FULL PAPER

Laboratory Animal Science

JTE-852, a novel spleen tyrosine kinase inhibitor, blocks antigen-induced allergic reactions in rats

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ABSTRACT. Conventional clinical treatments for allergy management remain suboptimal; new, orally available medications that improve a wide range of allergic signs have been desired. We previously demonstrated that JTE-852, a novel spleen tyrosine kinase inhibitor, potently and simultaneously suppresses secretion of granule contents, arachidonate metabolites, and cytokines from mast cells stimulated by immunoglobulin E-crosslinking. In the present study, we investigated the effects of JTE-852 in four rat models (sneezing, rhinorrhea, airway constriction, and airway inflammation) as representatives of allergy models. Rats were sensitized and challenged with antigen. Allergic reactions developed after challenge were detected. JTE-852 and current anti-allergic drugs (ketotifen, pranlukast, and prednisolone) were administered orally before challenge. JTE-852 showed significant blocking effects on antigen-induced allergic reactions in all models, indicating that JTE-852 in oral dosage form would improve a wide range of allergic signs. The current anti-allergic drugs, on the other hand, failed to display significant suppression in several models. Because JTE-852 suppresses the secretion of all three groups of allergic mediators from mast cells, it would be capable of targeting signs that current drugs cannot sufficiently relieve. We anticipate JTE-852 to be a promising new anti-allergic drug that is potentially more effective than conventional drugs.

KEY WORDS: allergy, disease model, JTE-852, rat, spleen tyrosine kinase

Allergic diseases including allergic rhinitis and asthma occur as the result of type I allergic reactions, which develop according to a known pattern. First, antigen-specific immunoglobulin E (IgE) is produced by B cells and binds to its receptor (FcεRI) on mast cells, thereby sensitizing these cells to an antigen. Successive challenges by the same antigen result in IgE-crosslinking, leading to intracellular signal transductions that stimulate the secretion of allergic mediators (including granule contents, arachidonate metabolites, and cytokines) from the mast cells. These mediators subsequently cause symptoms or signs such as vasodilation, vascular hyperpermeability, edema, mucus hypersecretion, smooth muscle contraction, pain, pruritus, cell infiltration, and tissue destruction etc [1, 7, 12, 16, 22].

These three groups of mediators—granule contents, arachidonate metabolites, and cytokines—are key players in type I allergic reactions. Indeed, many conventional drugs clinically used for allergic diseases target these mediators. Histamine H1-receptor antagonists and cysteinyl leukotriene 1 receptor antagonists are the examples [12, 17]. However, most conventional drugs block only a single mediator, requiring the combined administration of several different drugs to treat the entire spectrum of signs [2, 17]. While steroidal drugs can provide relief from a wide range of signs due to their potent and broad anti-inflammatory effects (partly by blocking cytokine production) [1, 12, 18], these drugs are associated with poor compliance due to patients' aversion to adverse effects and the need for topical application of some medications [5, 23]. Hence, the allergy treatment options that are currently available are not optimal, leaving a clear unmet need for new, orally available anti-allergic drugs that are capable of simultaneously suppressing all three groups of mediators.

In a previous study, we identified JTE-852 as a novel spleen tyrosine kinase (Syk) inhibitor; and characterized its pharmacological profile and mechanism of action [11]. JTE-852 inhibited the kinase activity of Syk, with an inhibition constant (Ki) value of 0.4 nM and in an adenosine 5′-triphosphate (ATP)-competitive fashion. Moreover, JTE-852 blocked the secretion of histamine, leukotriene $C_4/D_4/E_4$ (LTC$_4$/D$_4$/E$_4$), and interleukin-13 (IL-13) from mast cells that had been stimulated by IgE-crosslinking; its 50% inhibitory concentration (IC$_{50}$) values were in 34–64 nM range. It was also confirmed that oral JTE-852

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attenuated passive cutaneous anaphylaxis (PCA) reactions in rats, with a 50% effective dose (ED$_{50}$) value of approximately 3 mg/kg. These results demonstrated that JTE-852 potently and simultaneously suppresses the secretion of granule contents, arachidonate metabolites, and cytokines; thereby preventing subsequent *in vivo* reactions caused by these mediators. Therefore, JTE-852 shows potential for becoming a new anti-allergic drug capable of addressing the currently unmet needs in allergic diseases. In the present study, we investigated the effects of JTE-852 in animal models of allergic diseases to verify the potential.

