fig. 4. Effects of YM158, compared with those of separate or simultaneous administration of pranlukast and seratrodast, on increased airway resistance (RL) induced by intravenous injected ovalbumin in actively sensitized guinea pigs pretreated with 1 mg/kg indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. sensitized control using Dunnett's multiple range test.

accurately reports unstable thromboxane $A_2$ levels), was determined by ELISA (Fig. 1). In antigen-challenged guinea pigs not treated with indomethacin, the level of intra-pulmonary cysteinyl-leukotrienes increased about 5.0-fold, and the level of thromboxane $B_2$ increased about 8.8-fold compared with non-sensitized group values (Fig. 1a). In guinea pigs treated with 1 mg/kg of indomethacin, the level of cysteinyl-leukotrienes increased about 6.8-fold but the level of thromboxane $B_2$ increased only about 4.4-fold compared with non-sensitized group values (Fig. 1b), showing that absolute cysteinyl-leukotrienes levels increase in indomethacin-treated animals since cyclooxygenase activity is partially blocked. In animals treated with 5 mg/kg of indomethacin, which completely blocks cyclooxygenase activity, the level of cysteinyl-leukotrienes was about 6.3-fold greater in sensitized animals than in non-sensitized animals, while thromboxane $B_2$ levels were negligible (Fig. 1c).

### *3.2. Effects of orally administered pranlukast, seratrodast and YM158 on antigen-induced asthmatic response in the absence of indomethacin*

Suppression of antigen-induced airway resistance due to treatment with antagonists orally administered 1 h before challenge was examined. Comparison of AUC values showed that the leukotriene $D_4$ receptor antagonist pranlukast (30 mg/kg) had no effect, while the thromboxane $A_2$ receptor antagonist seratrodast (10 mg/kg) significantly suppressed resistance. YM158, the dual antagonist, did not suppress resistance at a dose of 10 mg/kg, but it significantly suppressed resistance at a dose of 30 mg/kg (Fig. 2).

### *3.3. Effects of orally administered pranlukast, seratrodast and YM158 on antigen-induced asthmatic response in the presence of indomethacin (5 mg / kg)*

Comparison of AUC values showed that seratrodast (10 mg/kg) had no effect on, while pranlukast (30 mg/kg) significantly suppressed, airway resistance. YM158 did not suppress resistance at a dose of 10 mg/kg, but did significantly suppress resistance at a dose of 30 mg/kg (Fig. 3).

### *3.4. Effects of orally administered pranlukast, seratrodast and YM158 on antigen-induced asthmatic response in the presence of indomethacin (1 mg / kg)*

Both pranlukast (30 mg/kg) and seratrodast (10 mg/kg) administered alone, significantly suppressed increased airway resistance induced by antigen challenge (Fig. 4). The coadministration of these antagonists produced a synergistic suppression of resistance (Fig. 4). YM158 at a dose of 30 mg/kg suppresses resistance, eliciting a response almost as strong as that obtained by the coadministration of pranlukast and seratrodast (Fig. 4). As Fig. 5 shows, interestingly, only thromboxane $B_2$ level in the YM158-treated group showed a statistically significant decrease from the sensitized untreated control level. Additionally, pranlukast and YM158 decreased the level of cysteinyl-

