

Natural Vanadium-Containing Jeju Groundwater Inhibits Immunoglobulin E-Mediated Anaphylactic Reaction and Suppresses Eicosanoid Generation and Degranulation in Bone Marrow Derived-Mast Cells

Xian Li, Yue Lu, Ju Hye Yang, Ye Jin, Seung-Lark Hwang, and Hyeun Wook Chang*

College of Pharmacy, Yeungnam University, Gyeongsan 712–749, Republic of Korea.

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The high-affinity receptor for immunoglobulin E (IgE) (FcεRI)-mediated activation of mast cells plays an important role in various allergic diseases. To assess the anti-allergic activity of natural vanadium-containing Jeju groundwater (JW), an *in vivo* passive cutaneous anaphylaxis (PCA) animal model and *in vitro* mouse bone marrow-derived mast cells (BMMCs) was used. JW inhibited cyclooxygenase-2 (COX-2)-dependent prostaglandin D₂ (PGD₂) generation in a dose-dependent manner, with a concomitant reduction of COX-2 protein expression in IgE-induced BMMCs. In addition, JW inhibited 5-lipoxygenase (5-LOX)-dependent generation of leukotriene C₄ (LTC₄) as well as degranulation in a dose-dependent manner. These results demonstrate that JW has dual COX-2/5-LOX inhibitory activity. In addition, vanadium pentoxide (V₂O₅), which is the major vanadium component of JW, also inhibited PGD₂ and LTC₄ generation as well as degranulation in IgE-induced BMMCs. Furthermore, oral administration of JW dose-dependently inhibited mast cell-dependent passive anaphylactic reaction in IgE-sensitized mice. Taken together, these results suggest that JW may be useful in regulating mast cell-mediated allergic response through the suppression of eicosanoid generation and degranulation in mast cells.

Key words vanadium-containing Jeju groundwater; prostaglandin D₂; leukotriene C₄; degranulation; bone marrow-derived mast cell; passive cutaneous anaphylaxis

In recent years, there has been a growing realization that mast cells play a central role in the induction of allergic and inflammatory reactions. Mast cells are activated by allergen-specific immunoglobulin E (IgE) binding; upon interaction of high-affinity IgE receptor (FcεRI) or stem cell factor (SCF, also known as c kit-ligand, KL) with c-Kit, mast cells release various preformed chemical mediators such as histamine, serotonin and proteoglycans, as well as newly synthesized inflammatory mediators, including prostaglandin D₂ (PGD₂) and leukotriene C₄ (LTC₄), through the activation of cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and various cytokines.^{1,2)} In mouse bone marrow-derived mast cells (BMMCs), a biphasic PGD₂ biosynthetic response occurs over time. Immediate PGD₂ generation, defined as occurring within 2 h, is associated with the activation of COX-1, while delayed PGD₂ generation, observed following several hours of culturing (2–10 h), is associated with COX-2 after stimulation with either IgE/antigen (Ag) or a combination of cytokines and lipopolysaccharide (LPS).^{3,4)} It is well known that PGD₂ is the major prostanoid secreted from activated mast cells and has long been implicated in manifestation of inflammation and allergic diseases.⁵⁾ Leukotriene C₄ (LTC₄), another class of inflammatory lipid mediator, is synthesized from arachidonic acid by the action of 5-LOX.⁶⁾

Previously, another group reported that natural vanadium-containing Mt. Fuji groundwater improves hyperglycemia by ameliorating liver insulin receptor activity.⁷⁾ Vanadate shows immunotoxicity through the production of inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and PGE₂ in LPS-stimulated WEHI-3 cells.⁸⁾ In addition, sodium orthovanadate induces release of histamine from rat and mouse mast cells as well as vanadium-stimulated histamine release from mast cells and basophils at late phase

in the presence of hydrogen peroxide.^{9–12)} However, the anti-allergic activities and underlying mechanisms of natural vanadium-containing groundwater have not been reported. We here describe for the first time the anti-allergic activity of natural vanadium-containing Jeju groundwater (JW) and the mechanism of its action in BMMCs. In our findings, JW clearly inhibited eicosanoid production and degranulation in mast cells *in vitro* and attenuated the IgE-mediated anaphylactic reaction through LTC₄ and PGD₂ production *in vivo*, suggesting that a low concentration of natural vanadium-containing JW has beneficial effects on the regulation of various allergic responses.

MATERIALS AND METHODS

Chemicals and Reagents JW containing vanadium components (V⁵⁺: 26.0±2.0 μg/L, Na⁺: 5.06±1.0 mg/L, Ca²⁺: 3.4±0.5 mg/L, Mg²⁺: 3.0±1.0 mg/L, K⁺: 3.0±0.5 mg/L) was provided by the Jeju Special Self-Governing Province Development Corp. (Jeju, Korea).