MATERIALS AND METHODS

Articles and vehicle

JTE-852 was synthesized and cysteinyl leukotriene-1 receptor antagonist pranlukast was extracted from ONON® capsules (Ono Pharmaceutical Co., Ltd., Osaka, Japan) in the Central Pharmaceutical Research Institute of Japan Tobacco, Inc. (Osaka, Japan). The chemical structure of JTE-852 has been previously reported [11]. Histamine H1-receptor antagonist ketotifen and steroidal drug prednisolone were purchased from Sigma-Aldrich, Co. (St. Louis, MO, U.S.A.). Metolose® SM-1500 (methylcellulose) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and its 0.5% aqueous solution was used as a vehicle.

Animals

Brown-Norway (BN) rats and Sprague-Dawley (SD) rats were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). Husbandry conditions were maintained as follows: temperature of 23.0 ± 3.0°C, humidity of 55 ± 15%, 12 hr lighting time (lights on at 8 a.m., lights off at 8 p.m.), CRF-1 pelletized diet (Oriental Yeast Co., Ltd., Tokyo, Japan) supplied *ad libitum*, and UV-irradiated tap water supplied *ad libitum*. Animals were treated in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals*. Animal study protocols were approved by the Institutional Animal Care and Use Committee of the Central Pharmaceutical Research Institute, Japan Tobacco Inc.

Sneezing model

Toluene 2,4-diisocyanate (TDI) was purchased from Honeywell (Morris Plains, NJ, U.S.A.) and used as a hapten. Six-week-old male BN rats were intranasally dosed with 10% TDI solution at a volume of 5 μl/each nose on Day 1. This treatment was repeated on Days 2–5 and Days 8–12; a total of 10 administrations were performed to achieve sensitization. On Day 22, the sensitized rats were weighed and allocated to 6 groups with 14 rats in each group; body weights were balanced between groups. On the next day, Day 23, JTE-852 was administered orally to the rats in a suspension of vehicle at a volume of 5 ml/kg (1, 3, 10 and 30 mg/kg). After 1 hr, 5% TDI solution was administered intranasally to rats in the test groups at a volume of 5 μl/each nose. Ethyl acetate was administered to rats in the sham group in the same manner. Immediately after this challenge, sneezing was counted for 30 min in a blind fashion.

Rhinorrhea model

Albumin from chicken egg white (OVA) was purchased from Sigma-Aldrich, Co. and used as an antigen. Imject® Alum (40 mg/ml of aluminum hydroxide and magnesium hydroxide) was purchased from Pierce Biotechnology, Inc. (Rockford, IL, U.S.A.) and used as an adjuvant. Chicago Sky Blue 6B dye was purchased from Alfa Aesar (Heysham, U.K.).

Seven-week-old male SD rats were administered intraperitoneally with OVA/Alum mixture (a saline-based suspension consisting of 10 μg/ml OVA and 10 mg/ml Alum) at a volume of 1 ml for 3 consecutive days to achieve sensitization. Fifteen days after the first intraperitoneal (IP) injection, the sensitized rats were weighed and allocated to 5 groups with 12 rats in each group, with balanced body weights across groups. Pranlukast and prednisolone were used as reference articles, and ketotifen was used as the positive control article. The four articles were suspended in vehicle and administered orally to rats at a dose of 30 mg/kg (5 ml/kg). After 1 hr, the rats were anesthetized by intravenous (IV) injection of pentobarbital, and 40 mg/ml Chicago Sky Blue 6B dye was injected to the tail vein at a volume of 3 ml/kg. Five min after dye injection, the trachea of each rat was exteriorized and cannulated in the direction of the nasal cavities. Perfusion was then performed for 20 min to wash the rats' nasal cavities by infusing warmed saline with the EICOM EP-60 syringe pump (Eicom Corp., Kyoto, Japan) at a flow rate of 0.1 ml/min; fluid drained from the nares was discarded. Next, the nasal cavities of rats were perfused for 10 min in the same way, and the drained fluid was collected as the 0-min sample. Subsequently, perfusion with 6 mg/ml OVA solution was performed for 10 min, and the drained fluid was collected as the 10-min sample. After centrifugation of the samples, the absorbance of the supernatants at a wavelength of 620 nm was measured with the VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA, U.S.A.).

