

Inhibitory Effect of DA-9201, an Extract of *Oryza sativa* L., on Airway Inflammation and Bronchial Hyperresponsiveness in Mouse Asthma Model

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Asthma is one of the major public health problems worldwide and the morbidity and mortality of asthma has increased in the past two decades. Accumulating data suggest that unnecessary immune responses and inflammation should be suppressed to treat asthma. The purpose of this study is to investigate the anti-asthmatic effects of DA-9201, an ethanolic extract of black rice (*Oryza sativa* L. var *japonica*), on an ovalbumin (OVA)-induced mouse model of asthma. Balb/c mice immunized with OVA were administered with DA-9201 (30, 100 or 300 mg/kg, *p.o.*) or dexamethasone (3 mg/kg, *p.o.*) and challenged with 1% aerosolized OVA for 30 min. The effects on airway inflammation, airway hyperresponsiveness (AHR), antibody profiles and cytokines were evaluated. DA-9201 treatment significantly reduced the number of eosinophils in bronchoalveolar lavage fluid (BALF) and ameliorated the AHR. Lung histological features also showed that DA-9201 reduced airway inflammation. Furthermore, DA-9201 treatment decreased IFN- γ as well as IL-4, IL-5 and IL-13 levels in the supernatant of cultured splenocytes, and suppressed the level of OVA-specific IgG, IgG2a, IgG1 and total IgE in plasma. DA-9201 showed anti-asthmatic effects by suppressing unnecessary immune responses, airway inflammation, eosinophilia, AHR and IgE level. These results suggest DA-9201 might be beneficial for the treatment of asthma.

Key words *Oryza sativa* L.; DA-9201; airway inflammation; airway hyperresponsiveness; asthma

Asthma is a clinical syndrome characterized by intermittent episodes of wheezing and coughing.¹⁾ The pathological manifestations of asthma include airway inflammation, chronic pulmonary eosinophilia, airway hyperresponsiveness, elevated plasma IgE level, *etc.*^{1–5)} Elevated Th2 cell immune responses are also observed in a lot of asthmatics and asthmatic animal models. Therefore, it has been largely accepted that unbalanced and aberrant Th2 cells play a pivotal role in the pathogenesis of asthma. Through the release of cytokines such as IL-4, IL-5 and IL-13, these cells orchestrate the recruitment and activation of mast cells and eosinophils, the primary effector cells of the allergic response.³⁾ In addition, it has been also revealed that Th1 cells secreting cytokines such as IFN- γ are also involved in asthma.^{6,7)} Thus, it seems important that unnecessary immune responses should be suppressed to treat asthma.

In the past decade, the treatment of asthma has emphasized long-term suppression of airway inflammation and relief of symptoms with quick-acting bronchodilators such as aerosolized β_2 agonists. Inhaled corticosteroids are the most effective agents available for the symptomatic control of asthma and improvement in pulmonary function. However, despite the availability of effective treatments, some of asthmatics remain poorly controlled.⁸⁾ Furthermore, corticosteroids do not consistently abrogate airway inflammation and they have potential side effects when used in escalating doses.⁴⁾ For this reason, other approaches that modulate immunologically-mediated inflammatory responses and IgE-associated responses are in use or under development.^{9–11)}

The *Oryza sativa* L. var *japonica* (Family: Poaceae (syn, Gramineae)), which is commonly known as 'Heuk-Mi' (black rice) in S. Korea, is a dark purple-colored seed and a major rice crop in the south Asia and China. It is broadly known as enriched rice and has been used as a traditional medicine for various allergic disorders such as chronic der-

matitis and bronchitis.¹²⁾ This study was performed to investigate the therapeutic potential of DA-9201, an ethanolic extract of the black rice, on the airway inflammation and AHR in an OVA-induced mouse model of asthma.

MATERIALS AND METHODS

Mice Female Balb/c mice (6 weeks) were obtained from Charles River Japan. They were housed under a 12-h light/dark cycle (lighting: 07:00 to 19:00), in the temperature range of $23 \pm 2^\circ\text{C}$, at a relative humidity of $60 \pm 10\%$, with ventilation (turnover frequency of 15–20 times per hour) and a light intensity of 150–600 Lux. All experiments were performed in accordance with the institutional Standard Operation Procedure for Animal Care and Experiments (SOP-ANC) of the Dong-A Pharmaceutical Company and with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Reagents Acetyl- β -methylcholine chloride (methacholine, Mch) and dexamethasone-water soluble (DEXA) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). OVA (chicken egg albumin grade V) and aluminum hydroxide gel (alum) were also purchased from Sigma-Aldrich. All other chemicals or reagents were commercial and of the highest quality available.

