



Protective effects of allantoin against ovalbumin (OVA)-induced lung inflammation in a murine model of asthma

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ABSTRACT

Asthma is characterized by difficulty in breathing because of the constriction of the smooth muscles of the bronchi, as a result of inflammation. In the present study, we focus on the protective effects of allantoin against ovalbumin (OVA)-induced lung inflammation in a murine allergic model, and assess cytokine release, eosinophilia, and mucus hypersecretion. Allantoin treatment led to significant reduction in the levels of Ig (immunoglobulin)E and T-helper-2-type cytokines, such as IL(interleukin)-4 and IL-5, in bronchoalveolar lavage (BAL) fluid. Airway inflammatory-cell infiltration was remarkably alleviated in allantoin-treated asthma groups, compared with the control group. Moreover, allantoin-treated asthma groups exhibited a marked decrease in cytokine mRNA expression in lung tissues, compared with the control group. The effectiveness of allantoin was similar to that of montelukast, used as a positive control. These results support the utility of allantoin as a protective agent against asthma.

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1. Introduction

Allergic asthma is a chronic inflammatory disease of the airways characterized by eosinophilia, reversible airway obstruction, airway hyperresponsiveness (AHR), increased mucus production, and remodeling. Escalating environmental pollution, changing food habits, and stressful working conditions have led to the growing incidence of several diseases which affect the immune system. One of the most common chronic diseases is allergic asthma. The prevalence of this disease is rising rapidly, and is of increasing severity in recent years [1]. Protein factors and cytokines involved in the development and perpetuation of inflammation associated with asthma have received considerable attention over the past decade. A number of cellular components and cytokines promote immune cell recruitment and activation, and induce epithelial cell involvement. Although the detailed role(s) of several cytokines identified in the asthmatic lung remain to be established, the predominance and functional importance of T-helper cell type 2 (Th2) cytokines, in particular, and the Th2/T-helper cell type 1 (Th1) cytokine ratio, is rather well established [2,3]. The actions of inflammatory cells in airway allergy are tightly regulated by a network of Th2 cytokines, such as IL(interleukin)-4, IL-5, and IL-13. These cytokines assist in the recruitment and activation of eosinophils [4,5], one of the hallmarks of allergic asthma. Allergic asthma is mediated by a dominant Th2 immune response and characterized by AHR, airway inflammation, increased Ig(immunoglobulin)E levels, and

mucus hypersecretion. In particular, IL-4 is required for differentiation of naive T cells into the Th2 phenotype, and is involved in several critical pathobiological mechanisms, including epithelial injury and mucus secretion [6]. Lymphocytes (predominantly activated CD4+ T cells) increase in number after allergen challenge. These white blood cells play important roles in allergic inflammation by inducing B cells to produce IgE, and additionally release cytokines, such as IL-4 and IL-5, essential for eosinophil recruitment [7].

Allantoin is present in botanical extracts of the comfrey plant and urine from cows and most mammals. Animals and plants convert allantoin to glyoxylic acid and urea via allantoic acid [8]. Allantoin is one of the best-known wound-healing agents, exerting keratolytic and astringent effects and stimulating new tissue formation [9]. Corticosteroids are frequently used to treat patients with asthma, but have some limitations because of adverse reactions. Therefore, novel drugs exerting minimal side-effects are required for asthma therapy. During the course of identification of safe lead compounds from natural sources, we discovered a new therapeutic drug, allantoin. To the best of our knowledge, this is the first study to provide experimental evidence that allantoin inhibits OVA-induced airway inflammation in a murine model of asthma.

2. Materials and methods

2.1. Animals

Female BALB/c (8 weeks) weighing 22–24 g were maintained in specific pathogen-free conditions, and routinely screened serologically for the relevant respiratory pathogens. The animals were

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purchased from Daehan Biolink Co. Ltd. (Seoul, Korea). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to experiments, and provided with water and standard chow *ad libitum*. All experimental procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the protocols of the National Animal Welfare Law of Korea.

2.2. Sensitization and airway challenge

Groups of mice ($n = 7$ or $n = 8$) received the following treatments: (1) sham sensitization plus challenge with phosphate buffered saline (PBS; ipNeb), (2) sensitization plus challenge with OVA (Sigma A5503; Sigma, St. Louis, MO) (ipNeb), and (3) sensitization with OVA (i.p.) plus challenge with OVA (Neb) and 25 or 50 mg/kg allantoin ([Fluka, Germany]) or montelukast (30 mg/kg) p.o. Briefly, mice were sensitized by intraperitoneal injection with 20 μ g OVA emulsified in 2 mg aluminum hydroxide in 200 μ L PBS buffer (pH 7.4) on days 0 and 14. Each mouse was challenged through the airway with OVA (1%, w/v, in PBS) for 30 min using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 21, 22, and 23 after initial sensitization. Mice were orally administered allantoin (25 or 50 mg/kg/day) each day from days 18 to 23 consecutively. Animals were sacrificed 48 h after the last challenge (day 25) to assess the suppressive effects of allantoin. A schematic diagram of the treatment schedule is depicted in Fig. 1.