Concentration of dye in each supernatant was determined from the standard curve which was created with Chicago Sky Blue 6B and saline. Dye leakage during the 10-min challenge was calculated by subtracting 0-min values from 10-min values.

Airway constriction model

Six-week-old male BN rats were given intraperitoneally OVA/Alum mixture, a saline-based suspension consisting of 10 μg/ml OVA and 10 mg/ml Alum, at a volume of 1 ml for 3 consecutive days to achieve sensitization. Ten days after the first IP injection, the sensitized rats were weighed and allocated to 6 groups with 8 rats in each group so as to balance the body weights. Ketotifen and prednisolone were used as reference articles, and pranlukast was used as the positive control article. On the next day (11 days after the first IP injection), the four articles were suspended in vehicle and administered orally to the rats at a dose of 30 mg/kg (5 ml/kg). After 1 hr, rats were given 2% OVA solution by inhalation for 15 min with the NE-U17 ultrasonic nebulizer (OMRON

Corp., Kyoto, Japan). In the sham group, saline was inhaled in the same manner. One hour after inhalation, enhanced pause (Penh) was measured [19]. The PLY3215 whole body plethysmograph (Buxco Electronics, Inc., Wilmington, NC, U.S.A.), a device for quantifying respiratory function in unrestrained animals, was used for the measurement of Penh.

Airway inflammation model

OVA/Alum mixture, a saline-based suspension consisting of 10 mg/ml OVA and 10 mg/ml Alum, was intraperitoneally injected to six-week-old male BN rats at a volume of 1 ml for 3 consecutive days to achieve sensitization. Two weeks after the first IP injection, the sensitized rats were weighed and allocated to 6 groups with 12 rats in each group, with balanced body weights across the groups. After group assignment, the rats were orally dosed with the four articles suspended in vehicle at a dose of 30 mg/kg (5 ml/kg). After 1 hr, 0.5% OVA solution was administered to the rats by inhalation for 15 min with the NE-U17. In the sham group, saline was inhaled in the same way. Twenty-four hours after inhalation, the challenged rats were euthanized by exsanguination under anesthesia with pentobarbital. The trachea of each rat was exteriorized and cannulated in the direction of the bronchi, and subsequently, bronchoalveolar lavage fluids (BALFs) were collected. Briefly, 2.5 ml of phosphate-buffered saline (PBS) containing 2 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% bovine serum albumin (BSA) were gently delivered into the airways and alveoli, and then recovered; this process was repeated 5 more times for a total of 6 lavages.

The concentrations of total leukocytes in the BALFs were measured with the ADVIA120 hematology analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.). BALF smears were also prepared with the Cytospin 2 cytocentrifuge (Thermo Fisher Scientific K.K., Yokohama, Japan), and Wright-Giemsa stains were performed with the Diff-Quik stain[TM] kit (Funakoshi Co., Ltd., Tokyo, Japan). Eosinophils, neutrophils, and mononuclear cells on the smears were counted in a blind manner. The remaining BALFs were centrifuged, and concentrations of IL-13 in the supernatants were determined by Rat IL-13 ELISA kit (Biosource International, Inc., Camarillo, CA, U.S.A.).

The numbers of total leukocytes and amounts of IL-13 were calculated by multiplying concentration by BALF volume. The ratio of eosinophils and neutrophils on the smears were also calculated, and the number of each type of cell was then calculated by multiplying the ratio by the number of total leukocytes.

Statistical analyses

For body weights at group allocation in each model, significance tests were performed as follows: Bartlett's test was performed to confirm homogeneity of variance, and one-way analysis of variance (ANOVA) was then performed to confirm that there were no significant differences between all groups. A two-tailed significance level of 5% was used.

For readouts of allergic reactions in each model, significance tests were performed using the following procedures: for comparisons between two groups, homogeneity of variance was tested with an *F*-test; subsequently, Student's *t*-test or Aspin-Welch's *t*-test was performed for homoscedastic data or heteroscedastic data, respectively. For comparisons between three or more groups, homogeneity of variance was tested with Bartlett's test; subsequently, the Steel test was performed because the data were judged to be heteroscedastic. A two-tailed significance level of 5% was used. SAS System version 8.2 and SAS Preclinical Package version 5.0 (SAS Institute Japan Inc., Tokyo, Japan) were used for all statistical analyses.

