



Genipin inhibits allergic responses in ovalbumin-induced asthmatic mice

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ABSTRACT

Genipin is a natural compound isolated from the fruit of *Gardenia jasminoides* with various pharmacological effects. In this study, we investigated whether genipin effectively alleviates allergic responses in a murine model of ovalbumin (OVA)-induced asthma. The mice were administered an intraperitoneal injection of OVA on day 0 and 14 to boost the immune response; genipin was then administered from day 18 to 23 by oral gavage. On days 21 to 23, mice were OVA-challenged using an ultrasonic nebulizer, and airway hyperresponsiveness (AHR) was determined on day 24 by plethysmography. Genipin significantly reduced the inflammatory cell count in bronchoalveolar lavage fluids (BALF) and AHR, which were accompanied by lower interleukin-5 (IL-5), IL-13 and OVA-specific immunoglobulin (Ig) E levels in the BALF or serum from OVA-induced asthmatic mice. In histology, genipin significantly decreased airway inflammation and mucus hypersecretion in OVA-induced asthmatic mice. Additionally, genipin inhibited OVA-induced increases in the expression of inducible nitric oxide synthase and cyclooxygenase-2 proteins. Further, genipin reduced the activity and protein levels of matrix metalloproteinase-9 in lung tissue from OVA induced asthmatic mice. Overall, genipin effectively alleviated the asthmatic inflammatory response in an OVA-induced asthmatic model. Therefore, our results suggest that genipin has therapeutic potential for treating asthma.

1. Introduction

Asthma is an important pulmonary disease that has markedly increased in recent decades [1] and is commonly induced by allergen exposure, including house dust, pollen, animal dander, and smoking [2,3]. Further, asthma is characterized by eosinophil accumulation, mucus hypersecretion, and airway hyperresponsiveness (AHR) [4]. These pathophysiological changes are closely associated with elevated allergen-specific type 2 T helper (Th2) cytokines, including interleukin (IL)-4, -5, and -13. Th2 cytokines are involved in eosinophil maturation, infiltration, and activation, as well as IgE and mucus production, which eventually induce the asthmatic responses [5]. Other signaling molecules related to the inflammatory response, such as inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2 and matrix metalloproteinase (MMP)-9, also play a critical role in the progression of airway inflammation in allergic asthma [6,7]. In addition to inducing airway inflammation, COX-2 and iNOS aggravate the inflammatory response in damaged lesions, while increased MMP-9 expression plays a role in the development of asthma by inducing inflammatory cell infiltration into lung tissue, and finally, airway remodeling [7].

Therefore, suppression of the inflammatory response is considered an important step for controlling asthma.

Genipin is an aglycon of geniposide and a natural iridoid compound extracted from the fruit of *Gardenia jasminoides* that has various pharmacological properties, including antioxidative, anti-inflammatory, anticancer, and antifungal activities [8,9]. According to previous studies, the anti-inflammatory effects of genipin are exerted via iNOS and COX-2 suppression [10]. It has also been reported that MMP-9, a protein related to inflammation and tissue remodeling, is regulated by genipin [9] and the protective effects of genipin against acute lung injury have been previously investigated [11,12]. Despite the known anti-inflammatory and pharmacological properties of genipin against pulmonary disease, the antiasthmatic of genipin has not been previously explored.

Therefore, we investigated the protective effects of genipin in an ovalbumin (OVA)-challenged asthmatic mouse model by measuring inflammatory cells, cytokine levels and mucus secretion. Additionally, we elucidated the underlying mechanisms associated with the effects of genipin by focusing on the anti-inflammatory response, specifically the expression of iNOS, COX-2 and MMP-9.

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2. Materials and methods

2.1. Animals

BALB/c female mice (6-week-old, Koatech Co., Pyeongtaek, Republic of Korea) were used after 1 week of quarantine and acclimatization. The mice were housed under standard condition and provided sterilized tap water and standard rodent chow (Samyang Feed, Wonju, Republic of Korea) *ad libitum*. The Institutional Animal Care and Use Committee of Chonnam National University approved the protocols for the animal study (CNU IACUC-YB-R-2016-19), and the animals were cared for in accordance with the National Institutes of Health Guidelines for the care and use of laboratory animals and Korean national laws for animal welfare.