2.3. Bronchoalveolar lavage fluid (BALF) and accumulation of inflammatory cells

Mice were sacrificed using an overdose of 50 mg/kg of pentobarbital (Sigma) 48 h after the last challenge, and tracheotomy was performed. After instillation of ice-cold PBS (0.6 mL) into the lungs, BAL fluid was obtained by three aspirations (total volume 1.8 mL) via tracheal cannulation. Each aliquot of BAL fluid was centrifuged, and the supernatant collected and stored at -70°C . Total inflammatory cell numbers were assessed by counting cells in at least five squares of a hemocytometer after exclusion of dead cells stained with trypan blue. Cell pellets were washed three times with PBS, resuspended in 1.0 mL PBS and 100 μ L of BAL fluid placed on a slide, and centrifuged (200 g, 4°C , 10 min) for fixation using a cytospin machine (Hanil Science Industrial, Seoul, Korea). After slides were dried, cells were fixed and

stained using Diff-Quik[®] stain reagents (B4132-1A; Dade Behring Inc., Deerfield, IL) according to the manufacturer's instructions.

2.4. Measurement of total and OVA-specific IgE in BAL fluid

Total and OVA-specific IgE levels were measured using Enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 100 μ L/well IgE (2 μ g/mL; Serotec, Oxford, UK) in PBS–Tween 20. Antibodies were detected in BAL fluid with isotype-specific secondary antibodies (anti-mouse IgE; Serotec, Oxford, UK). After washing four times, 200 μ L of *o*-phenylenediamine dihydrochloride (Sigma, St. Louis, MO, USA) was added to each well. Plates were incubated for 10 min in the dark, and absorbance measured at 450 nm. Total and OVA-specific IgE concentrations were calculated from a standard curve generated using 250 ng/mL recombinant IgE (Serotec, Oxford, UK).

2.5. Measurement of Th2 cytokines in BALF

ELISA was performed according to the manufacturer's instructions. IL-4 and IL-5 levels in BAL fluid were measured using specific mouse IL-4 and IL-5 ELISA kits (BioSource International, Camarillo, CA).

2.6. Semi-quantitative RT-PCR

RT-PCR was performed to estimate the relative quantities of IL-4 and IL-5 mRNA in the lung. Total RNA was extracted from the lung tissue using TRIzol reagent, as described by the manufacturer. For cDNA preparation, total RNA was incubated at 37°C for 90 min using the first-strand cDNA synthesis kit (Promega, Madison, WI). The following primer sequences were used: mouse IL-4, 5'-TCT CTA GAT CAT GGG CAT TTT GAA CGA GGT C-3' and 5'-TGC ATG ATG CTC TTT AGG CTT TCC-3', and mouse IL-5, 5'-ATG ACT GTG CCT CTG TGC CTG GAG C-3' and 5'-CTG TTT TTC CTG GAG TAA ACT GGG G-3'. Amplification was performed as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C (IL-4, IL-5, and GAPDH), extension at 72°C for 1 min, and final extension at 72°C for 10 min. GAPDH was used as the internal control for each reaction. The final PCR products were separated on 1% agarose gels, and visualized by ethidium bromide staining. Densitometric analysis was performed using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

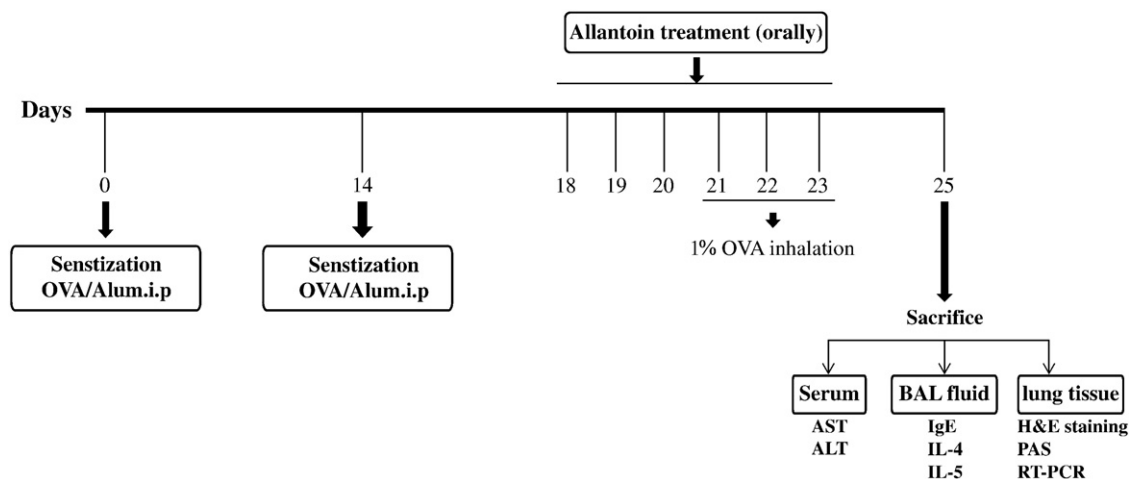


Fig. 1. Mouse model of airway inflammation, and treatment with allantoin.