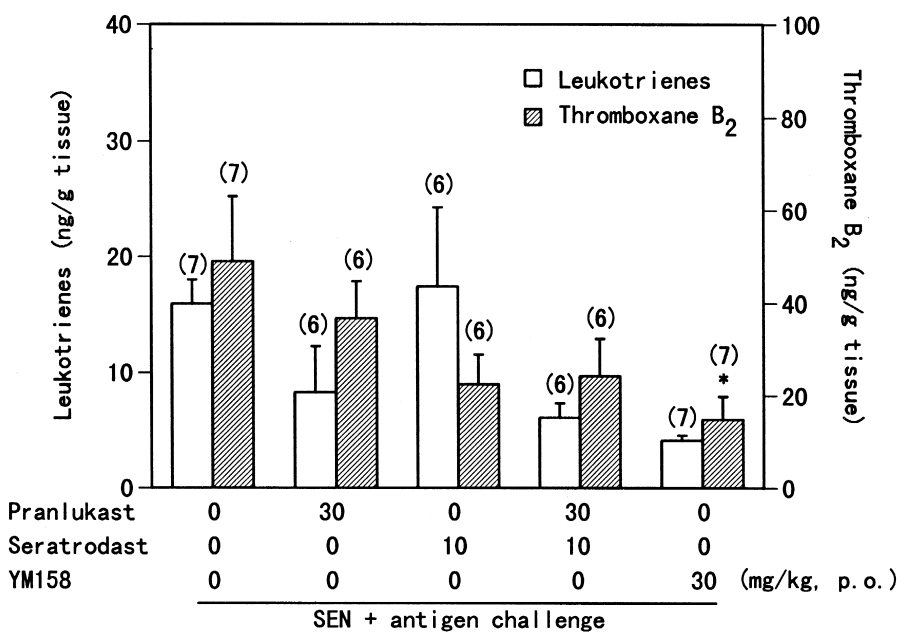


Fig. 5. Effects of YM158, compared with those of separate or simultaneous administration of pranlukast and seratrodast, on lipid mediator content in lung tissue isolated from antigen-challenged guinea pigs pretreated with 1 mg/kg indomethacin. Data represent the means ± S.E.M. of indicated number of animals. Statistical significance: $^{*}p < 0.05$ vs. sensitized control using Dunnett's multiple range test.

leukotrienes and seratrodast decreased the level of thromboxane $B_2$ in lungs, although these decreases were not statistically significant (Fig. 5).

Pranlukast dose-dependently inhibited the increase in airway resistance when administered orally at doses of 0.3–30 mg/kg. The inhibitory effect was significant at doses of 3 mg/kg or greater, and the $ED_{50}$ value was 3.9 mg/kg. Increasing the dose of pranlukast to 30 mg/kg did not increase the degree of inhibition; the degrees of inhibition at doses of 10 and 30 mg/kg were 68% and 66%, respectively (Fig. 6). Seratrodast also dose-dependently inhibited increased airway resistance when administered orally at doses from 0.3 to 10 mg/kg, and the degrees of inhibition at doses of 3 and 10 mg/kg were 62% and 63%, indicating that the maximum inhibitory action was achieved. The inhibitory effect was significant at doses of 1 mg/kg and more, and the $ED_{50}$ value was 2.1 mg/kg (Fig. 7). In both cases, the inhibitory effects on increased airway resistance induced by antigen challenge peaked at the highest doses of the compounds that were used, indicating that the maximal inhibition of the single receptor antagonist given alone did not approach the effectiveness