2.2. Ovalbumin-induced asthma and experimental groups

The animals were treated with 20 µg OVA (Sigma-Aldrich Co., St. Louis, MO, USA) with 2 mg aluminum hydroxide (Sigma-Aldrich Co.) by intraperitoneal injection on day 0 and 14 and then airway challenged using an OVA solution (1% w/v) with a nebulizer (NE-U12, Omron Co., Tokyo, Japan) for 1 h from day 21 to 23. Dexamethasone and genipin were dissolved in phosphate buffered saline (PBS) and administered to the mice by oral gavage from day 18 to 23. The animals were grouped into five groups ($n = 7$) as follows: NC (normal control, PBS sensitization/challenge), OVA (OVA sensitization/challenge); DEX (OVA sensitization/challenge + dexamethasone 3 mg/kg); and GEN-10 or 20 (OVA sensitization/challenge + genipin 10 or 20 mg/kg, respectively).

2.3. Airway hyperresponsiveness

AHR was evaluated by whole-body plethysmography (OCP3000, Allmedicus, Seoul, Republic of Korea) 24 h after the final OVA challenge. Each animal was placed on a plastic chamber and exposed to methylcholine (Sigma-Aldrich) aerosols via a nebulizer at increasing concentrations (0, 10, 20, and 30 mg/mL) for 3 min. Finally, enhanced pause (Penh) value was measured for 3 min after the methylcholine exposure.

2.4. Analysis of bronchoalveolar lavage fluid

The animals were anesthetized with Zoletil 50® (10 mg/kg, *i.p.*; Virbac Laboratories, Carros, France) 24 h after measuring AHR, and blood was collected via the caudal vena cava. To separate serum, blood was incubated for 1 h at room temperature and the supernatant collected after centrifugation at 200g for 10 min. BALF was collected as previously described [13]. Specifically, a tracheotomy was performed, and an endotracheal tube was inserted into the trachea. Ice-cold PBS (0.7 mL) was infused into the lung and withdrawn two times (total volume 1.4 mL). To count inflammatory cells, 100 µL BALF was centrifuged (200g, 4 °C, 10 min) onto slides using a cytospin (Hanil Science Industrial, Seoul, Korea). The slides were dried, fixed, and stained with Diff-Quik® reagent (Sysmex Co., Kobe, Japan). The remaining BALF was centrifuged (200g, 4 °C, 10 min), and its supernatant was stored at –80 °C for Th2 cytokine analysis. IL-5 and IL-13 levels were determined in BALF via a commercial enzyme-linked immunosorbent assay (ELISA, R & D System Inc., Minneapolis, MN, USA).

2.5. Analysis of OVA-specific immunoglobulin (Ig) E in serum

The production of OVA-specific IgE in serum was evaluated using an ELISA kit (BioLegend, San Diego, CA, USA). Specifically, 96-well microtiter plates were coated overnight with OVA (10 µg/mL in PBS-Tween-20). After incubation, the 100 times diluted samples were added to each well and the plates were washed, blocked and incubated for an

additional 2 h. The plates were then washed, horse-radish peroxidase (HRP)-conjugated goat anti-mouse IgE antibodies were added, and the plates were washed again. *o*-Phenylenediamine dihydrochloride (200 µL, Sigma-Aldrich) was then added, and the plates were incubated for 10 min in the dark. Absorbance was measured at 450 nm using an ELISA plate reader (Bio-Rad Laboratories, Hercules, CA, USA). The sigmaplot (Systat software, Inc., San Jose, CA) was used to plot the logistic, 4-parameter graph which was used to calculate the measured absorbances.

2.6. Histopathological analysis of the lung tissue

After BALF collection, the lung tissue was fixed in neutral buffered formalin, embedded in paraffin, and sectioned 4-µm thick. The tissues were stained with hematoxylin and eosin (H & E) solution (Sigma-Aldrich) to evaluate airway inflammation and periodic acid Schiff (PAS) (IMB Inc., San Marcos, CA, USA) to measure mucus production. Each slide was examined manually in a totally blinded manner using a light microscope (Leica, Wetzlar, Germany) with 10 and 20 × objective lenses and a 100 × oil immersion lens. Ten randomly selected non-overlapping areas per slide were captured with digital camera (IMTcamCCD5; IMT Inc., Daejeon, Republic of Korea), and quantitative analyses of airway inflammation and mucus production were performed using an image analyzer (IMT i-Solution software, Vancouver, BC, Canada).