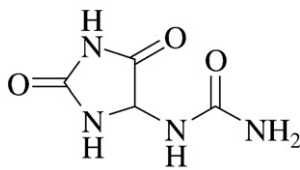


Fig. 2. Chemical structure of allantoin.

2.7. Lung tissue histopathology

After BALF, lungs were fixed by immersion in 10% (v/v) neutral buffered formalin, trimmed and embedded in paraffin. For observation, lungs were cut into 4 μ m sections, and stained with H&E solution (Hematoxylin, Sigma MHS-16, and Eosin, Sigma HT110-1-32) and periodic acid Schiff (PAS) (IMEB Inc., San Marcos, CA) to measure mucus production. Tissues were subsequently mounted and coverslipped using Dako mounting medium (Dakocytomation, Denmark, CA). The degree of cell infiltration in the airway was scored in a double-blind screen by two independent investigators [19]. The peribronchiole and perivascular inflammation was evaluated by using a scoring of 0–5, 0, no cells; 1, a few cells; 2, a ring of cells 1 cell-layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells; and 5, cells deep. Goblet-cell hyperplasia in the airway epithelium was quantified based on a five-point system: 0, no goblet cells; 1, <25% of the epithelium; 2, 25–50% of the epithelium; 3, 50–75% of the epithelium; and 4, >75% of the epithelium. For each mouse, five airway sections that were randomly distributed throughout the left lung were analyzed, and their average scores were calculated. Quantitative analysis of mucus production was performed using an image analyzer (Leica Microsystem Imaging Solution Ltd., Cambridge, UK).

2.8. Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

ALT and AST levels were determined to assess liver function using an autoanalyzer (BECKMAN CX4, CA, USA) and commercial kits (BECKMAN Coulter, Inc., Fullerton, CA, USA).

2.9. Image capture and photomicrography

Photomicrographs were captured using a Photometric Quantix digital camera running a Windows program, and montages assembled

in Adobe Photoshop 7.0. Images were cropped and corrected for brightness and contrast, but not otherwise manipulated.

2.10. Statistical analysis

Data are expressed as means $\pm$ standard deviations (SD). Statistical significance was determined using Student's two-tailed *t*-test and ANOVA test to compare independent mean values, employing Microsoft Excel. The critical level for significance was set at $P < 0.05$.

3. Results

3.1. Effect of allantoin on OVA-induced eosinophilia in BAL fluid

To assess the effects of allantoin (Fig. 2) on eosinophilia in OVA-challenged mice, cells recruited to the BAL fluid were counted 48 h after the last challenge. In PBS-challenged mice, the number of total cells and eosinophils in the BAL fluid remained essentially unchanged. In contrast, OVA-challenged mice displayed a significant increase both in total cells and eosinophils. Oral administration of 25 or 50 mg/kg allantoin 1 h before OVA challenge significantly suppressed the number of eosinophils and total inflammatory cells in a dose-dependent manner, compared to OVA-challenged mice ($p < 0.05$) (Fig. 3). Our results indicate that allantoin has inhibitory effects on lung inflammation in an asthma model.

3.2. Effect of allantoin on inflammatory-cell accumulation in lung tissue

The anti-inflammatory effects of allantoin were further confirmed by histological examination. Inflammatory cells were not detected in peribronchial/peribronchiolar lung areas of PBS-challenged mice. In contrast, OVA-challenged mice displayed severe infiltration of leukocytes into peribronchiole and perivascular connective tissue. Treatment with 25 or 50 mg/kg allantoin led to a significant reduction in inflammatory cells (Fig. 4A and C).

3.3. Effect of allantoin on airway goblet-cell hyperplasia and mucus production

To evaluate airway hypersecretion of mucus and goblet-cell hyperplasia, we stained lung sections with PAS. Goblet-cell hyperplasia was significantly induced in the airway (mucus overproduction was