Induction of IgE-Mediated Passive Cutaneous Anaphylaxis (PCA) in Mice Seven ICR mice (Hyochang Science, Daegu, Korea) were placed in each cage within a laminar airflow cabinet and were fed with standard laboratory chow (Purina, Seoul, Korea) and water *ad libitum*. The mice were kept at a temperature of 22±1°C and at a relative humidity of 55±10% and 12 h/12 h (light/dark) cycle for at least 7 d prior to the experiments throughout the study. For PCA, 80 ng of mouse anti-dinitrophenyl (DNP) IgE (Sigma-Aldrich, St. Louis, MO, U.S.A.) was intradermally injected into the ears of 6-week-old male ICR mice (Hyochang Science, Daegu, Korea), followed 1 h later by oral administration of 400 μL of JW or 0.5–2 mg/kg vanadium pentoxide (V₂O₅) or 50 mg/kg

* To whom correspondence should be addressed. e-mail: hwchang@yu.ac.kr

fexofenadine-HCl (Fexo), a histamine H1 receptor antagonist (Korea Pharma, Seoul, Korea). One hour later, the mice were intravenously challenged with 60 μ g of antigen (Ag) (DNP-human serum albumin (HSA); (Sigma-Aldrich, St. Louis, MO, U.S.A.) in 200 μ L of phosphate buffered saline (PBS) containing 0.8% (w/v) Evans blue. The mice were euthanized 1 h after treatment with Ag, after which their ears were removed and dissolved with 500 μ L of formamide at 63°C overnight. The amount of dye extravasation was determined colorimetrically at 630 nm. Serum LTC₄ and PGD₂ concentrations were determined by respective assay kits (Cayman Chemicals, Ann Arbor, MI, U.S.A.). Experiments using mice were approved by the Institutional Animal Care and Use Committee of Yeungnam University.

Preparation and Activation of Mouse Bone Marrow-Derived Mast Cells (BMMCs) Bone marrow cells from male Balb/cJ mice (purchased from Sam Taco, Inc., Seoul, Korea) were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics, and 10% fetal calf serum) and 20% pokeweed mitogen-stimulated spleen condition medium (PWM-SCM) as a source of interleukin-3 (IL-3). After 3 weeks, >98% of the cells were verified to be BMMCs according to a previously described procedure.¹³ For cell stimulation, BMMCs (5 $\times$ 10⁵ cells/mL) were sensitized overnight with 100 ng/mL of anti-DNP and then stimulated for 15 min with 25 ng/mL of DNP-HSA. To examine the effects of JW or vanadium pentoxide (V₂O₅), they were added 1 h prior to the addition of DNP-HSA. β -hexosaminidase (β -hex), a marker of mast cell degranulation, was quantified by a spectrophotometric method as described previously.¹⁴ 5-LOX-dependent LTC₄ was quantified using a LTC₄ assay kit (Cayman Chemicals, Ann Arbor, MI, U.S.A.). To assess COX-2-dependent PGD₂ synthesis, BMMCs were preincubated with 1 μ g/mL of aspirin for 2 h to irreversibly inactivate preexisting COX-1. After washing, BMMCs were activated with DNP-HSA for 7 h with or without JW or V₂O₅ or licoferone (Toronto Research Chemicals Inc., North York, ON, Canada). PGD₂ in the supernatants were quantified using a PGD₂ assay kit (Cayman Chemicals, Ann Arbor, MI, U.S.A.). All data were the arithmetic mean of triplicate determinations.

Measurement of Intracellular Ca²⁺ Levels Intracellular Ca²⁺ levels were determined with FluoForte™ Calcium assay kit (Enzo Life Sciences, Ann Arbor, MI, U.S.A.). IgE-sensitized BMMCs were preincubated with FluoForte™ TM Dye-Loading Solution for 1 h at room temperature. After washing the dye from the cell surface with PBS, BMMCs (5 $\times$ 10⁴) were seeded into 96-well microplates. Then, cells were pretreated with JW for 30 min before adding DNP-HSA. The fluorescence was measured using a fluorometric imaging plate reader with excitation of 485 nm and emission at 520 nm (BMG Labtechnologies FLUOStar OPITIMA plate reader, Offenburg, Germany). All experiments were performed separately at least three times.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)/Immunoblot Analysis IgE-sensitized mast cells were stimulated with Ag for the indicated times with or without JW and V₂O₅. The lysate (1 $\times$ 10⁵ cells equivalent) were applied to 8% (w/v) SDS-PAGE under reducing conditions, transferred onto nitrocellulose membranes, and subjected to immunoblotting. After running the gel, the protein

bands were blotted onto nitrocellulose membranes (Schleicher and Schull, Dassel, Germany) using a semi-dry blotter (MilliBlot-SDE system, Millipore, Bedford, MA, U.S.A.) according to the manufacturer's instructions. Membranes were then washed once with 10 mM Tris-buffered saline (TBS, pH 7.2) containing 0.1% tween-20 (TBS-T), and then blocked for 1 h in TBS-T containing 3% skim milk. After washing the membranes with TBS-T, antibody directed against COX-2 was added at a dilution of 1:3000–5000 in TBS-T. After incubation for 2 h followed by washing three times, membranes were treated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed, South San Francisco, CA, U.S.A.) (diluted to 1:7000) in TBS-T. The protein bands were visualized using an enhanced chemiluminescence (ECL) system (Pierce Biotechnology, Rockford, IL, U.S.A.).