The Preparation of DA-9201 The black rice (*Oryza sativa* L. var *japonica*) was purchased from the National Agricultural Cooperative Federation (Geochang, S. Korea). The black rice (8.00 kg) was processed by using a household rice-polishing machine (Bio SangSang, Korea) to obtain the rice bran (1.348 kg). Then, the rice bran was extracted with 70% ethanol (6.74 l) for 24 h at room temperature with agitating. The extract was filtered through filter paper (Advantec, Japan) and the filtrate (5.13 l) was concentrated at 60°C under vacuum using an evaporative system and lyophilized at

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–40 °C to dryness; 77.80 g (yield: 5.77%) of extract powder was obtained. The dried extract, DA-9201, was suspended in a 1% hydroxypropylmethylcellulose (HPMC) solution for oral administration.

The HPLC Analysis of DA-9201 The dried ethanol extract, 10 mg of DA-9201, was dissolved in 1 ml of mobile phase followed by filtration using a 0.45 μ m syringe filter. Standards of several anthocyanins including cyanidine-3-*O*- β -glucopyranoside, peonidine-3-*O*- β -glucopyranoside, delphinidine-3-*O*- β -glucopyranoside and pelargonidine-3-*O*- β -glucopyranoside were obtained from Polyphenols Laboratories (Hanabryggene Technology Centre, Norway). Standards of phenolic acids such as protocatechuic acid and ferulic acid were obtained from Sigma-Aldrich.

The chromatographic systems consisted of Dionex (Sunnyvale, CA, U.S.A.) equipped with a P580 pump, ASI-100 automated sample injector and UV detector. Separations were performed using 5 μ m Inertsil ODS column (250 $\times$ 4.6 mm ID; GL Science Inc., Japan) with 1 mM phosphoric acid/methanol (85:15, v/v) for protocatechuic acid, and 0.5% trifluoroacetic acid/acetonitrile (85:15, v/v) was used for anthocyanins at a flow rate of 1 ml/min. Wavelengths used for the identification of protocatechuic acid and anthocyanins were 254 nm and 520 nm, respectively. To quantify the ferulic acid, the HPLC-MS analyses were carried out using a Waters Alliance 2975 LC system (Waters Co., U.S.A.) coupled to a mass spectrometer. DA-9201 was separated on an Atlantis C18 column (5 μ m, 2.1 mm i.d. $\times$ 100 mm, Waters Co., U.S.A.) using an isocratic elution of acetonitrile/1 mM Ammonium Formate (pH 3.0) (50:50, v/v) at a flow rate of 0.2 ml/min. The temperatures for the column and autosampler tray were 40 °C and 15 °C, respectively. The eluent was introduced directly into the negative ionization electrospray source of a tandem quadrupole mass spectrometer (Quattro Micro, Micromass UK Ltd., U.K.). The ion source and desolvation temperature were held at 120 °C and 250 °C, respectively. The optimum cone voltages and collision energy were 20 eV and 18 V, respectively. Selected reaction monitoring (SRM) using the precursor product ion transitions of m/z 193 > 133.9 was used to quantify ferulic acid.

Experimental Design Airway eosinophilia was elicited by a modified protocol (Fig. 1).¹³ Mice were immunized intraperitoneally with 50 μ g OVA mixed with 1 mg alum as an adjuvant in a total volume of 0.1 ml per mouse. Sensitized mice were immunized again 10 d after the primary immunization. Normal control mice were injected intraperitoneally with phosphate buffer saline (PBS, pH 7.0). DA-9201 was suspended in 1% HPMC solution and orally administered once a day at dose levels of 30, 100 or 300 mg/kg (each $n=9$) from the next day of primary immunization for 23 d. DEXA (3 mg/kg) was also orally administered once a day during the last 2 weeks. All mice were challenged with aerosolized 1% OVA for 30 min/d during the last 4 d. OVA challenging was performed by placing mice in a Plexiglas box (29 $\times$ 22 $\times$ 18 cm) and aerosolizing OVA using an ultrasonic nebulizer (NE-U12; Omron, Japan) driven by compressed air for 30 min. Normal control mice were challenged with aerosolized saline. OVA control mice were immunized and challenged with OVA.