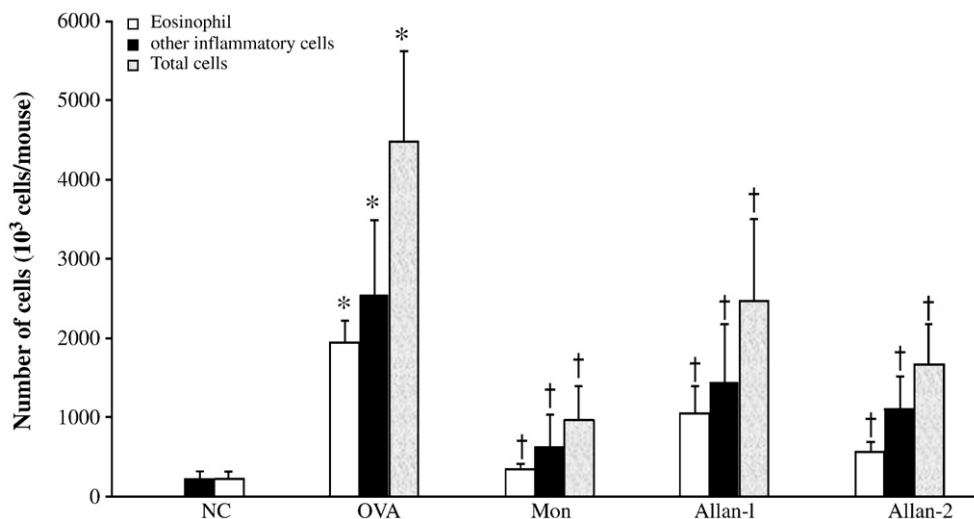


Fig. 3. Effect of allantoin on recruitment of inflammatory cells in bronchoalveolar lavage (BAL) fluid of mice 48 h after the final OVA challenge. Cells were isolated by cytopinning and stained with Diff-Quik. Cell numbers was assessed by direct counting under a light microscope in at least five squares of a hemocytometer after exclusion of dead cells staining with trypan blue. NC, normal control mice (PBS only); OVA, OVA-sensitized/challenged mice; allan-25, allantoin (25 mg/kg) + OVA-sensitized/challenged mice; allan-50, allantoin (50 mg/kg) + OVA-sensitized/challenged mice; Mon, montelukast (30 mg/kg) + OVA-sensitized/challenged mice. Allantoin or montelukast treatment was performed 1 h before challenge. Values are expressed as means $\pm$ SD ($n = 7$ /group). *Significant differences from NC, $P < 0.05$, and †significant differences from OVA, $P < 0.05$.