RESULTS

Effect of JTE-852 on sneezing

The result of our rat model of sneezing is shown in Fig. 1. Approximately 100 counts of sneezing were observed in the vehicle group, which was significantly higher than the sham group. JTE-852 suppressed sneezing counts in a dose-related manner, and its suppressive effect was significant at doses of 3, 10 and 30 mg/kg.

Effect of JTE-852 on rhinorrhea

In our rat model of rhinorrhea, we assessed JTE-852 in addition to three anti-allergic drugs: ketotifen, pranlukast, and prednisolone. As shown in Fig. 2, JTE-852 at a dose of 30 mg/kg significantly attenuated the dye leakage that was observed in the vehicle group. This effect was comparable to that of ketotifen and prednisolone, both of which exhibited significant suppression of dye leakage at 30 mg/kg. Pranlukast, however, did not show a significant suppression of dye leakage.

Effect of JTE-852 on airway constriction

The result of our rat model of airway constriction is shown in Fig. 3. Penh value in the vehicle group was significantly higher than that in the sham group. JTE-852 significantly and almost completely blocked this increase in Penh. Pranlukast also significantly attenuated the increase in Penh; however, the efficacy was lower than that of JTE-852. Prednisolone suppressed the increase in Penh, but without statistical significance. Ketotifen had a negligible effect on Penh, even at a dose of 30 mg/kg.

Effect of JTE-852 on airway inflammation

In this model, we examined the effects of JTE-852 and three standard anti-allergic drugs on antigen-induced airway inflammation, which was defined as inflammatory cell infiltrations and cytokine secretion in BALFs. The total leukocytes, eosinophils, neutrophils, and IL-13 in BALFs were significantly higher in the vehicle group than in the sham group (Fig. 4), confirming the presence of antigen induced airway inflammation. JTE-852 significantly and almost completely blocked the increase
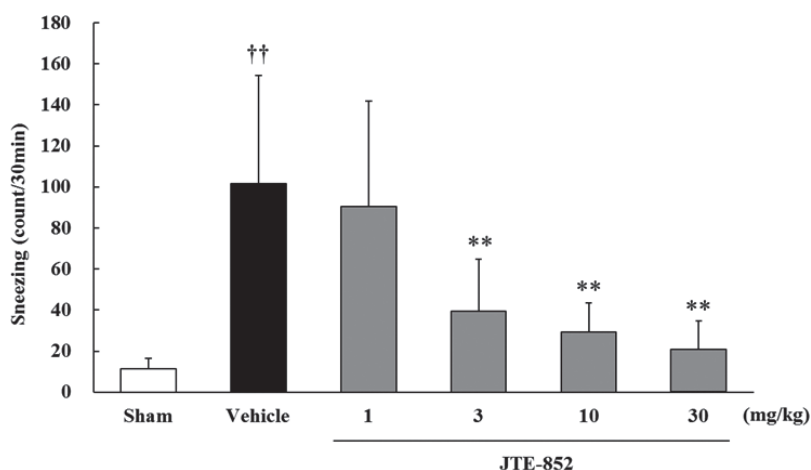
Fig. 1. Effect of JTE-852 in sneezing model. TDI was administered intranasally to Brown-Norway rats at a volume of 5 μl/each nose once daily from Day 1 to Day 5 and from Day 8 to Day 12. On Day 23, the sensitized rats were challenged by intranasal administration of TDI. Ethyl acetate was administered to rats in the sham group. Immediately after challenge, the number of sneezes in 30 min was counted in a blind manner. JTE-852 was administered orally 1 hr before challenge. Data express the mean + standard deviation; n=14 rats per group. $^{\dagger\dagger}P<0.01$ vs. sham group (Aspin-Welch's t-test); $**P<0.01$ vs. vehicle group (Steel test). TDI, toluene 2,4-diisocyanate.
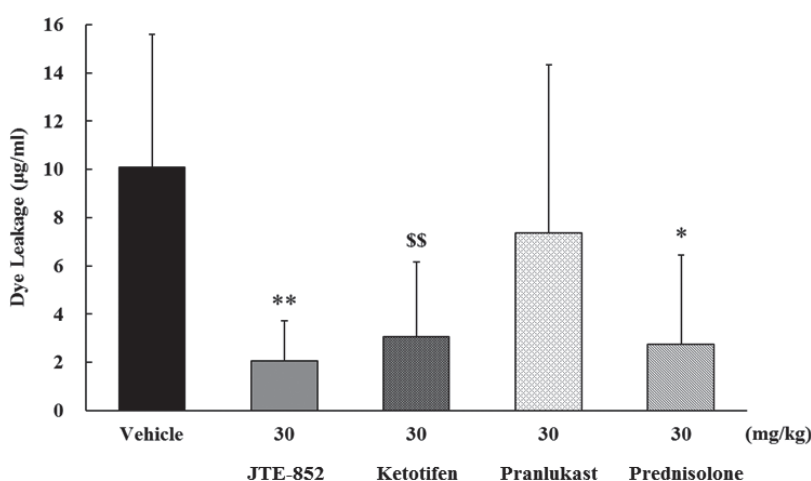