2.7. Analysis of MMP-9 in lung tissue

To estimate MMP-9 activity, gelatin zymography was carried out as described previously [14]. Briefly, equal amounts of total cellular protein (30 µg) were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) containing 1% gelatin (Sigma-Aldrich) as an MMP substrate. Quantitative analysis of MMP-9 activity was performed via a ChemiDoc (Bio-Rad Laboratories).

To determine MMP-9 expression, we performed immunohistochemistry (IHC) per a previous report [13]. Paraffin-embedded sections were deparaffinized, dehydrated, washed with PBS containing 0.3% Triton X-100, and incubated for 10 min at room temperature with 10% goat serum to block non-specific staining. The slides were incubated overnight with primary mouse MMP-9 antibodies (1:200 dilution, Santa Cruz Biotechnology, Dallas, TX, USA). After incubating with the primary antibodies, the slides were washed and incubated with biotinylated secondary antibodies at 37 °C for 1 h and then incubated with an avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. The excess complex was removed and slides were washed with PBS prior to incubating in 0.05% diaminobenzidine (1:200, Millipore Co., Bedford, MA) for 10 min. The slides were counterstained, rinsed with PBS to terminate the reaction, and protected with coverslips prior to microscopic examination. Each slide was examined manually in a totally blinded manner using a light microscope (Leica) with 10 and 20 × objective lenses and a 100 × oil immersion lens. Ten randomly selected non-overlapping areas per slide were captured with digital camera (IMTcamCCD5; IMT Inc.), and quantitative image analysis of MMP-9 expression was conducted via IMT i-Solution software.

2.8. Western blot analysis

Lung tissue was homogenized with tissue lysis reagent (Sigma-Aldrich) containing a protease inhibitor cocktail (Sigma-Aldrich). Protein concentrations in the samples were determined using Bradford reagent (Bio-Rad Laboratories). Western blotting was performed as described previously [13], and following the antibodies were used: iNOS (Cell Signaling, Danvers, MA, USA), COX-2 (Cell Signaling), β-actin (Cell Signaling) and MMP-9 (Santa Cruz Biotechnology). To determine protein expression, band densities were quantified using a ChemiDoc (Bio-Rad Laboratories).

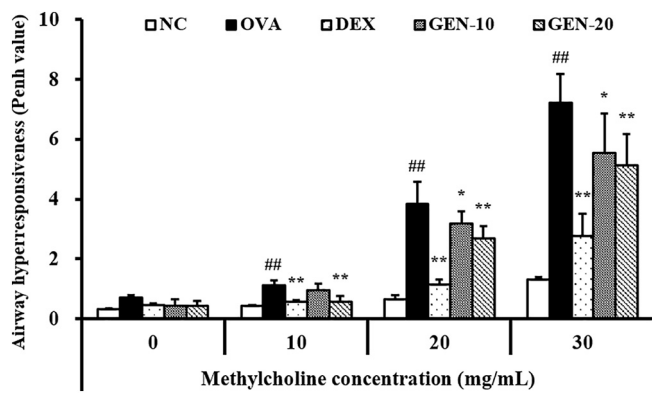


Fig. 1. Genipin reduces airway hyperresponsiveness (AHR) induced by an ovalbumin (OVA) challenge. AHR was determined using whole-body plethysmography (Allmedicus). NC, normal control mice treated with phosphate-buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD ($n = 7$). ^{##}Significantly different from NC, $p < 0.01$; ^{****} significantly different from OVA, $p < 0.05$ and < 0.01 , respectively.

2.9. Statistical analysis

Data are expressed as the means $\pm$ standard deviation (SD). The statistical significance of the results was determined by analysis of variance, followed by a multiple comparison test with Dunnett's adjustment. Statistical analyses were performed using Graph Pad InStat v 3.0 (GraphPad, La Jolla, CA, USA). p -Values < 0.05 were considered to indicate a statistically significant difference.

3. Results

3.1. Genipin reduces AHR in OVA induced asthmatic mice

AHR significantly increased with increasing concentrations of methylcholine in OVA-induced asthmatic mice (Fig. 1); however, this effect was attenuated by treatment with genipin. In particular, AHR was significantly lower in genipin-treated mice than that in untreated OVA-induced asthmatic mice at exposed 20 and 30 mg/mL methylcholine.