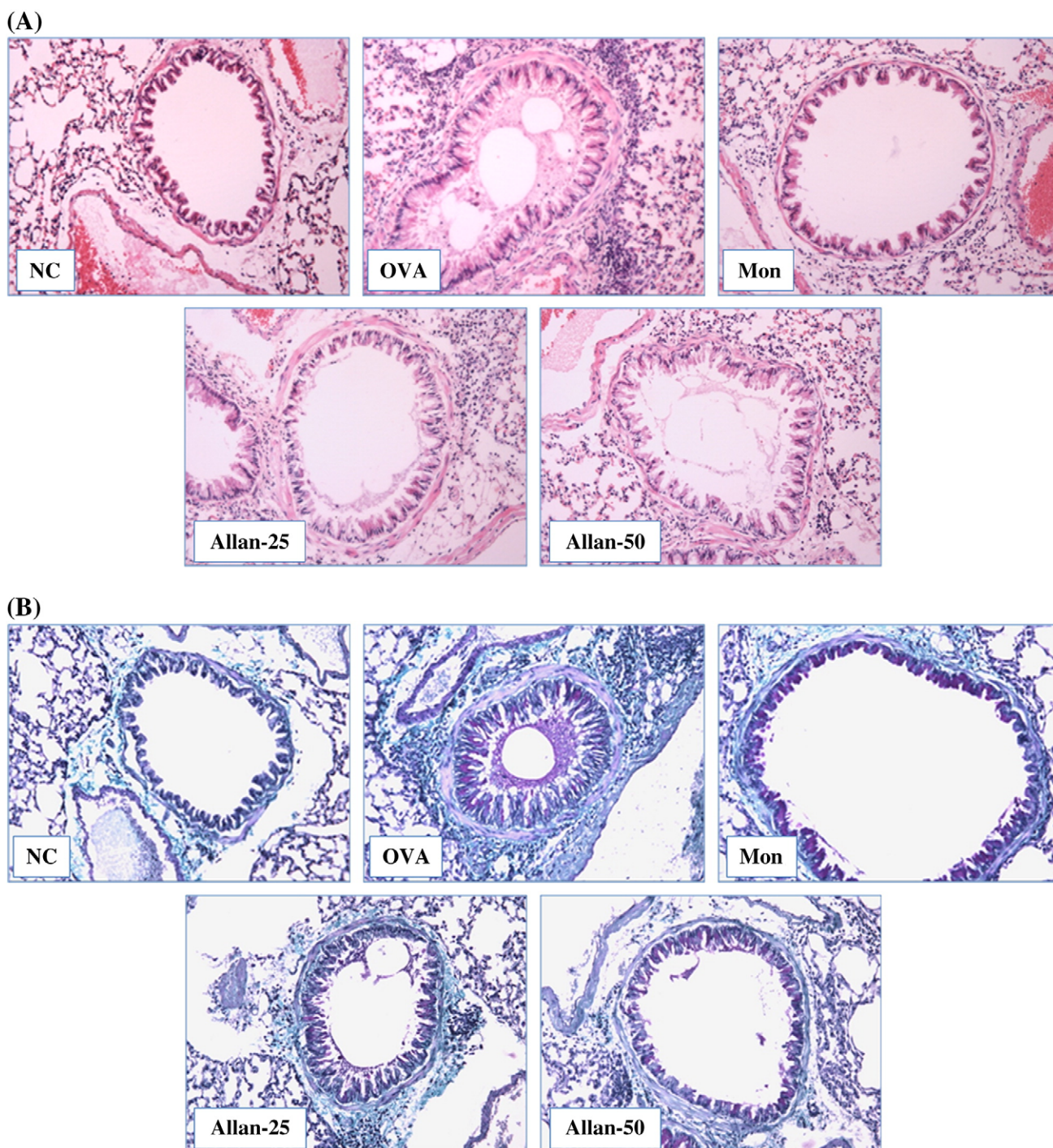


Fig. 4. Effects of allantoin on recruitment of leukocytes and mucus production in lung tissue. Histological examination of lung tissue was performed 48 h after the last OVA challenge. Lung tissues were fixed, sectioned at 4 μ m thickness, and stained with (A) H&E solution and (B) periodic acid Schiff (PAS) for mucus production. (Magnification $\times 200$). (C) Scoring the extent of inflammation by quantitative analysis of inflammatory-cell infiltration in lung sections. NC, normal control mice (PBS only); OVA, OVA-sensitized/challenged mice; allan-25, allantoin (25 mg/kg) + OVA-sensitized/challenged mice; allan-50, allantoin (50 mg/kg) + OVA-sensitized/challenged mice; Mon, montelukast (30 mg/kg) + OVA-sensitized/challenged mice. Allantoin or montelukast treatment was performed 1 h before challenge. *Significant difference from NC, $P < 0.05$, and †significant difference from OVA, $P < 0.05$.

clearly observed as a violet color) in OVA-challenged mice, compared with PBS-challenged control animals. Treatment with allantoin suppressed mucus hypersecretion in the lung tissue in a dose-dependent manner (Fig. 4B). The mucus area was scored as 3.41 ± 0.34 in the OVA-treated mice compared with PBS-treated mice and it was significantly decreased to 2.58 ± 0.33 in the allantoin (50 mg/kg) + OVA-treated mice, which was even lower than the positive reference, montelukast (2.90 ± 0.24) (Fig. 4D). This finding is consistent with the

above results, clearly supporting the theory that allantoin inhibits airway inflammation in asthma.

3.4. Effect of allantoin on the Th2 cytokine level in BAL fluid

To determine the possible inhibitory mechanisms underlying allantoin activity in airway inflammation, we examined the levels of T-helper type 2 (Th2) cytokines associated with asthma in BAL fluid

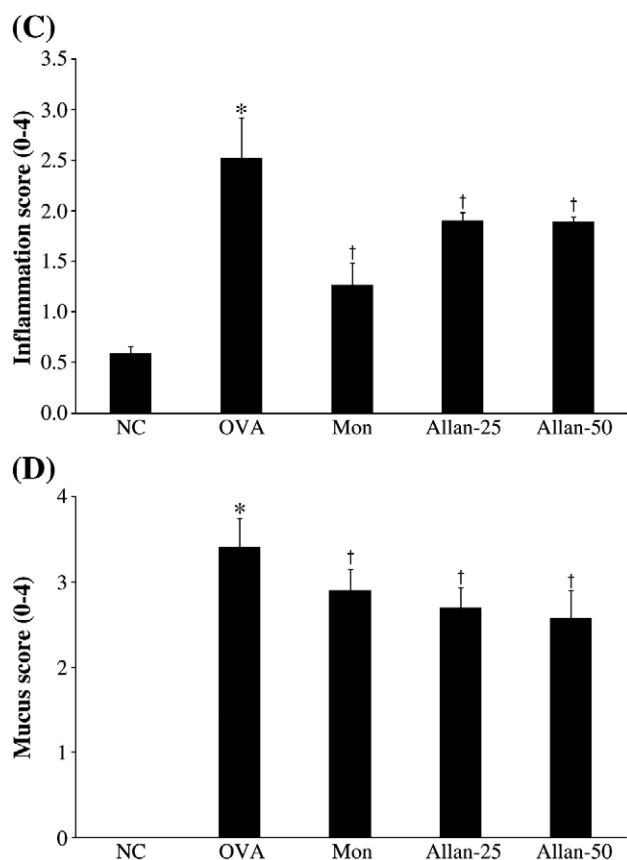


Fig. 4. (continued).