Measurement of AHR Responsiveness to methacholine was assessed 24 h after the last OVA challenge. AHR was

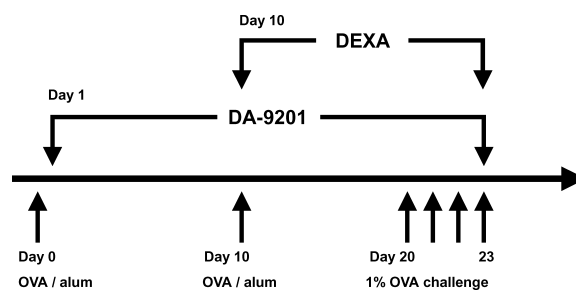


Fig. 1. Schematic Diagram of the Experimental Protocol

Mice were sensitized on day 0 and 10 by intraperitoneal injection of 50 μ g OVA emulsified in 1 mg alum. On day 20, 21, 22 and 23 after the primary sensitization, the mice were challenged for 30 min with an aerosol of 1% OVA solution or saline (control) using an ultrasonic nebulizer. DA-9201 and DEXA were administered orally from day 1 to day 23 and from day 10 to day 23, respectively.

measured in conscious mice by using barometric whole-body plethysmography (OCP-3000, Allmedicus Co., Ltd., U.S.A.). Mice were placed in separate plethysmographic chambers. The enhanced pauses (Penh) were recorded during 3 min prior to and after exposure to aerosolized saline or MCh inhalation (5, 10, 15, 20 mg/ml in saline, 3 min). It was calculated by the following; $\text{Penh} = [(Te - Tr)/Tr] \times (PEP/PIP)$, where Te is the expiratory time (seconds), Tr is the relaxation time (time of the pressure decay to 36% of total box pressure during expiration), PEP is the peak expiratory pressure (milliliters per second), and PIP is the peak inspiratory pressure (milliliters per second). Results are expressed as the percent increase in Penh after challenging with each concentration of Mch.

Bronchoalveolar Lavage Under the ether anesthesia mice were bled and sacrificed. BALF was collected by lavaging the trachea by three times with total 1.2 ml of pyrogen-free PBS containing 0.05 mM EDTA. The lavage fluid was centrifuged at 400 g for 3 min at 4 °C, and the cells were separated from the fluid. The fluid was stored at –20 °C until it was analyzed. The cells were re-suspended in PBS containing 0.05 mM EDTA, and the total number of viable cells was determined using a hemacytometer. Differential cell numbers were determined with cytopsin preparations, followed by Giemsa staining.

Preparation of Splenocytes Single cell suspensions were prepared from spleens placed in RPMI 1640 medium and erythrocytes were lysed with RBC lysis buffer (NH_4Cl 0.16 M 90%, Tris 0.17 M 10%, pH 7.2). After washing, the cells were re-suspended in RPMI 1640 containing 10% FBS and 100 U/ml of penicillin–streptomycin. Two-hundred microliters of splenocytes suspension was plated at 10^6 cell per well with 50 μ g/ml of OVA in 96-well round-bottom microtiter plates. The final volume of each well Cells were cultured at 37 °C with 5% CO_2 for 60 h.

Enzyme-Linked Immunosorbent Assay for Cytokines and Total-IgE IL-4, IL-5, IL-13 or IFN- γ in BALF and in the supernatant of cultured splenocytes were determined by enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, MN, U.S.A.). Total IgE in plasma, BALF and the supernatant of cultured splenocyte from each animal were also analyzed according to the manufacturer's instructions (SHIBAYAGI Co. Ltd., Japan).