Statistical Analysis All experiments described were performed three or more times. Average values were expressed as mean $\pm$ S.E.M. The Student's *t*-test was used for the comparison of two independent groups. For all tests, a *p*-value < 0.05 was considered statistically significant.

RESULTS

JW or V₂O₅ Suppresses Mast Cell-Dependent Anaphylactic Reaction in Mice To evaluate the anti-allergic inflammatory activity of JW or V₂O₅ *in vivo*, the effect of JW or V₂O₅ on IgE-induced PCA was examined using a mouse model. Oral administration either JW at concentrations of 100, 200, and 400 μ L to IgE-sensitized mice 1 h before injection of Ag attenuated the mast cell-dependent PCA reaction by approximately 5%, 15%, and 28%, respectively (Fig. 1A). In addition, V₂O₅ also inhibited PCA reaction by approximately 6%, 18%, and 31% at 0.5, 1 and 2 mg/kg, respectively (Fig. 4A). 50 mg/kg of Fexo also strongly reduced the PCA reaction by about 55%. Furthermore, in Ag-challenged mice, there were marked increases in serum levels of LTC₄ (Figs. 1B, 4B) and PGD₂ (Figs. 1C, 4C). As shown in Figs. 1B and C, JW dose dependently inhibited both PGD₂ and LTC₄ generation. Under the same experimental condition, V₂O₅ also attenuated eicosanoid generation in a dose-dependent manner (Figs. 4B,C). However, under the same conditions, IgE or Ag (DNP-HSA) alone did not affect the PCA reaction as well as PGD₂ and LTC₄ generation.

JW Inhibits Degranulation and Ca²⁺ Mobilization in Mast Cells The *in vivo* results described above led us to evaluate the mechanism of the anti-allergic activity of JW or V₂O₅ and therefore, we determined the effects of JW or V₂O₅ on mast cells, a central player of the anaphylactic reaction. We first examined the effect of JW on degranulation in mast cells. To investigate the effect of JW on degranulation of IgE/Ag-activated BMMCs, we measured the release of β -hex in the presence or absence of JW. As shown in Fig. 2A, JW reduced β -hex release by approximately 13%, 16%, and 21% with 10, 20, and 40 μ L of JW, respectively. Under the same experimental condition, 40 μ L of JW alone did not induce degranulation in BMMCs (data not shown). Since the increase in the concentration of cytosolic Ca²⁺ is essential for degranulation of mast cells,¹⁵ we investigated the effect of JW on Ca²⁺ influx. Interestingly, JW reduced Ca²⁺ influx by approximately 19.5%, 25.8% and 28.6% at 10, 20, and 40 μ L, respectively (Fig. 2B).