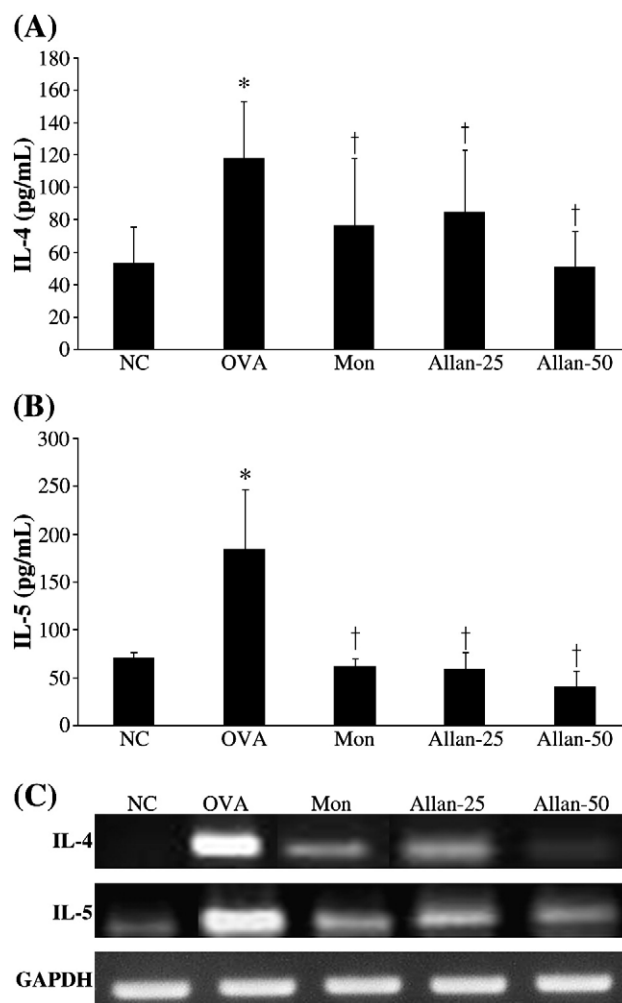


Fig. 5. Effects of allantoin on cytokine levels in BAL fluid and lung tissue. BAL fluid and lung tissue were collected 48 h after the last ovalbumin (OVA) challenge. Each sample was analyzed with ELISA or RT-PCR. (A) IL-4 level in BAL fluid; (B) IL-5 level in BAL fluid; and (C) mRNA level of interleukin (IL)-4 and 5 in lung tissue. NC, normal control mice (PBS only); OVA, OVA-sensitized/challenged mice; allan-25, allantoin (25 mg/kg) + OVA-sensitized/challenged mice; allan-50, allantoin (50 mg/kg) + OVA-sensitized/challenged mice; Mon, montelukast (30 mg/kg) + OVA-sensitized/challenged mice. Allantoin or montelukast treatment was performed 1 h before challenge. Values are expressed as means $\pm$ SD ($n = 7$ /group). *Significant difference from NC, $P < 0.05$, and †significant difference from OVA, $P < 0.05$.

and lung tissue. IL-4 and IL-5 in BAL fluid were markedly increased in OVA-sensitized/challenged mice, compared with PBS-challenged mice. Administration of allantoin (25 or 50 mg/kg) led to a significant reduction in the levels of these Th2 cytokines in BAL fluid and mRNA expression in lung tissue as compared to those in the OVA-sensitized/challenged group (Fig. 5A and B).

3.5. Effect of allantoin on release of total and OVA-specific IgE into BAL fluid

Total and OVA-specific IgE levels were determined by ELISA in each experimental group. IgE levels in BAL fluid were dramatically elevated in OVA-sensitized/challenged mice, compared with PBS-treated animals. Moreover, administration of allantoin led to a reduction in the total and OVA-specific IgE levels (in BAL fluid), compared with those of the OVA-sensitized/challenged group (Fig. 6A and B).

3.6. Cytotoxicity of allantoin in a mouse model of allergic asthma

Specific kits were employed to measure AST and ALT levels in serum, according to the manufacturer's instructions. Allantoin (25 or 50 mg/kg) did not affect the activities of serum toxicity marker enzymes (ALT, AST), indicating that normal liver function was preserved (Fig. 7).

4. Discussion

Current management guidelines describe asthma as a chronic inflammatory disorder of the airways [10,11]. Following OVA sensitization/challenge in mice, classic signs of allergic airway disease were observed, including increased inflammatory cell numbers (lymphocytes, eosinophils, and neutrophils) in BAL fluid, histological evidence of

perivascular and peribronchial inflammation, and increased IL-4, IL-5, and IgE in BAL fluid.