ELISA for Ag-Specific IgG, IgG2a and IgG1 in Plasma

Microtiter plates were coated with 100 μ l of OVA solution (5 μ g/ml in PBS) overnight at 4 °C. After washing with PBS, the plates were blocked with 150 μ l of 1% bovine serum albumin (BSA, Sigma-Aldrich) for 30 min at room temperature. Serially diluted plasma samples (1:50 for IgG2a, 1:5000 for IgG and IgG1) were added to the wells and incubated for 2 h at room temperature. Alkaline phosphatase-conjugated goat anti-mouse IgG, IgG2a or IgG1 was added and incubated for 2 h at room temperature (The professor Chang-Yuil Kang (College of Pharmacy, Seoul National University) kindly donated goat anti-mouse IgG, IgG2a, IgG1, and anti-OVA IgG). The plates were washed and then 100 μ l of phosphatase substrate (*p*-nitrophenyl-phosphate in a carbonate buffer, pH 9.6) was added to each well. The absorbance was read at 405 nm using an Emax microplate reader (Molecular Devices, Menlo Park, CA, U.S.A.). Concentration of OVA-specific IgG in plasma was determined from standard curves constructed using immuno-affinity purified anti-OVA IgG. The level of IgG2a or IgG1 was expressed as % of normal control (relative ratio compared with that of normal control).

Lung Histology Histopathological examination was performed on lungs that were removed and fixed with 10% phosphate-buffered formalin. The tissues were embedded in paraffin, sectioned at a thickness of 5 μ m, stained with hematoxylin and eosin (H&E), and analyzed through use of light microscope. The inflammatory score was obtained from the following criteria; inflammation including airway edema and cellular infiltration was graded as none (score 0), mild (score 0.5), moderate (score 1), or severe inflammation (score 1.5).

Statistical Analysis All statistical analyses were performed using SigmaStat® for Windows 2.0 software (Jandel corporation, U.S.A.). The ANOVA test was used for the comparisons between the experimental groups and within each test group. All the data is expressed as a mean $\pm$ standard error of the mean (S.E.M.). The comparison between the group means was accomplished using a Dunnett's multiple range test at a significance level of $p=0.05$.

RESULTS

DA-9201 DA-9201, ethanolic extracts of black rice bran, was black to purple colored-powder. The amount of the standard materials contained in DA-9201 is shown in Table 1. Cyanidine-3-*O*- β -glucopyranoside, peonidine-3-*O*- β -glucopyranoside, pelargonidine-3-*O*- β -glucopyranoside, protocathechuic acid and ferulic acid were contained 1.49%, 0.30%, 0.47% and 0.24%, respectively.

Effect of DA-9201 on the Total and Differential Cell Numbers in BALF The number of total cells in BALF was significantly increased in OVA control mice (Fig. 2A). However, the cell number was reduced in a dose-dependent manner by the treatment with DA-9201. Furthermore, while the proportion of eosinophils in OVA control was significantly increased (Fig. 2B), treatment with DA-9201 induced a dose-dependent decrease in the ratio of eosinophil. The administration of DEXA at the dose of 3 mg/kg also significantly reduced the total and differential number of eosinophils in BALF.

Effects of DA-9201 on Pathological Changes in Lung The scores of total lung inflammation including airway edema and cellular infiltration were significantly increased in

Table 1. The Amounts of Standard Materials in DA-9201

Standard materials	Quantity (% w/w)	Retention time
Cyanidine-3- <i>O</i> - β -glucopyranoside	1.49	5.63—5.65
Peonidine-3- <i>O</i> - β -glucopyranoside	0.30	9.15—9.53
Delphinidine-3- <i>O</i> - β -glucopyranoside	0.00	3.95—4.05
Pelargonidine-3- <i>O</i> - β -glucopyranoside	0.30	8.15—8.45
Protocatechuic acid	0.47	2.8
Ferulic acid	0.24	6.1

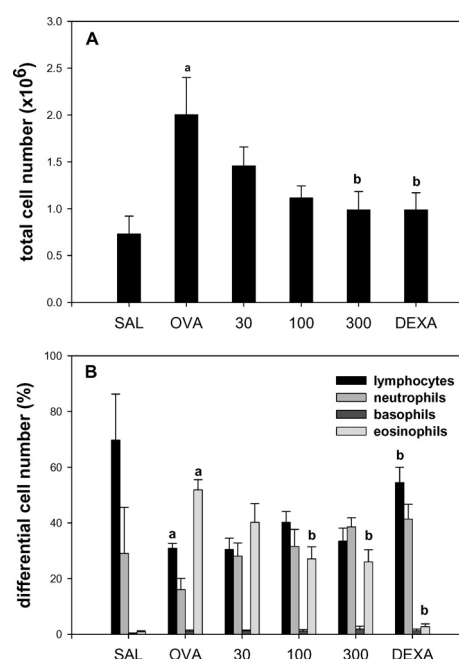


Fig. 2. Effect of DA-9201 on the Total and Differential Cell Numbers in BALF

The total (A) and differential (B) cell numbers in BALF were counted at 24 h after the last OVA challenge. Bars represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 30; DA-9201 30 mg/kg, 100; DA-9201 100 mg/kg, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