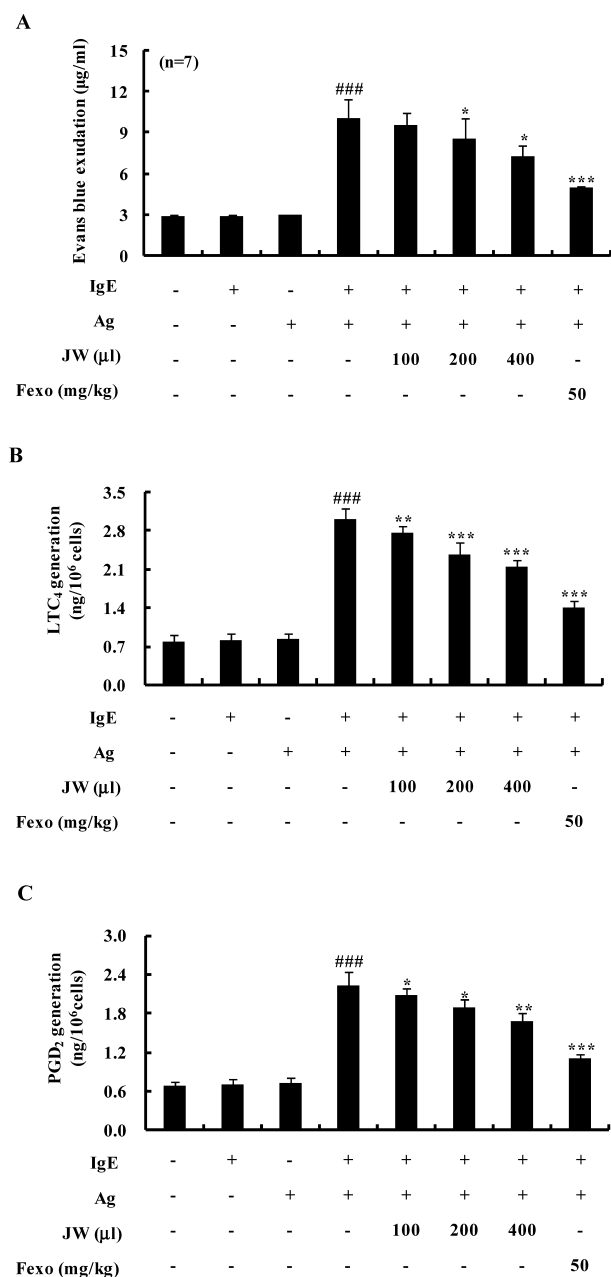


Fig. 1. JW Inhibits IgE/Ag-Mediated PCA Reaction and Eicosanoid Generation

(A) The ears of ICR mice were injected intradermally with 80 ng of anti-DNP. After 24 h, the mice were intravenously injected with 60 µg of DNP-HSA in 200 µL of PBS containing 0.8% Evans blue. To evaluate the effect of JW, it was orally administered 1 h before Ag administration. Dye extravasation in ears was quantified. (B, C) Blood was collected 1 h after Ag challenge, and serum LTC₄ and PGD₂ concentrations were determined by EIA kit. $n=7$ animals in three independent experiments, mean $\pm$ S.E.M. ### $p<0.001$ were compared to non-treated mice. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ were compared to IgE/DNP-HSA sensitized mice. Fexofenadine (50 mg/kg) was used as an atypical anti-histamine control drug.

JW Inhibits COX-2 Dependent PGD₂ and 5-LOX Dependent LTC₄ Generation in IgE-Induced Mast Cells

Next, to further characterize the role of JW in the allergic response, we investigated the effect of JW on the generation of another allergic inflammatory mediator, PGD₂ in IgE/Ag-induced BMMCs. PGD₂ is a major product of arachidonic acid (AA) metabolism via the COX pathway in IgE-induced mast cells. PGD₂ synthesis occurs in a biphasic manner. The immediate phase of PGD₂ generation (within 2 h) depends on

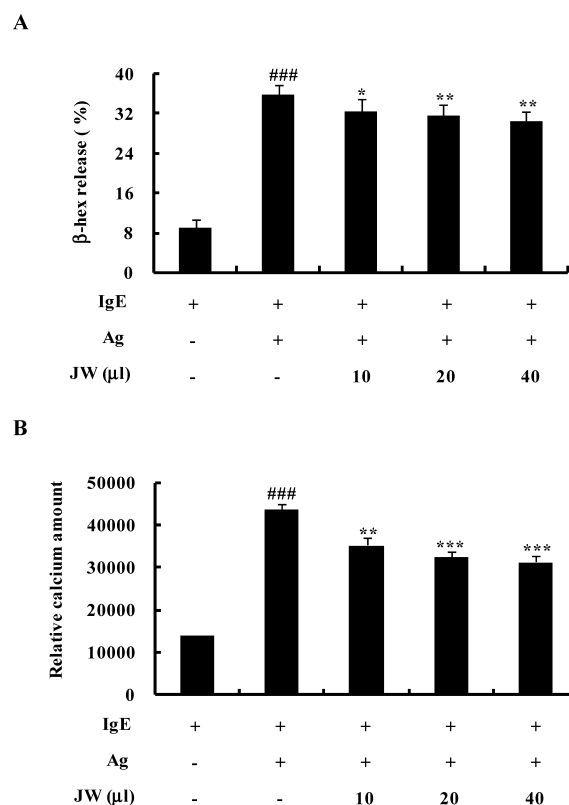


Fig. 2. JW Inhibits Degranulation and Ca²⁺ Mobilization in IgE/Ag-Activated BMMCs

After BMMCs were sensitized overnight with anti DNP-IgE and challenged with DNP-HSA with or without JW, β-hex release (at 15 min) (A) and Ca²⁺ mobilization (at 5 min) (B) were evaluated. The values indicate the mean $\pm$ S.E.M. from three independent experiments. ### $p<0.001$ was compared to non-treated BMMCs, and * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ were compared to IgE/Ag sensitized BMMCs.

preexisting COX-1 while delayed PGD₂ generation following several hours of culturing (2–10 h), is associated with COX-2 after stimulation with IgE/Ag or cytokines.^{3,4,16} To assess COX-2-dependent PGD₂ generation, IgE-sensitized BMMCs were pretreated with aspirin to abolish any preexisting COX-1 activity and then stimulated with Ag for 7 h with or without JW. As shown in Fig. 3A upper panel, delayed PGD₂ generation was dose-dependently inhibited by JW (approximately 9, 21, and 30% with 10, 20, and 40 µL of JW, respectively), with a concomitant suppression of COX-2 protein expression (lower panel). Under these conditions, the levels of β-actin (internal controls for the cytosolic fraction) were unchanged. AA can be converted into leukotrienes (LTs) by the action of 5-LOX in BMMCs. Thus, we further investigated the effect of JW on 5-LOX-dependent LTC₄ generation. As shown in Fig. 3B, JW dose-dependently inhibited LTC₄ generation by approximately 12, 20, and 24% at doses of 10, 20, and 40 µL, respectively. Under the same condition, 1 µM licofelone, a COX-2/5-LOX dual inhibitor,¹⁷ almost completely inhibited both PGD₂ and LTC₄ generation. Under the same experimental condition, 40 µL of JW alone induced neither PGD₂ nor LTC₄ generation in BMMCs (data not shown). Further, COX-2-dependent PGD₂ and 5-LOX-dependent LTC₄ generation reached approximately 7 and 56 ng/10⁶ cells, respectively.