Fig. 2. Effects of JTE-852 and anti-allergic drugs in rhinorrhea model. Sprague-Dawley rats were sensitized by intraperitoneal injections of OVA/Alum for 3 consecutive days. Fifteen days after the first intraperitoneal injection, the rats were given intravenous injection of Chicago Sky Blue 6B dye and were then challenged by intranasal perfusion with OVA for 10 min. The fluid drained from rat nares was collected and absorbance of the dye was then measured. All articles were administered orally approximately 2 hr before challenge. Data express the mean + standard deviation; n=12 rats per group. $^{\$\$}P<0.01$ vs. vehicle group (Student's t-test); $*P<0.05$; $**P<0.01$ vs. vehicle group (Steel test). OVA, albumin from chicken egg white.

in cells and IL-13. Prednisolone also blocked the allergic responses in similar manner to JTE-852. Pranlukast showed significant suppression in total leukocytes and eosinophils only; however, these effects were smaller than those of JTE-852 or prednisolone. Ketotifen did not display a significant suppressive effect.

DISCUSSION

In our previous study, we demonstrated that the Syk inhibitor JTE-852 potently and simultaneously blocks the secretion of granule contents, arachidonate metabolites, and cytokines from mast cells stimulated by IgE-crosslinking [11]. The aim of our present study was to further define the preclinical properties of JTE-852 in animal models of allergic diseases. A total of four models, two of allergic rhinitis and two of asthma, were used to represent allergic disease models. JTE-852, at doses of 1, 3, 10 and 30 mg/kg, showed dose-related attenuation of PCA reaction in our prior study, achieving almost complete attenuation at a dose of 30 mg/kg [11]. In the present study, we revisited this dose-related effect of JTE-852 in the sneezing model, using the same range of
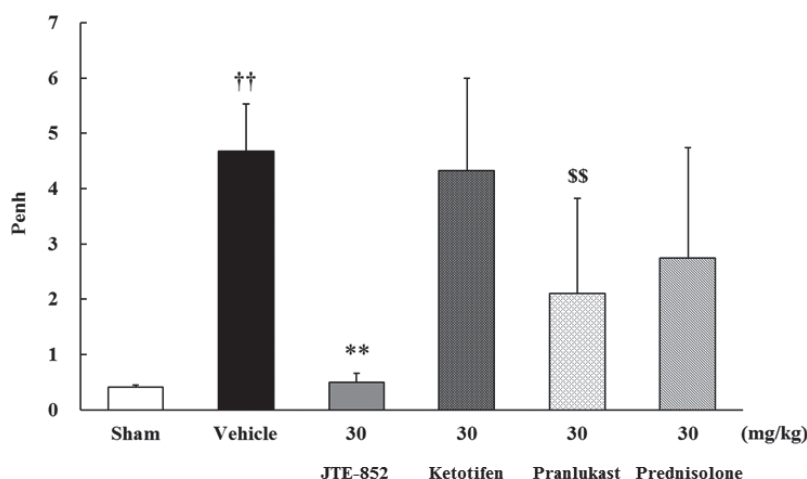
Fig. 3. Effects of JTE-852 and anti-allergic drugs in airway constriction model. Brown-Norway rats were sensitized by intraperitoneal injection of OVA/Alum for 3 consecutive days. Eleven days after the first intraperitoneal injection, the rats were challenged by inhalation of OVA. In the sham group, rats inhaled saline. After 1 hr, Penh of each rat was measured. All articles were administered orally 1 hr before challenge. Data express the mean + standard deviation; n=8 rats per group. $^{††}P<0.01$ vs. sham group (Aspin-Welch's t-test); $^{$$}P<0.01$ vs. vehicle group (Student's t-test); $^{**}P<0.01$ vs. vehicle group (Steel test). OVA, albumin from chicken egg white; Penh, enhanced pause.