Low levels of mucus are produced in the normal lung, and metaplasia with mucus hypersecretion is a characteristic feature of airway disorders, such as asthma. Considerable evidence supports the theory that inflammatory-cell infiltration and mucus hypersecretion are associated with Th2 cytokine action, which regulates immune functions in mice. IL-4 plays a key role in mucus production through recruitment of Th2 cells to the lungs, and induction of inflammation [12]. Blockage of the IL-4 receptor in mice sensitized and challenged with antigen markedly decreased airway goblet-cell metaplasia. The Th2 cytokines, IL-4 and IL-5, were significantly reduced in response to allantoin administered 1 h before OVA challenge. In contrast, no effect of allantoin on PBS-challenged mice was observed. The dose-related reduction in mucus hypersecretion may be attributed to a decrease in IL-4 production mediated by allantoin. Eosinophils infiltrating the airway also affect mucus secretion by epithelial goblet cells [13]. IL-4 and IL-13 are important in directing B cell growth, differentiation, and secretion of IgE in mice [14]. Considerable evidence supports the characterization as an allergic inflammatory

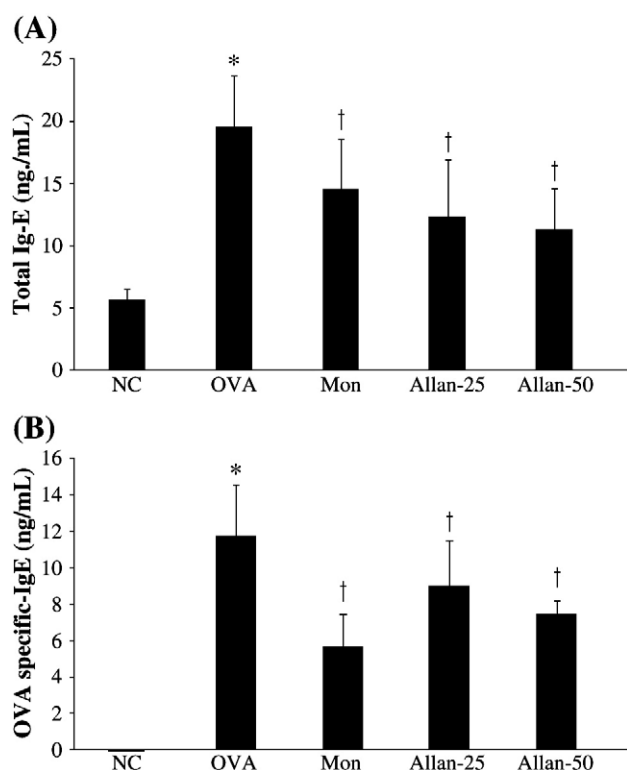


Fig. 6. Effects of allantoin on total and OVA-specific IgE levels in BAL fluid. BAL fluid was collected 48 h after the final ovalbumin (OVA) challenge in mice. Each sample was analyzed using ELISA ($n=7$ /group). (A) Total IgE level in BAL fluid; (B) OVA-specific IgE level in BAL fluid; NC, normal control mice (PBS only); OVA, OVA-sensitized/challenged mice; allan-25, allantoin (25 mg/kg) + OVA-sensitized/challenged mice; allan-50, allantoin (50 mg/kg) + OVA-sensitized/challenged mice; Mon, montelukast (30 mg/kg) + OVA-sensitized/challenged mice. Allantoin or montelukast treatment was performed 1 h before challenge. Values are expressed as means $\pm$ SD ($n=7$ /group). *Significant difference from NC, $P<0.05$, †significant difference from OVA, $P<0.05$.

disease in which immunoglobulin E (IgE) plays a prominent role [15,16]. Our data indicate that the protective effects of allantoin on IL-4 and IL-5 production, and eosinophilia, in BAL fluid, and IL-4 and IL-5 mRNA expressions in lung tissue, result in attenuation of mucus hypersecretion. Histological analyses additionally confirmed that allantoin inhibited inflammatory-cell infiltration into the lung tissue.

Eosinophilia is a significant pathological feature of allergic diseases, possibly contributing to airway damage through release of a myriad of mediators and cytokines [17]. Infiltration of eosinophils into the airways is linked to the production of IL-5, which is important for eosinophil proliferation, activation, and migration [18]. In the present study, we

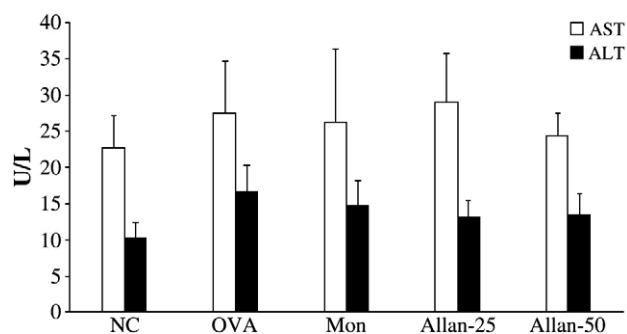


Fig. 7. Levels of AST and ALT in serum. Serum was collected 48 h after the last OVA challenge of mice. Each sample ($n=7$) was analyzed using commercial kits (Beckman Coulter, Inc., Fullerton, CA). Allantoin or montelukast treatment was performed 1 h before challenge.