3.2. Genipin decreases eosinophilia in BALF from OVA-induced asthmatic mice

The number of inflammatory cells in BALF from OVA-induced asthmatic mice, particularly eosinophils, was higher than that in the normal controls (Fig. 2). In contrast, the inflammatory cell count in

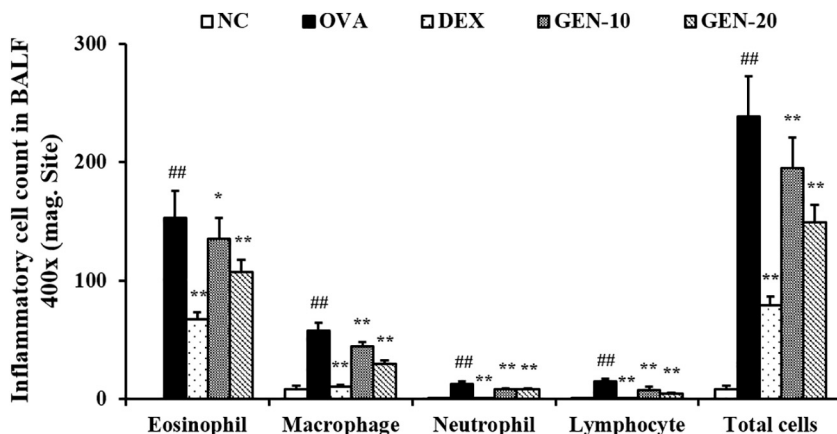


Fig. 2. Genipin decreases the number of inflammatory cells in bronchoalveolar lavage fluid (BALF) from ovalbumin (OVA)-induced asthmatic mice. The cells were isolated via centrifugation and stained with Diff-Quik® stain reagent. NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD ($n = 7$). ^{##}Significantly different from NC, $p < 0.01$; ^{****} significantly different from OVA, $p < 0.05$ and < 0.01 , respectively.

BALF from genipin-treated mice was significantly lower than that in untreated OVA-induced asthmatic animals; reductions were markedly observed in mice administered 20 mg/kg genipin.

3.3. Genipin suppresses the elevation in Th2 cytokines and OVA-specific IgE observed in OVA-induced asthmatic mice

IL-5 levels in BALF were significantly higher in OVA-induced asthmatic mice than that in the normal controls (Fig. 3A). In contrast, IL-5 levels in BALF from genipin-treated mice were markedly lower than those in untreated OVA-induced asthmatic mice. Although IL-5 levels decreased in mice treated with 10 mg/kg genipin, the effect was not significant. Similar to the IL-5 results, IL-13 levels in BALF from OVA-induced asthmatic mice were markedly elevated (Fig. 3B). Further, IL-13 levels were significantly lower in genipin-treated mice than those in untreated OVA-induced asthmatic mice. OVA-specific IgE levels were markedly higher in OVA-induced asthmatic mice than those in the normal controls, whereas OVA-specific IgE levels were significantly lower in genipin-treated mice than those in untreated OVA-induced asthmatic mice (Fig. 3C).

3.4. Genipin attenuates airway inflammation and mucus production in OVA-induced asthmatic mice

A marked increase in inflammatory cell accumulation was observed in the lung tissue from OVA-induced asthmatic mice (Fig. 4A); however, airway inflammation was significantly lower in genipin-treated mice than that in untreated OVA-induced asthmatic mice. Further, bronchial lesions, which are representative of mucus production, remarkably increased in OVA-induced asthmatic mice (Fig. 4B). In contrast, mucus production was significantly less in genipin-treated mice than that in untreated OVA-induced asthmatic mice.

3.5. Effects of genipin on the expression of iNOS, COX-2, and MMP-9 in lung tissue from OVA-induced asthmatic mice

Inflammatory proteins, including iNOS and COX-2, were markedly higher in OVA-induced asthmatic mice than those in the normal controls (Fig. 5). However, the expression of iNOS and COX-2 was significantly less in genipin-treated mice than that in untreated OVA-induced asthmatic mice. Consistent with the expression results for iNOS and COX-2, the expression of MMP-9 was markedly higher in OVA-induced asthmatic mice than that in the normal controls; however, MMP-9 was considerably lower in mice administered genipin than that in untreated OVA-induced asthmatic mice.