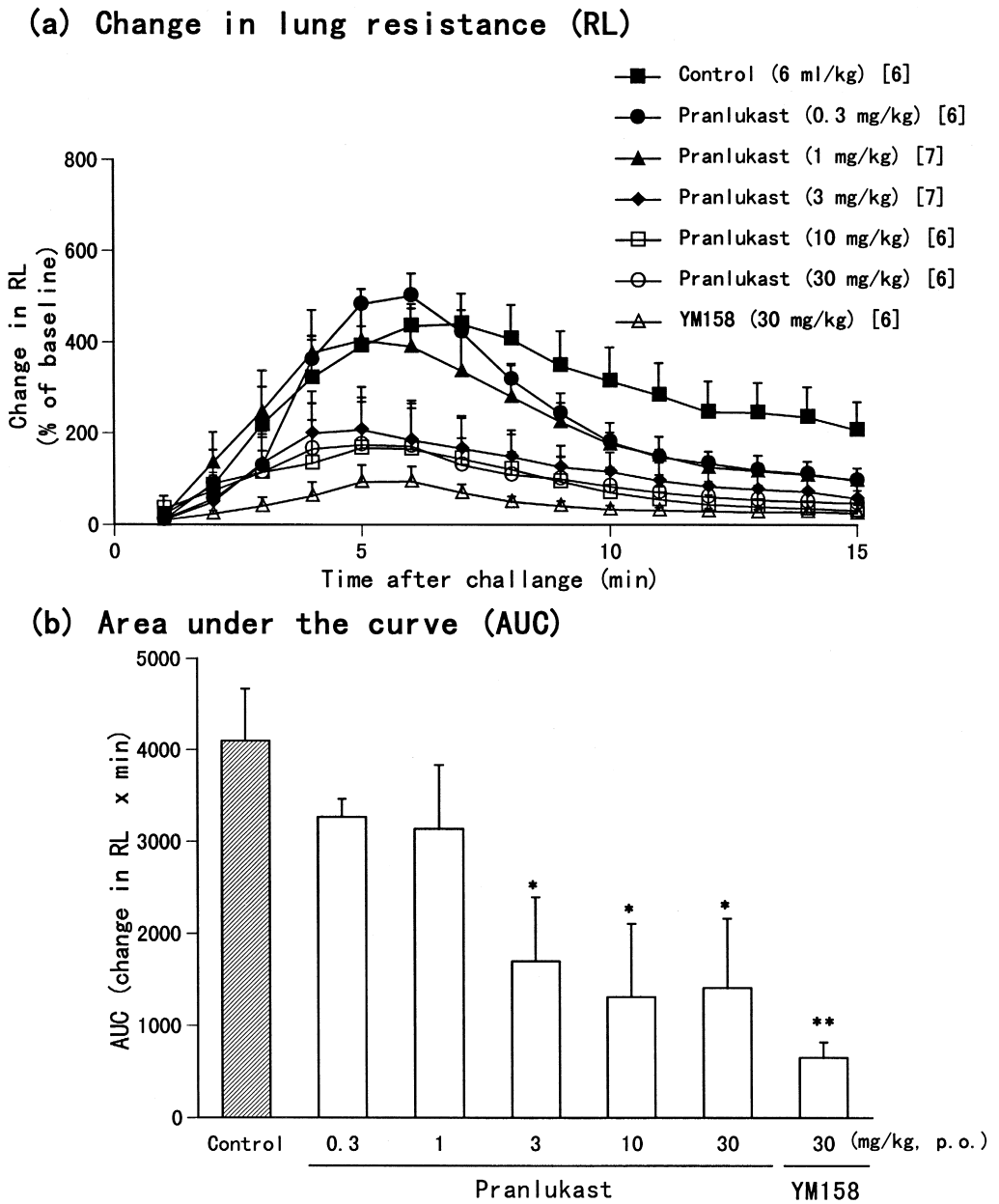


Fig. 6. Dose dependency of pranlukast effects on increased airway resistance (RL) induced by intravenous injected ovalbumin in actively sensitized guinea pigs pretreated with 1 mg/kg indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. sensitized control using Dunnett's multiple range test.