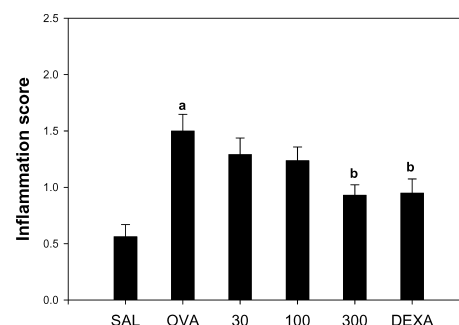


Fig. 3. Effect of DA-9201 on Pathologic Changes in Lung Tissues

Lungs were sampled at 24 h after the last OVA challenge and pulmonary inflammation was scored under the light microscope at a magnification of $\times 100$. Bars represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 30; DA-9201 30 mg/kg, 100; DA-9201 100 mg/kg, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

OVA control mice when compared with the normal control mice (Fig. 3). These responses were ameliorated in a dose-dependent manner by the administration of DA-9201. Inflammatory score was significantly decreased at the dose level of

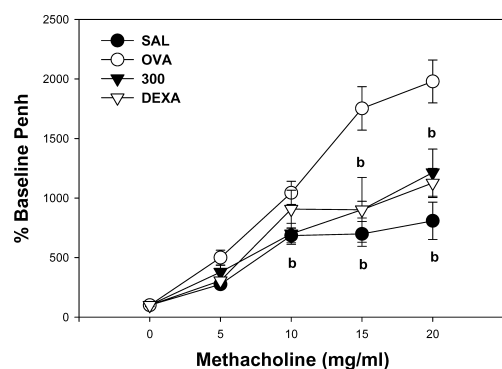


Fig. 4. Effect of DA-9201 on Airway Hyperresponsiveness

AHR to aerosolized methacholine was measured in unrestrained, conscious mice. Mice were placed into the main chamber and were nebulized with saline or methacholine (5, 10, 15, 20 mg/ml) for 3 min for each nebulization. Recording of Penh were taken for 3 min after each nebulization. Data represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

DA-9201 300 mg/kg, which was comparable to that of the DEXA-treated group.

Effects of DA-9201 on AHR In OVA control mice, the percent Penh produced by the exposure to methacholine was significantly increased when compared with that of the normal control mice. DA-9201 treatment showed a significant and dose-dependent decrease in AHR. While the dose of level of DA-9201 30 mg/kg or 100 mg/kg did not show the statistical significance (data not shown), 300 mg/kg of DA-9201 significantly reduced the AHR, which was comparable to that of the mice treated with DEXA (Fig. 4).

Effects of DA-9201 on Levels of IL-4, IL-5, IL-13 and IgE in BALF Enzyme immunoassay revealed that levels of IL-4, IL-5, IL-13 and IgE in BALF were significantly increased at 24 h after the last OVA inhalation in the OVA control mice (Figs. 5A–D). The levels of these cytokines and IgE were decreased by the treatment with DA-9201.

Effects of DA-9201 on Levels of IL-4, IL-5, IFN- γ and IgE in the Supernatant of Cultured Splenocytes While the levels of IL-4 and IL-5 were significantly increased in OVA control, the levels of IL-4 and IL-5 in DA-9201-treated groups were significantly decreased in a dose-dependent manner (Figs. 6A, B). The level of IFN- γ was not significantly decreased by the administration of DA-9201 when compared with OVA control (Fig. 6C). Furthermore, DA-9201 treatment significantly and dose-dependently decreased the IgE level (Fig. 6D).

Effects of DA-9201 on Antibody Responses in Plasma As shown in Fig. 7, DA-9201 at the dose of 300 mg/kg showed a significant suppression in levels of plasma OVA-specific IgG, IgG2a, IgG1 and total IgE.