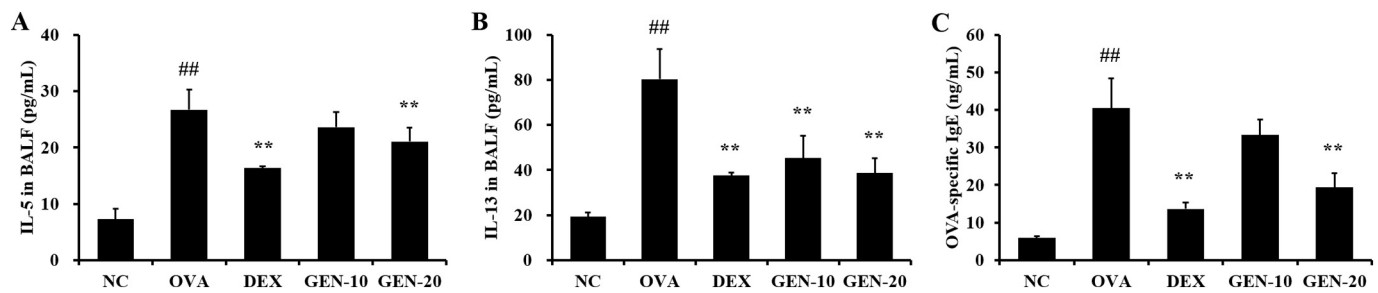


Fig. 3. Genipin reduces interleukin (IL)-5, IL-13, and ovalbumin (OVA)-specific immunoglobulin (Ig)E in OVA-induced asthmatic mice. Levels of IL-5, IL-13, and OVA-specific IgE were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. (A) IL-5 levels, (B) IL-13 levels, and (C) OVA-specific IgE level are shown. NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD (n = 7). ^{##}Significantly different from NC, $p < 0.01$; ^{**}significantly different from OVA, $p < 0.01$.

3.6. Effects of genipin on the expression and activity of MMP-9 in lung tissue from OVA-induced asthmatic mice

MMP-9 was excessively expressed in the lung tissue of OVA-induced asthmatic mice (Fig. 6). However, MMP-9 expression was markedly lower in the lung tissue from genipin-treated mice than that in untreated OVA-induced asthmatic mice. Consistent with the MMP-9 expression results, MMP-9 activity was markedly elevated in OVA-induced asthmatic mice; however, it was meaningfully reduced in mice administered genipin (Fig. 7).

4. Discussion

Asthma is a chronic airway inflammatory disease that threatens

human health, and its incidence has been growing steadily in recent years. The characteristics of asthma include chronic airway inflammation, mucus overproduction, and AHR, which finally results in airway obstruction and limited flow of the normal airway [1]. In the present study, we explored the effects of genipin on airway inflammation using an OVA-induced asthma model. Genipin effectively decreased inflammatory cell infiltration, IL-5, and IL-13 levels in BALF, as well as OVA-specific IgE in serum of OVA-induced asthmatic mice; these phenomena were accompanied by reduced inflammatory cell accumulation and mucus production in the lung tissue. Additionally, genipin markedly reduced the expression of iNOS, COX-2, and MMP-9 in the lung tissue from OVA-induced asthmatic mice.

Eosinophilia, which is associated with airway inflammation, and mucus overproduction are regarded as crucial characteristics of asthma