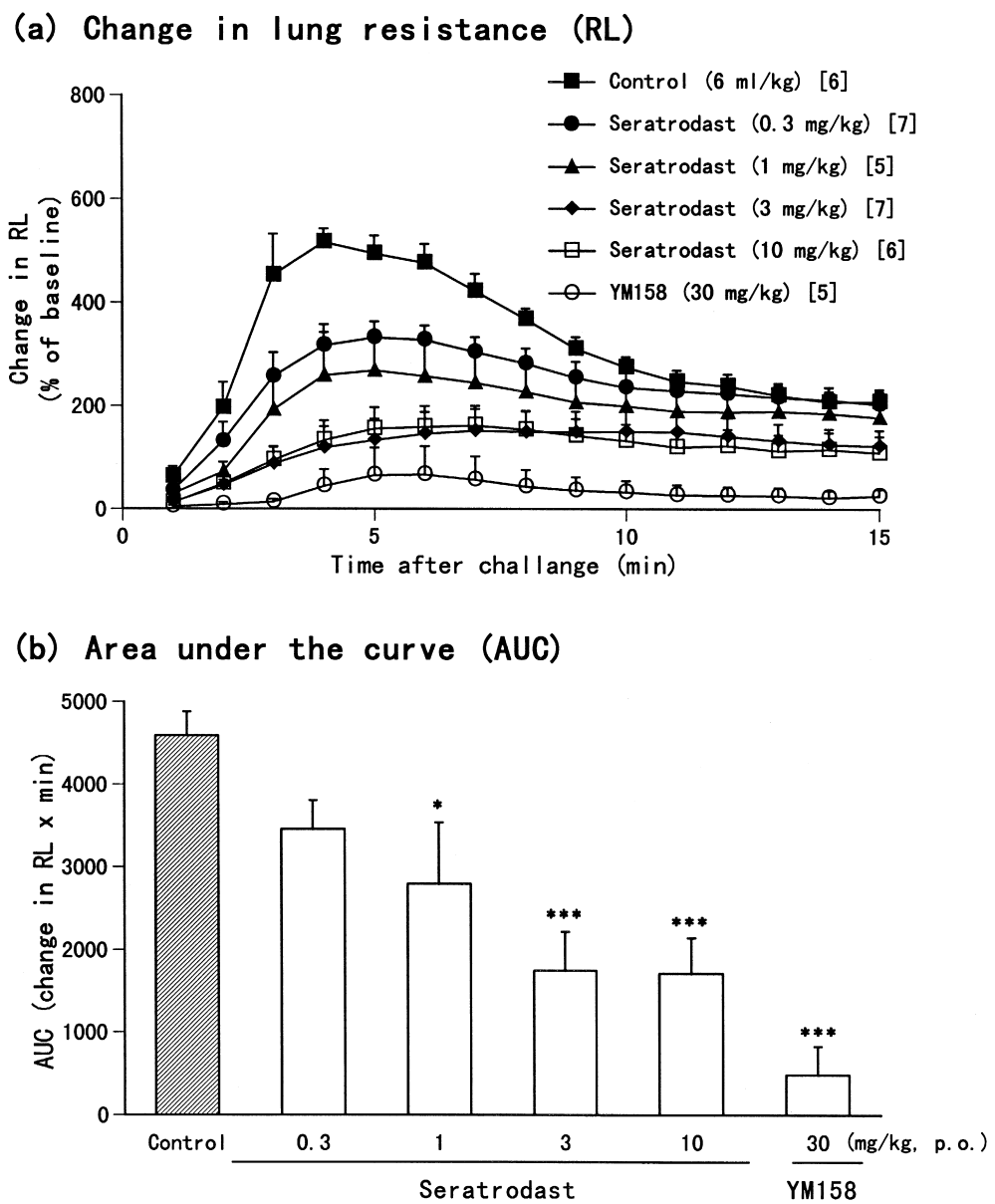


Fig. 7. Dose dependency of seratrodast effects on increased airway resistance (RL) induced by intravenous injected ovalbumin in actively sensitized guinea pigs pretreated with 1 mg/kg indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{*}p < 0.05$ and $^{***}p < 0.001$ vs. sensitized control using Dunnett's multiple range test.

of YM158 at a dose of 30 mg/kg (84%: Fig. 6; 89%: Fig. 7). YM158 at doses of 3, 10 and 30 mg/kg also suppressed increased airway resistance dose-dependently. This suppression was statistically significant at doses of 10 mg/kg or greater, with $ED_{50}$ value of 8.3 mg/kg (Fig. 8).