DISCUSSION

Accumulating data have shown that unbalanced and aberrant Th2 inflammation and subsequent eosinophilic infiltration into lung are the main cause of allergic asthma.¹⁴ In fact, IL-4, one of the representative Th2 cytokines, has been shown to promote B cell switching to IgE production with IL-13, which enhance mucus production and AHR. IL-5, another Th2 cytokines, is also the primary determinant of eosinophil priming, activation, recruitment, growth, differen-

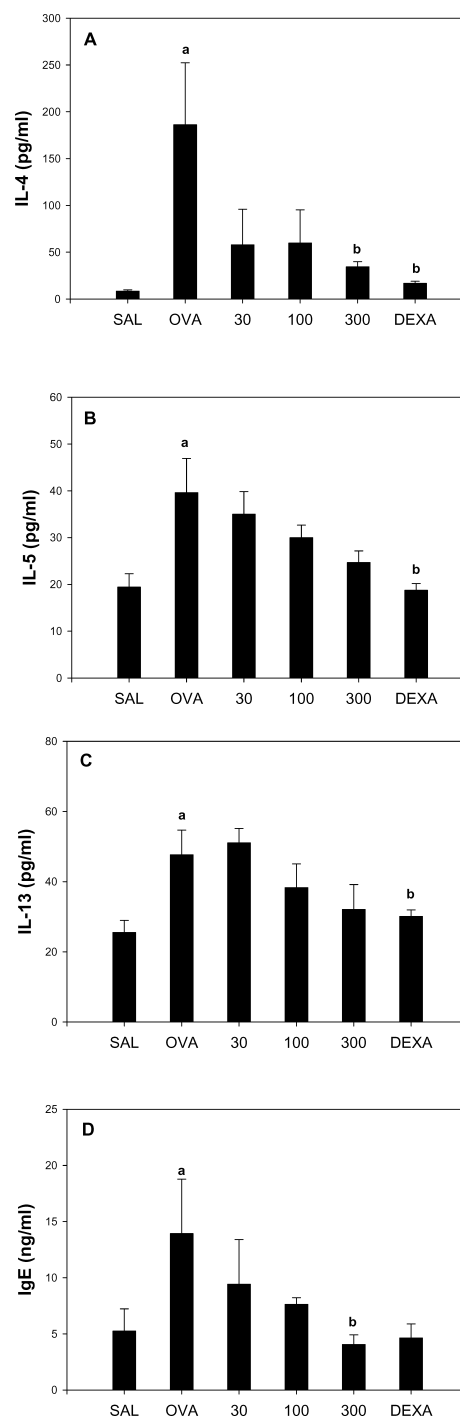


Fig. 5. Effects of DA-9201 on Levels of IL-4, IL-5, IL-13 and IgE in BALF

The levels of IL-4 (A), IL-5 (B), IL-13 (C) and IgE (D) in BALF were quantified by ELISA at 24 h after the last OVA challenge. Bars represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 30; DA-9201 30 mg/kg, 100; DA-9201 100 mg/kg, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

tiation and survival.¹⁴ Moreover, infiltrated eosinophils have dense intracellular granules that are sources of inflammatory proteins. Major basic protein, one of those proteins, can directly damage airway epithelium, intensify bronchial responsiveness, and cause degranulation of basophils and mast cells.⁴ Therefore, it appears evident that the inflammation caused by Th2 cells and eosinophils and subsequent responses initiate and maintain asthma. From this point of