V₂O₅ Inhibits Degranulation, Ca²⁺ Mobilization and Eicosanoid Generation in IgE-Induced Mast Cells Since V₂O₅ is a major component of vanadium in JW, we investigat-

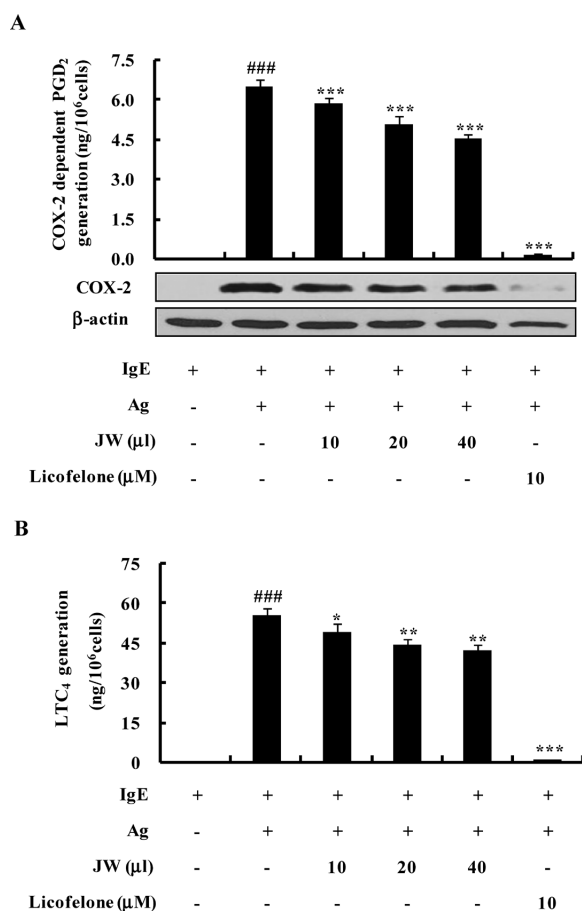


Fig. 3. Effect of JW on COX-2-Dependent PGD₂ Generation (A) and 5-LOX-Dependent LTC₄ Generation (B) in IgE/Ag-Activated BMMCs

(A) Activated BMMCs were preincubated with 1 μg/mL of aspirin for 2 h to abolish preexisting COX-1 activity, followed by a brief washing and then stimulated with DNP-HSA for 7 h with or without JW and 10 μM licofelone. PGD₂ released into the supernatant was quantified using a PGD₂-MOX enzyme immunoassay kit (upper) and BMMCs were then taken for immunoblotting of COX-2 protein (lower). (B) IgE-sensitized BMMCs were preincubated for 1 h with the indicated volume of JW, after which BMMCs were challenged with DNP-HSA for 15 min and LTC₄ generation was measured. The values indicate the mean ± S.E.M. from three independent experiments. ###*p* < 0.001 was compared to non-treated BMMCs, and **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 were compared to IgE/Ag sensitized BMMCs

ed the effect of V₂O₅ on degranulation and AA-metabolizing enzymes in IgE/Ag-induced BMMCs. We first examined the cytotoxicity of V₂O₅ on BMMCs using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We found that V₂O₅ neither affected cell viability nor stimulate degranulation or eicosanoid generation even at 100 μM (data not shown). Therefore, V₂O₅ at concentrations below 1 μM was used for subsequent experiments. We found that V₂O₅ inhibited degranulation in a dose-dependent manner by approximately 11, 19, and 24% at concentrations of 0.25, 0.5, and 1 μM, respectively (Fig. 5A). As described above, Ca²⁺ is essential for degranulation of mast cells,¹⁵ thus, we examine the effect of V₂O₅ on Ca²⁺ influx. As shown in Fig. 5B, V₂O₅ also reduced Ca²⁺ influx by approximately 16, 22 and 25.26% at 0.25, 0.5, and 1 μM, respectively. In addition, Fig. 6A upper panel showed that V₂O₅ also dose-dependently suppressed COX-2-dependent PGD₂ generation (approximately 23, 29, and 35% at concentrations of 0.25, 0.5, and 1 μM, respectively) through the suppression of COX-2 protein expression like that of JW (Fig. 6A lower panel). Furthermore, V₂O₅ also inhibited