doses. In the remaining three models, 30 mg/kg was used as a dose at which JTE-852 can fully perform its mechanism of action, Syk inhibition. We considered the PCA model as the most appropriate model to evaluate Syk inhibition of JTE-852 *in vivo*, and selected 30 mg/kg as the dose because JTE-852 at 30 mg/kg attenuated the PCA reaction almost completely. Notable side effects were not observed even at a dose of 30 mg/kg.

Allergic rhinitis is a type I allergic disease on the nasal mucosa, and its main symptoms are characterized by sneezing, rhinorrhea, itchy nose, and nasal congestion [12, 20]. These symptoms are caused by the granule contents, arachidonate metabolites, and cytokines that are secreted from nasal mast cells activated by IgE-crosslinking in response to reinvasion of antigens such as dust, mites, and pollen [2]. In clinical practices, histamine H1-receptor antagonists, cysteinyl leukotriene 1 receptor antagonists, dual antagonists of thromboxane A_2 receptor and prostaglandin D_2 receptor (e.g., ramatroban), and steroidal drugs are used for the treatment of allergic rhinitis [10, 12].

As described above, sneezing is one of the main symptoms of allergic rhinitis. Sneezing is a reflexive response caused by exposure of histamine to trigeminal nerve, the histamine is secreted from mast cells stimulated by IgE-crosslinking and/or neuropeptides such as substance P [20]. In our rat model of sneezing, JTE-852 displayed the preventive effect on antigen-induced sneezing response. Rhinorrhea is another primary symptom of allergic rhinitis. Histamine secretion from IgE-crosslinked mast cells leads to increased vascular permeability in the nasal mucosa, resulting in rhinorrhea. It is thus reasonable that histamine H1-receptor antagonists have traditionally been used to block this symptom [8, 12, 20, 21]. In our rhinorrhea model, we observed the suppressive effect of JTE-852 on antigen-induced rhinorrhea reaction. The results of these two models reveal that JTE-852 is effective in disease model of allergic rhinitis. To our knowledge, this is the first report demonstrating the suppressive effect of Syk inhibitor in animal models of allergic rhinitis. JTE-852, therefore, shows promise as a useful drug for alleviating symptoms and signs of allergic rhinitis.

Asthma is a respiratory disease characterized by airway constriction/obstruction, chronic airway inflammation, and airway hyperreactivity [4, 23]. Antigen-specific IgE and pulmonary mast cells are detected in asthma patients [1, 3]; therefore, it is suggested that many of the above airway signs, if not all, are caused by mediators secreted from mast cells with IgE-crosslinking [1, 12, 23]. In fact, cysteinyl leukotriene 1 receptor antagonists and mast cell stabilizers such as disodium cromoglycate are clinically used for the treatment of asthma [1, 6]. Omalizumab (XOLAIR®), a humanized neutralizing antibody against IgE, is also used in the clinical setting [12, 23]. Preclinical animal studies have shown that Fcε receptor-deficient mice exhibited deletion of mediator secretion from IgE-crosslinked mast cells and remarkable reductions in allergic airway inflammation [13]. Collectively, the IgE-crosslinking/mast cell/mediator axis plays important roles in the pathogenesis of asthma. As described above, airway constriction and obstruction are primary features of asthma. The result of our animal model showed that JTE-852 blocked antigen-induced airway constriction.