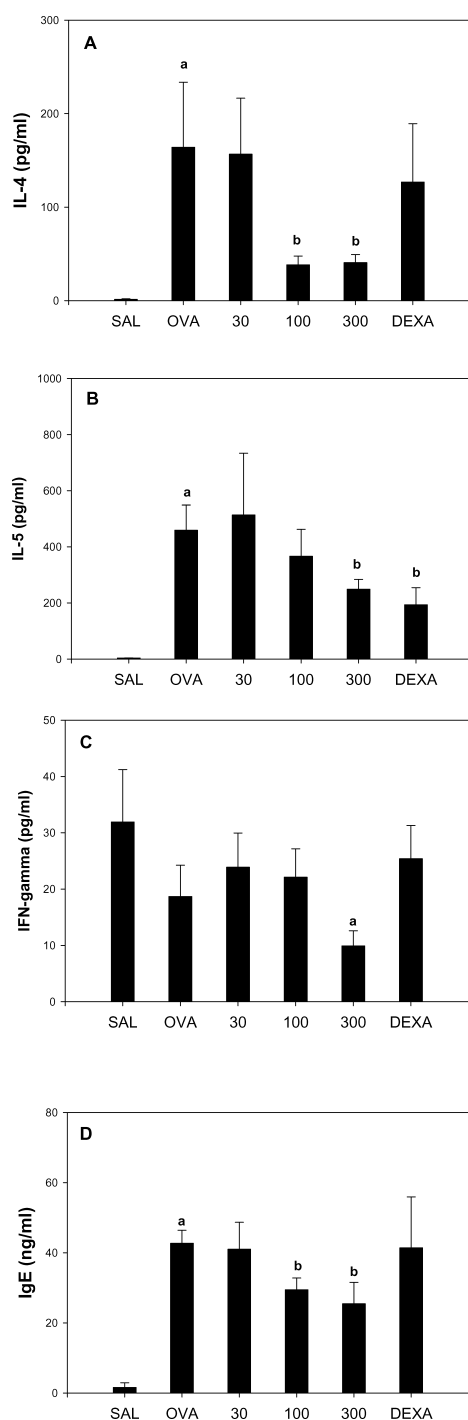


Fig. 6. Effects of DA-9201 on Levels of IL-4, IL-5, IFN- γ and IgE in the Supernatant of Cultured Splenocytes

The levels of IL-4 (A), IL-5 (B), IFN- γ (C) and IgE (D) in the supernatant of cultured splenocytes were quantified by ELISA after culture with OVA for 60 h. Bars represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 30; DA-9201 30 mg/kg, 100; DA-9201 100 mg/kg, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

view, anti-inflammatory therapies have become important therapeutic strategies for asthma treatment. Corticosteroids are the most potent, non-specific anti-inflammatory agents and they are known to produce substantial improvement in lung functions of asthmatic subjects. However, frequently reported side effects make the compliance poor. Furthermore, taking it into consideration that asthmatics should have long-term anti-inflammatory therapies, there is a need for safe and

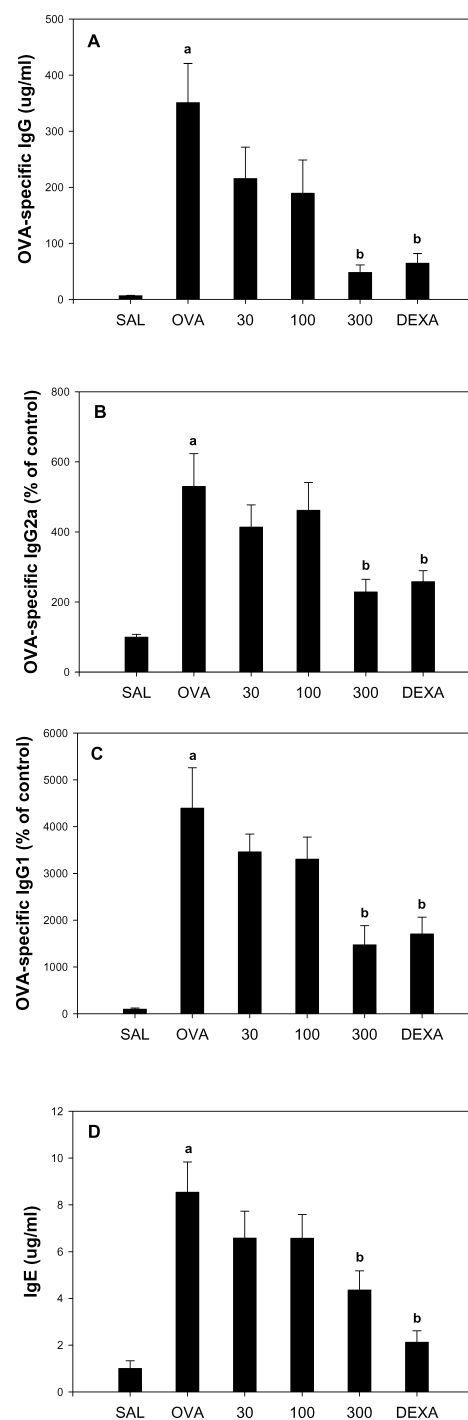


Fig. 7. Effects of DA-9201 on OVA-Specific IgG, IgG2a, IgG1 and Total IgE Levels in Plasma