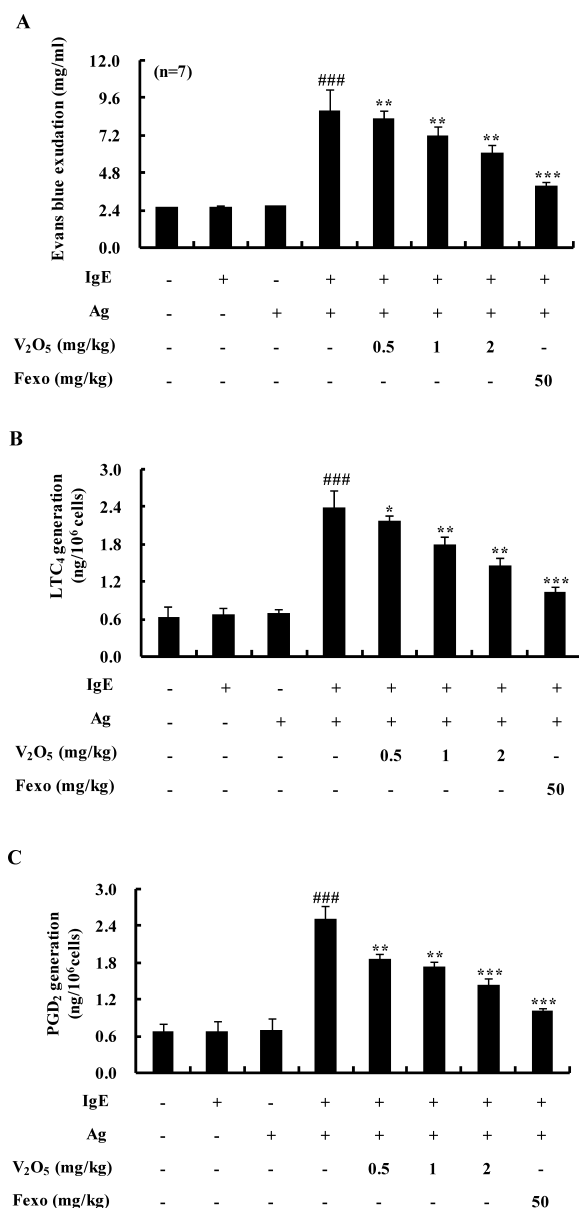


Fig. 4. V₂O₅ Inhibits IgE/Ag-Mediated PCA Reaction and Eicosanoid Generation

(A) The ears of ICR mice were injected intradermally with 80 ng of anti-DNP. After 24 h, the mice were intravenously injected with 60 μg of DNP-HSA in 200 μL of PBS containing 0.8% Evans blue. To evaluate the effect of V₂O₅, it was orally administered 1 h before Ag administration. Dye extravasation in ears were quantified. (B, C) Blood was collected 1 h after Ag challenge, and serum LTC₄ and PGD₂ concentrations were determined by EIA kit. *n* = 7 animals in three independent experiments, mean ± S.E.M. ###*p* < 0.001 were compared to non-treated BMMCs. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 were compared to IgE/DNP-HSA sensitized mice. Fexofenadine (50 mg/kg) was used as an atypical anti-histamine control drug.

5-LOX-dependent LTC₄ generation by approximately 2, 9, and 29% at concentrations of 0.25, 0.5, and 1 μM, respectively (Fig. 6B). Under the condition employed, COX-2-dependent PGD₂ and 5-LOX-dependent LTC₄ generation reached approximately 2.2 and 47.8 ng/10⁶ cells, respectively

DISCUSSION

In the present study, we demonstrated for the first time the anti-allergic activity JW using an *in vitro* BMMC model and *in vivo* allergic animal model. Mast cells are one of the

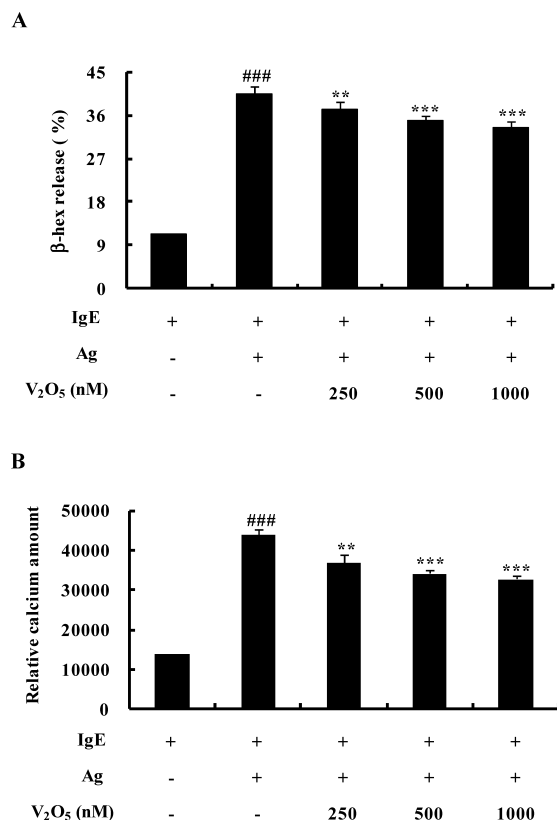


Fig. 5. V₂O₅ Inhibits Degranulation in Ca²⁺ Mobilization in IgE/Ag-Activated BMMCs