## 4. Discussion

The degree of contribution of cysteinyl-leukotrienes and thromboxane $A_2$ to antigen-induced increased airway resistance can be altered by indomethacin pretreatment in passively sensitized guinea pigs (Arakida et al., 1999). In the present study, experimental conditions, which more closely resemble clinical asthma, namely actively sensitized guinea pigs, were used to assess this technique. Although both histamine and catecholamines were likely modulate allergic airway responses in human subjects, endogenous histamine and β-stimulant were blocked in this study to clarify the lipid mediator-related components of asthmatic responses. Therefore, this experimental model has some differences from human asthma, and this model is still one of the experimental models of anaphylaxis. However, the amounts of lipid mediators in lung tissue

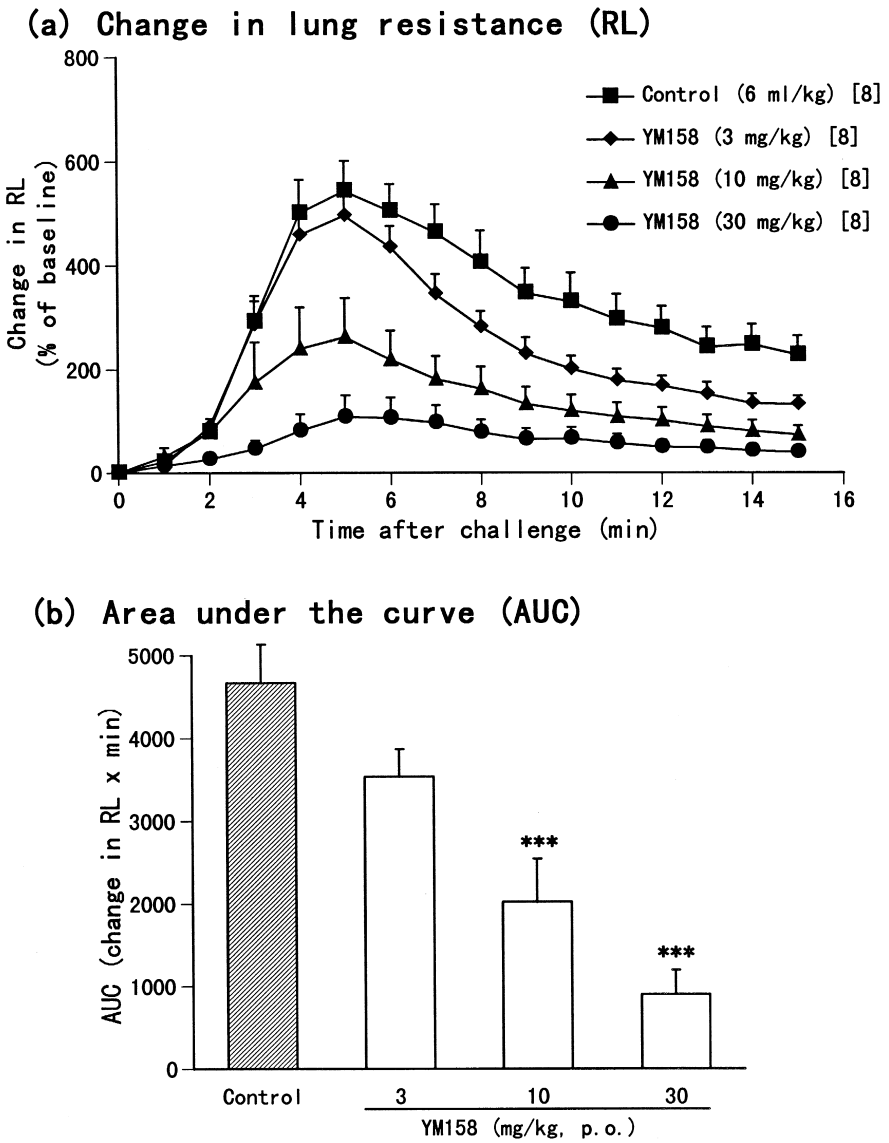


Fig. 8. Dose dependency of YM158 effects on increased airway resistance (RL) induced by intravenous injected ovalbumin in actively sensitized guinea pigs pretreated with 1 mg/kg indomethacin. Results are expressed as (a) the time course of the change in resistance and (b) as the area under the time–response curve (AUC). Data represent the means ± S.E.M. of the indicated number of animals (in brackets). Statistical significance: $^{***}p < 0.001$ vs. sensitized control using Dunnett's multiple range test.