Plasma was obtained at 24 h after the last OVA challenge. OVA-specific IgG (A), IgG2a (B), IgG1 (C) and total IgE (D) were determined by ELISA. Data represent mean $\pm$ S.E.M. ($n=9$). ^a $p<0.05$ vs. normal control, ^b $p<0.05$ vs. OVA control. SAL; negative control, OVA; positive control, 30; DA-9201 30 mg/kg, 100; DA-9201 100 mg/kg, 300; DA-9201 300 mg/kg, DEXA; dexamethasone 3 mg/kg.

potent drug which has fewer side effects.⁸⁾ In this study, we investigated possible therapeutic effects of DA-9201, an extract of black rice (*Oryza sativa* L. var. *japonica*), on allergic asthma using a mouse model. We found that the treatment of DA-9201 exhibited anti-inflammatory properties as evidenced by the reduction of eosinophil infiltration and Th2 cytokines. Lung histological examination also showed that DA-9201 reduced airway inflammation. Furthermore, it is ex-

pected that DA-9201 is particularly safe without side effects because DA-9201 induced no toxicological changes in several toxicological studies (data known shown), which was comparable to previous study showed that administration with black rice extract to rats induced no physiological differences up to 10 g/kg.¹²⁾ Thus, DA-9201 has advantages over other anti-inflammatory agents in that it is particularly safe and an effective oral treatment.

The idea that allergic inflammation in asthma arises from an imbalance between Th1 and Th2 cells has focused attention on the representative Th1 cytokine, IFN- γ . Since IFN- γ has been reported to inhibit the synthesis of IgE and the differentiation of precursor cells to Th2 cells, increased level of IFN- γ has been used to explain the anti-asthmatic effects of therapeutic agents.^{15,16)} However, other data have suggested that IFN- γ contribute to the activation of eosinophils and thus be likely to augment inflammation.^{6,7)} Thus it seems to be reasonable that unnecessary immune response caused by Th1 cells as well as inflammation mediated by Th2 cells and eosinophils should be suppressed to treat asthma. We revealed that DA-9201 down-regulated Th2 cytokine production both in cultured splenocytes and BALF in a dose-dependent manner. Especially, at the dose of 300 mg/kg, IL-4 and IL-5 production in cultured splenocytes and IL-4 in BALF was significantly decreased although IL-5 and IL-13 levels in BALF did not show statistical significance. Likewise, DA-9201 suppressed OVA-specific IgG1 produced by help of Th2 cells in a dose-dependent manner with the statistical significance at dose level of DA-9201 300 mg/kg. Furthermore, DA-9201 suppressed significantly OVA-specific IgG and IgG2a production, which was produced by help of Th1 cells. However, DA-9201 did not directly and significantly affect IFN- γ production in cultured splenocytes when compared with OVA control. Taking these results into consideration, immune responses mediated by Th2 cells and B cells were markedly suppressed by the administration of DA-9201. However, the effect of DA-9201 on immune response mediated by Th1 cells might be minimum. Further studies are needed to elucidate the exact mode of action of DA-9201.

Unlike other isotypes, IgE was formed by allergen binds to Fc(ϵ)R I on mast cells and basophils without allergen, and the cross-linking of mast cells or basophils-bound IgE by

allergen induces the activation of membrane and cytosolic pathways that cause the release of preformed mediators such as histamine and initiates the synthesis of arachidonic acid metabolites.⁴⁾ A recombinant humanized monoclonal antibody against IgE, omalizumab, has been used as an anti-asthmatic treatment.^{9–11)} DA-9201 also suppressed IgE levels in plasma, BALF and the supernatant of cultured splenocytes. Therefore, it is expected that DA-9201 can alleviate IgE-mediated subsequent responses as well as airway inflammation and eosinophilia.

Although the pharmacologically active components of DA-9201 have not yet been characterized and its chemical composition is also still not completely elucidated, DA-9201 suppressed airway inflammation and IgE level in this experimental model of asthma. This effect was ultimately accompanied by the reduction of airway hyperresponsiveness. Based on these results, we concluded that DA-9201 might be beneficial for the treatment of asthma.

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