BMMCs were sensitized overnight with anti DNP-IgE and challenged with DNP-HSA with or without the indicated concentration of V₂O₅. β-hex release (A) and Ca²⁺ mobilization (at 5 min) (B) were evaluated. The values indicate the mean ± S.E.M. from three independent experiments. ###*p* < 0.001 was compared to non-treated BMMCs, and ***p* < 0.01 and ****p* < 0.001 were compared to IgE/Ag sensitized BMMCs.

most important effector cells in immediate-type allergic reactions. Aggregation of the high-affinity IgE receptor (FcεRI) on mast cells triggers release of preformed mediators such as histamine and proteases as well as newly synthesized inflammatory mediators (e.g. lipid mediators, such as PGD₂ and LTC₄, and various cytokines).^{1,2,6} Histamine is one of the most important chemical mediators in pathologic allergic reactions. When mast cells are activated by IgE/Ag, the release of histamine bears a close resemblance to that of β-hex, which is a marker of degranulation. Therefore, the inhibitory effect of JW on IgE/Ag-induced β-hex release from BMMCs was examined. As shown in Fig. 2, JW dose-dependently inhibited β-hex release in BMMCs. As described above, AA was also rapidly metabolized to PGD₂, but this production was short-lived. However, upon prolonged exposure to antigen or cytokines, this initial phase was followed by sustained production of PGD₂. Thus, the kinetics of PGD₂ generation was examined in BMMCs stimulated with IgE/Ag. As shown Fig. 3A (upper panel), BMMCs stimulated with IgE/Ag produced *ca.* 7 ng/mL of PGD₂, and preincubation of BMMCs with JW resulted in the dose-dependent suppression of PGD₂ biosynthesis. Also, as seen in Fig. 3A (lower panel), COX-2 protein was not detected in the unstimulated BMMCs, whereas IgE/Ag strongly induced its formation. IgE/Ag-induced COX-2 protein expression was inhibited by JW in a dose-dependent manner. These results clearly show that inhibition of PGD₂ generation by JW

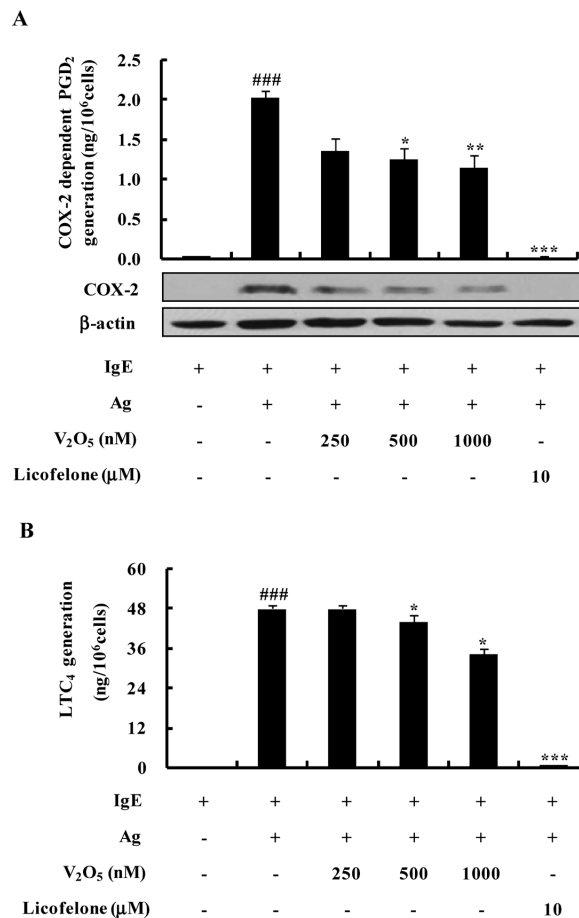


Fig. 6. Effect of V₂O₅ on COX-2-Dependent PGD₂ Generation (A) and 5-LOX-Dependent LTC₄ Generation (B) in IgE/Ag-Activated BMMCs

(A) Activated BMMCs were preincubated with 1 μg/mL of aspirin for 2 h to abolish preexisting COX-1 activity, followed by a brief washing and then stimulation with DNP-HSA for 7 h with or without indicated V₂O₅ and 10 μM licoferone. PGD₂ released into the supernatant was quantified using a PGD₂-MOX enzyme immunoassay kit (upper) and BMMCs were then taken for immunoblotting of COX-2 protein (lower). (B) IgE-sensitized BMMCs were preincubated for 1 h with the indicated volume of V₂O₅ or licoferone after which BMMCs were challenged with DNP-HSA for 15 min and LTC₄ generation was measured. The values indicate the mean ± S.E.M. from three independent experiments. ###*p* < 0.001 was compared to non-treated BMMCs, and **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 were compared to IgE/Ag sensitized BMMCs.

occurs through suppression of COX-2 protein expression.