Airway inflammation is also one of the characteristic signs of asthma. Inflammation arises from interactions between cytokines and inflammatory cells: cytokines secreted at inflammatory loci attract inflammatory cells that subsequently secrete additional mediators including more cytokines, resulting in aggravation and perpetuation of inflammation [12, 18, 20]. IL-5 and eosinophils are one of the examples of this exacerbation cycle [1, 20]. In our airway inflammation model, JTE-852 effectively suppressed the antigen-induced airway inflammation, which was determined by the presence of inflammatory cells and cytokine in BALFs. To our knowledge, this is the first report to demonstrate the suppressive effect of Syk inhibitor on cytokine secretion in BALFs in rats. The
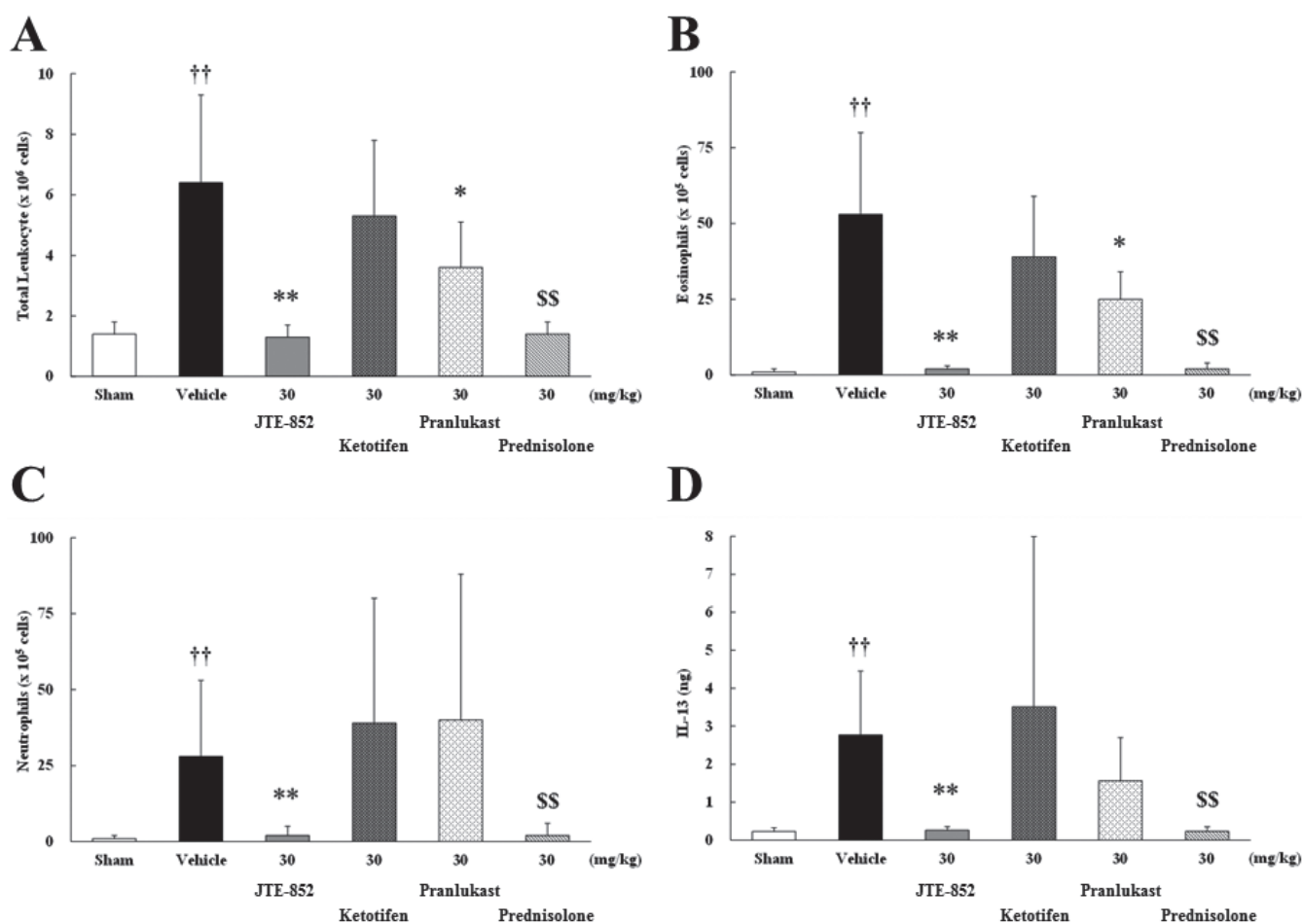
Fig. 4. Effects of JTE-852 and anti-allergic drugs in airway inflammation model. Brown-Norway rats were sensitized by intraperitoneal injection of OVA/Alum for 3 consecutive days. Two weeks after the first intraperitoneal injection, the rats were challenged by inhalation of OVA. In the sham group, rats inhaled saline. After 24 hr, BALFs were collected from the rats. The numbers of total leukocytes (A), eosinophils (B), neutrophils (C), and amount of IL-13 (D) in the BALFs were determined. All articles were administered orally 1 hr before challenge. Data express the mean + standard deviation; n=12 rats per group. $^{\dagger\dagger}P<0.01$ vs. sham group (Aspin-Welch's t-test); $^{\$\$}P<0.01$ vs. vehicle group (Aspin-Welch's t-test); $^{*}P<0.05$; $^{**}P<0.01$ vs. vehicle group (Steel test). BALF, bronchoalveolar lavage fluid; IL-13, interleukin-13; OVA, albumin from chicken egg white.