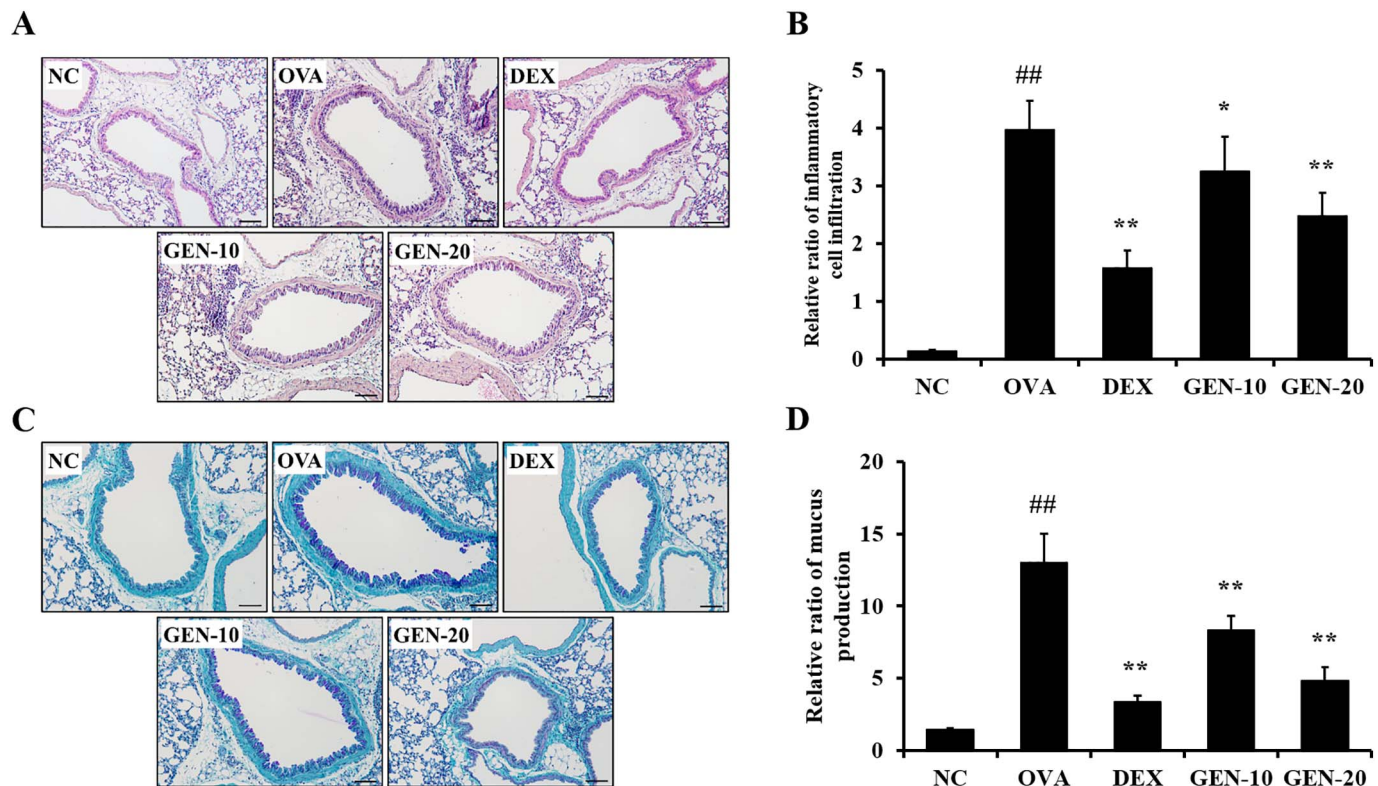


Fig. 4. Genipin decreases airway inflammation and mucus overproduction in lung tissues from ovalbumin (OVA)-induced asthmatic mice. To evaluate airway inflammation and mucus production, lung tissues were stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) reagents, respectively. Scale bar = 50 μ m. Quantitative analysis of the inflammatory response was performed using IMT i-Solution software (IMT i-Solution Inc.). (A) A representative figure of lung tissue stained with H&E solution, (B) quantitative analysis of airway inflammation, (C) a representative figure of lung tissue stained with PAS solution, and (D) quantitative analysis of mucus overproduction are shown. NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD (n = 7). ^{##}Significantly different from NC, $p < 0.01$; ^{***}significantly different from OVA, $p < 0.05$ and $p < 0.01$, respectively.