from challenged, actively immunized guinea pigs could be controlled by intravenous injection of indomethacin before antigen challenge. These findings can be summarized as follows: (1) thromboxane $A_2$ is the predominant mediator without indomethacin pretreatment, (2) cysteinyl-leukotrienes are the predominant mediators after pretreatment with 5 mg/kg indomethacin, and (3) both cysteinyl-leukotrienes and thromboxane $A_2$ equally participate after pretreatment with 1 mg/kg indomethacin. Thus, different degrees of contribution of cysteinyl-leukotrienes and thromboxane $A_2$, which are observed clinically, can be reproduced in actively sensitized animals.

It is interesting that the longer-lasting, more sustained increases in airway resistance in the animals treated with 5-mg/kg indomethacin (Fig. 3), a situation that gives a predominant leukotriene-mediated response, in comparison with the responses observed in untreated animals (Fig. 2) where thromboxane $A_2$ is the main bronchoconstrictive mediator as suggested by the inhibition by seratrodast. In addition, the peak increase in airway resistance is more rapid in animals not treated with indomethacin (Fig. 2) when compared with those mediated by leukotriene $D_4$ (Fig. 3). These results suggest that cysteinyl-leukotrienes are released more slowly than thromboxane $A_2$ following antigen challenge.

The degree of contribution of leukotriene $D_4$ and thromboxane $A_2$ varies in individual asthmatic patients, indicated by the observation that antagonism of only one of these mediator types produces only moderate improvement in approximately 50% of asthmatic cases. Moreover,

leukotriene $D_4$ receptor antagonists are more effective in pathologic conditions where leukotriene $D_4$ is the main mediator, such as aspirin-induced asthma, while some patients are more sensitive to thromboxane $A_2$. Therefore, it is likely that any given patient may be grouped as a type similar to one of the groups reproduced by indomethacin treatment in the present animal model. If asthmatic patients may be roughly classified into two types, in which either leukotriene $D_4$ or thromboxane $A_2$ is primarily responsible for asthmatic response, YM158 is expected to have therapeutic effects for both types of patients. More importantly, a new drug's effects can be assessed under conditions in which the participation of the major mediators is controlled, which will be quite useful in predicting the clinical effectiveness of the drug.

In this study, the inhibitory effects of lipid mediator antagonists were evaluated using 30 mg/kg of pranlukast and 10 mg/kg of seratrodast. These doses are sufficient to exert a complete antagonism, as previously assessed by agonist-induced bronchoconstriction in guinea pigs (Arakida et al., 2000). While pranlukast and seratrodast only inhibit the antigen-induced increased airway resistance elicited by their respective mediators, YM158 is an effective treatment regardless of the degrees of participation of leukotriene $D_4$ or thromboxane $A_2$. This indicates that YM158 is effective in both leukotriene $D_4$-predominant and thromboxane $A_2$-predominant types of asthma. Moreover, under conditions, which both leukotriene $D_4$ and thromboxane $A_2$ participate equally in eliciting an asthmatic response, YM158 administered alone, and pranlukast and seratrodast administered in combination, exhibited much stronger suppression on increased airway resistance than could be achieved by pranlukast or seratrodast given alone. The $ED_{50}$ values of the selective receptor antagonists, as indicator of potency, were 3.9 mg/kg for pranlukast and 2.1 mg/kg for seratrodast when leukotriene $D_4$ and thromboxane $A_2$ participate equally. These $ED_{50}$ values were more than 10-times less potent than the $ED_{50}$ values these antagonists exhibit when inhibiting asthmatic responses elicited by only their respective lipid mediator. In contrast, $ED_{50}$ values for YM158 inhibition of antigen-induced increased airway resistance was 8.3 mg/kg. This value was almost identical to the $ED_{50}$ values for leukotriene $D_4$- or U46619-induced increases in airway resistances, reported as 8.6 and 14 mg/kg, respectively (Arakida et al., 2000).