Leukotriens (LTs) are potent lipid mediators of asthma, inflammation, and various allergic responses.¹⁸ In addition, LTs are associated with atherosclerosis and osteoporosis.^{19–21} When mast cells are activated with IgE/Ag or various stimulants, mast cells release free AA through cytosolic PLA₂α (cPLA₂α), after which free AA can be converted into LTC₄ by the action of 5-LOX/LTC₄ synthase. LTC₄ is a family of eicosanoid lipid mediators that have been implicated in inflammation, proliferation, and allergic conditions like asthma.^{22,23} Thus, we investigated the effects of JW on 5-LOX-dependent LTC₄ generation in BMMCs. As shown in Fig. 3B, BMMCs stimulated with IgE/Ag for 15 min produced approximately 56 ng/10⁶ cells, and JW inhibited LTC₄ biosynthesis in a dose-dependent manner.

Vanadium is a trace mineral present in several kinds of foods, such as mushrooms, shellfish, grains, dill, parsley, black pepper, wine, beer, and sweetened drinks. It is considered essential for normal bone growth, although it is only

needed in very small amounts. It has been reported that vanadium exerts insulin-like effects in *in vitro* as well as *in vivo* systems.^{24,25} Previously, other groups have been reported that a high concentration of vanadate induces release of histamine.^{9–11} In addition, Kitani *et al.* reported the allergic and inflammatory potentials with high concentrations of (over 1 mM) V_2O_5 and orthovanadate (Na_3VO_4), based on the release of histamine from human basophils, rat mast cells, and rat basophilic leukemia (RBL-2H3) cells in the presence of hydrogen peroxide.¹² However, as far as we know, there has been no report on the anti-allergic or anti-inflammatory activity of vanadate at the present time. Our preliminary results show that even at a concentration up to $100\mu M$, V_2O_5 did not induce degranulation or eicosanoid generation in BMMCs (data not shown). Therefore, we assessed the effect of low concentration of V_2O_5 , which is a major component of vanadium in JW, on degranulation and eicosanoid generation in IgE-induced BMMCs. As shown in Fig. 5, V_2O_5 dose-dependently inhibited degranulation in IgE/Ag-induced BMMCs. In addition, V_2O_5 inhibited COX-2-dependent PGD_2 generation through the suppression of COX-2 protein expression (Fig. 6A) and V_2O_5 also dose-dependently inhibited 5-LOX-dependent LTC_4 generation in IgE/Ag-induced BMMCs (Fig. 6B). These results show that the inhibitory kinetics of degranulation and eicosanoid generation of V_2O_5 are similar to those of JW.

In order to evaluate anti-allergic activity of JW or V_2O_5 *in vivo*, a mouse PCA model was used in this study. When 100, 200, and $400\mu L$ of JW or 0.5, 1 and 2 mg/kg of V_2O_5 were orally administrated 1 h prior to Ag challenge, the PCA reaction was attenuated by JW and V_2O_5 in a dose-dependent manner (Fig. 1A and Fig. 4A). In addition, both JW and V_2O_5 inhibited LTC_4 and PGD_2 production in a dose dependent manner (Figs. 1B,C and Figs. 4B,C). These results clearly demonstrate that JW had a potent anti-allergic effect against mast cell-mediated anaphylactic reaction in mice. The precise mechanism for the anti-allergic activity of JW is not fully understood, but results from this study suggest that JW exerts anti-allergic activity *via* the inhibition of both eicosanoid generation and degranulation in activated mast cells at least, in part due to the trace amounts of vanadium in JW. However, it cannot be ruled out that anti-allergic effect of JW could be the additive effect of other trace elements including vanadium. Further experiments are required to confirm anti-allergic activity of other trace elements in JW. Quite recently, Nejime *et al.* reported that vanadate suppressed ATP-induced Ca^{2+} mobilization in HUVEC through the inhibition of the Ca^{2+} pump.²⁶ Our results showed that both JW and V_2O_5 inhibited intracellular Ca^{2+} influx in a dose-dependent manner (Figs. 2B and 5B). It is well known that calcium signal is essential for degranulation, generation of eicosanoid and optimal production of cytokines in mast cells in response to antigen and other stimulants.²⁷ Our data suggest that the inhibition of degranulation and eicosanoid generation by JW and V_2O_5 in IgE/Ag-induced mast cells may be associated with increase intracellular Ca^{2+} level and translocation of phospho-cPLA₂ and 5-LOX to the nuclear membrane. Therefore, the effect of JW or V_2O_5 on phosphorylation of cPLA₂ and translocation of phospho-cPLA₂ as well as 5-LOX in IgE/Ag-induced mast cells will be examined in the near future.

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