showed that allantoin inhibited the pulmonary accumulation of eosinophils upon injection in mice, in parallel with the IL-5 decrease in BAL fluid. Our results suggest that allantoin plays a key role in blocking mucus secretion and recruitment of eosinophils in the lung, by inhibiting IL-4 and IL-5-dependent pathways. In our experiments, montelukast was used as a positive control. Allantoin exerted effects similar to those of montelukast on IL-4 and IL-5 levels in BAL fluid. Specifically, upon treatment with allantoin before OVA challenge, we observed a protective effect of allantoin against inflammation, detected as a decrease in eosinophils and lymphocytes in BAL fluid and reduced inflammatory-cell influx into the lungs. Inhibition of the inflammatory response possibly occurs as a result of decreased cytokine levels. Asthmatic lung tissue, infiltrated inflammatory cells, and mucus generation of goblet cells were markedly induced by the airway challenge with OVA, whereas infiltrated inflammatory cells and mucus generation by goblet cells were significantly suppressed by allantoin. We additionally investigated the effects of allantoin on AST and ALT levels in the blood, to confirm the absence of liver toxicity effects. Notably, ALT and AST levels, known indicators of hepatocellular damage, did not change upon oral administration of allantoin. The data indicate that the dose of allantoin used in the present study had no cytotoxic effects on mice.

In summary, we show that allantoin significantly inhibited the increase in total inflammatory cell and eosinophil counts induced by OVA in BAL fluid, the levels of total and OVA-specific IgE in BAL fluid, OVA-induced eosinophilia in lung tissue, and mucus hypersecretion by airway goblet cells, via inhibition of Th2 cytokines (IL-4 and IL-5). Our results strongly support the utility of allantoin as a potential anti-inflammatory agent in asthma treatment.

Acknowledgments

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References

- [1] Liu LY, Coe CL, Swenson CA, Kelly EA, Kita H, Busse WW. School examinations enhance airway inflammation to antigen challenge. *Am J Respir Crit Care Med* 2002;165:1062–7.
- [2] Robinson DS, Hamid Q, Ying S, Tscipoulos A, Barkans J, Bentley AM, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;362:298–04.
- [3] Busse WW, Lemanske Jr RF. Asthma. *N Engl J Med* 2001;344:350–62.
- [4] Lemanske RF, Busse WW. Asthma. *J Allergy Clin Immunol* 2003;111(suppl 2):S502–19.
- [5] Ying S, Zhang G, Gu S, Zhao J. How much do we know about atopic asthma: where are we now? *Cell Mol Immunol* 2006;3:321–32.
- [6] Steinke JW, Borish L. Th2 cytokines and asthma – interleukin-4: its role in the pathogenesis of asthma, and targeting its for asthma treatment with interleukin-4 receptor antagonists. *Respir Res* 2001;2:66–70.
- [7] Durham SR. Mechanisms of mucosal inflammation in the nose and lungs. *Clin Exp Allergy* 1998;8(suppl 2):11–26.
- [8] LasKowsx M. Allantoinase and allantoinase. *Enzymes* 1951;1:946–50.
- [9] Willital GH, Heine H. Efficacy of Contractubex gel in the treatment of fresh scars after thoracic surgery in children and adolescents. *Int J Clin Pharmacol Res* 1994;14:193–202.
- [10] National Institutes of Health/National Heart, Lung, and Blood Institute. Global Initiative for Asthma. Global strategy for asthma management and prevention (revised 2007). Available at: <http://www.ginasthma.org>. Accessed 1 October 2009.
- [11] National Heart Lung and Blood Institute/National Asthma Education and Prevention Program NHLBI/NAEPP. Expert Panel Report 3: guidelines for the diagnosis and management of asthma. NIH Publication No. 07-4051. Bethesda, MD: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. 2007.
- [12] Steinke JW. Anti-interleukin-4 therapy. *Immunol Allergy Clin North Am* 2004;24:599–614.
- [13] Shimizu T, Shimizu S, Hattori R, Majima Y. A mechanism of antigen-induced goblet cell degranulation in the nasal epithelium of sensitized rats. *J Allergy Clin Immunol* 2003;112:119–25.
- [14] Emson CL, Bell SE, Jones A, Wisden W, McKenzie AN. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *J Exp Med* 1998;188:399–404.

- [15] Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005;115:459–65.
- [16] Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. *Nature* 1999;402(Suppl 6760):B12–7.
- [17] Gavett SH, Chen X, Finkelman F, Wills-Karp M. Depletion of murine CD4⁺T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994;10:587–93.
- [18] Foster PS, Hogan ST, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195–201.
- [19] Myou S, Leff AR, You S, Boetticher E, Tong J, Algelo YM, et al. Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase-TAT. *J Exp Med* 2003;198:1573–82.