Additionally, leukotriene $D_4$ and thromboxane $A_2$ were reported to have not only direct, but also indirect roles in several asthmatic responses. For example, leukotriene $D_4$- or platelet activating factor-induced asthmatic responses were inhibited by thromboxane $A_2$ receptor antagonists or synthetase inhibitors (Sakurai et al., 1994; Aizawa et al., 1996), and thromboxane $A_2$-analogue-induced responses were also reported to be partly mediated by cysteinyl-leukotrienes and platelet activating factor (Kawikova et al., 1996). From our study, the direct role of platelet activating factor in the pathogenesis of asthma is suggested by the results that the inhibition by leukotriene $D_4$ and thromboxane $A_2$ receptor antagonists were not complete in spite that histamine was also blocked. Differences in the efficacy of these mediators, such as the observation that leukotriene $D_4$ is a potent inducer of vascular permeability (Peck et al., 1981; Rinkema et al., 1984), while thromboxane $A_2$ is thought to have direct roles in airway hyperresponsiveness (Jones et al., 1992; Nagai et al., 1993; Fujimura et al., 1991), have also been reported. Thus, the antagonistic activity of YM158 against both leukotriene $D_4$ and thromboxane $A_2$ might be the reason why only YM158's $ED_{50}$ values were almost the same against antigen- and agonist-induced responses. This indicates that the dual receptor antagonism of YM158 will be useful for treating asthmatic conditions that arise from the complicated interplay among the various types of lipid mediators. If similar mechanisms exist in human subjects, then combined antagonism of $LTD_4$ and $TXA_2$ may prove more effective than antagonism of either of these receptor types in asthmatic subjects.

When leukotriene $D_4$ and thromboxane $A_2$ participate equally in eliciting an asthmatic response, intra-pulmonary cysteinyl-leukotrienes and thromboxane $B_2$ levels decreased after administration of the corresponding antagonists, suggesting that an autocrine-like stimulation of lipid mediator production in lungs may occur during induction of antigen-induced airway resistance increases. Although the roles of secondarily produced thromboxane $A_2$ in response to leukotriene $D_4$ and platelet-activating factor has previously been reported (Aizawa et al., 1996; Sakurai et al., 1994), the results of the present study interestingly suggest the presence of additional interactions among these lipid mediators such as roles of endogenous leukotriene $D_4$ and thromboxane $A_2$ induced by an autocrine stimulation. However, a statistically significant decrease was observed only for thromboxane $B_2$ when YM158 was administered, and more study is needed to determine the interactions among these mediators. Nonetheless, it is clear from the present results that the inhibition of intra-pulmonary mediators is greater if both receptor types are antagonized.

In summary, this study presented a system for the evaluation of anti-asthmatic drugs under different conditions, in which the participation of the major mediators is controlled. Briefly, the different degree of contribution of leukotriene $D_4$ and thromboxane $A_2$ could be reproduced in this study system. If asthmatic patients were roughly classified into some different lipid mediator related types, these created systems might correspond to the variety of the main related lipid mediators among the asthmatic patients. Thus, this technique may be useful in predicting the clinical effectiveness because it is important to evaluate a candidate compound under some different conditions that reflect the variety of asthmatic patients. In this system, the launched anti-asthmatic drugs, pranlukast and seratrodast, exerted therapeutic effects only on corresponding

mediator-related conditions, and the dual antagonist YM158 exhibited therapeutic effects regardless whether the major participating lipid mediator was leukotriene $D_4$ or thromboxane $A_2$, or whether neither was predominant. These results from our experiments using actively sensitized guinea pigs suggest that YM158, an orally active dual antagonist for leukotriene $D_4$ and thromboxane $A_2$ receptors, is expected to become a novel, anti-asthmatic agent to offer relief to a wide range of asthmatic patients.

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