results in both asthma models show the effectiveness of JTE-852 in disease model of asthma, indicating that JTE-852 would be a promising candidate in the search for new treatments for asthma.

As we discussed in the beginning, new anti-allergic drugs that are orally available and effective against a wide spectrum of allergic signs and symptoms have been required. In this study, orally administered JTE-852 demonstrated the blocking effects in all allergic models examined. These findings indicate that JTE-852 can potentially ameliorate a wide range of allergic signs and symptoms in an oral dosage form, thereby satisfying the key requirements for new anti-allergic drugs. Although further investigations, particularly in clinical trials, are needed; we anticipate JTE-852 to be a promising new anti-allergic drug.

In addition to JTE-852, three current anti-allergic drugs—ketotifen, pranlukast, and prednisolone—were assessed in our models of rhinorrhea, airway constriction, and airway inflammation. The aim of these examinations was to compare the efficacies of each drug. We therefore selected the doses of ketotifen, pranlukast, and prednisolone at which they can fully perform their mechanisms of action, histamine antagonism, $LTC_4/D_4/E_4$ antagonism, and anti-inflammatory effects, respectively. From our background data and other previous reports, the doses were all 30 mg/kg [9, 11, 14, 15, 24]. The suppressive effects of the four articles included in this study are summarized in Table 1. The anti-allergic drugs included in this study did not significantly prevent reactions in several of the allergic models, whereas JTE-852 did exhibit significant suppression of reactions in all the models. These allergic reactions are triggered by mediators secreted from IgE-crosslinking mast cells. Syk directly associates with the IgE receptor and plays essential roles in the signal transductions induced by IgE-crosslinking and subsequent mediator secretion. The Syk inhibitor JTE-852 blocks simultaneously the secretion of mediators from mast cells with IgE-crosslinking. The suppressive effects of JTE-852 on a wide range of allergic signs and symptoms would be attributed to its capability to block the secretion of mediators. Ketotifen, pranlukast, and prednisolone, on the other hand, are drugs which antagonize a small part of the mediators (histamine, $LTC_4/D_4/E_4$,

Table 1. Suppressive effects of JTE-852 and anti-allergic drugs in disease models of allergy

Model		Suppression of reaction (%) by each article			
		JTE-852	Ketotifen	Pranlukast	Prednisolone
Sneezing		90 [a]	N.E.	N.E.	N.E.
Rhinorrhea		80 [a]	70 [a]	27 [c]	73 [b]
Airway constriction		98 [a]	8 [c]	60 [a]	45 [c]
Airway inflammation	Total leukocyte	102 [a]	22 [c]	56 [b]	100 [a]
	Eosinophil	98 [a]	27 [c]	54 [b]	98 [a]
	Neutrophil	96 [a]	−41 [c]	−44 [c]	96 [a]
	IL-13	99 [a]	−29 [c]	48 [c]	100 [a]

Rats were sensitized and challenged with antigen or hapten. Allergic reactions displayed by the rats were observed and analyzed. Percent suppression values (%) and statistical significances (a) $P<0.01$, b) $P<0.05$ or c) Not significant) at 30 mg/kg of each article are shown in the table. IL-13, interleukin-13; N.E., not examined.

and cytokines, respectively). These differences between mechanisms of action of each agent seem to lead the differences between spectrums of effect on allergic reactions. JTE-852 is therefore anticipated to be a novel anti-allergic drug with the potential for greater effectiveness than conventional drugs.

In conclusion, oral JTE-852 showed significant blocking effects in various models of allergic diseases. Our findings indicate that JTE-852 can ameliorate a wide range of allergic signs in an oral dosage form. JTE-852 is thus expected to be a promising new anti-allergic drug.

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