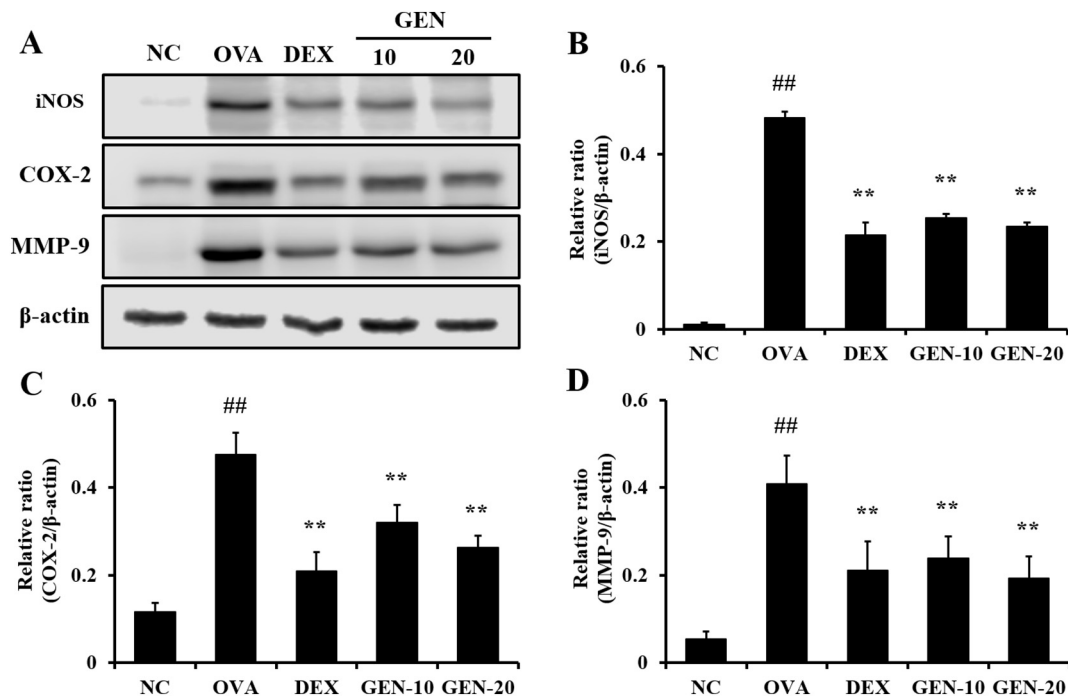


Fig. 5. Genipin reduces ovalbumin (OVA)-induced inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and matrix metalloproteinase-9 (MMP-9) expression in lung tissue. Protein expression was analyzed via western blot, and the relative protein expression ratios were evaluated. Densitometric values of protein expression were determined by means of a Chemi-Doc imaging system (Bio-Rad Laboratories). (A) Gel images showing protein expression, (B) the relative ratio of iNOS, (C) the relative ratio of COX-2, and (D) the relative ratio of MMP-9 are shown. NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD (n = 7). ^{##}Significantly different from NC, $p < 0.01$; ^{**}significantly different from OVA, $p < 0.01$, respectively.

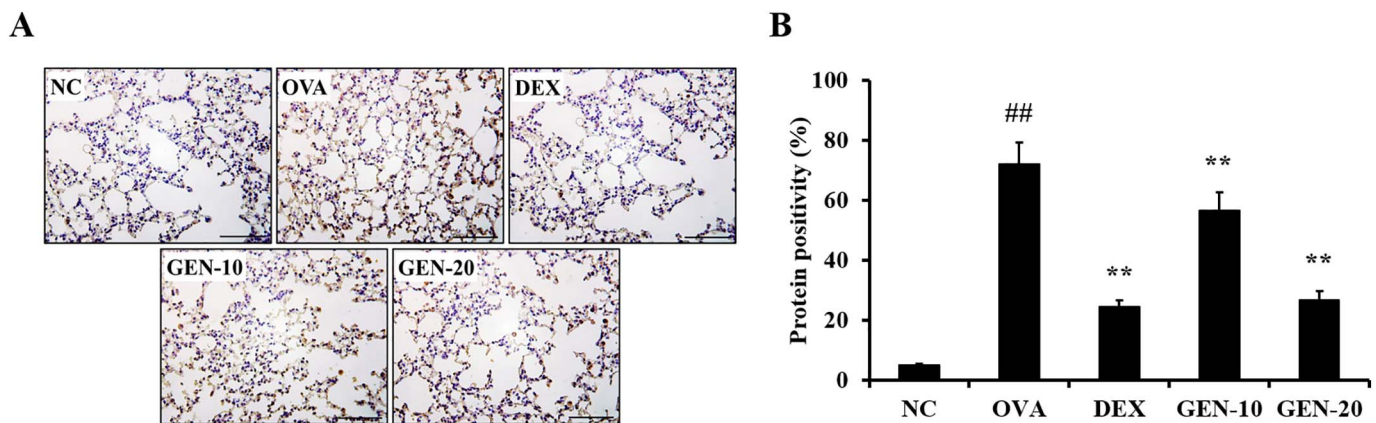


Fig. 6. Genipin decreases matrix metalloproteinase-9 (MMP-9) expression in lung tissues from ovalbumin (OVA)-induced asthmatic mice. Protein expression was analyzed via immunohistochemistry, and values of protein expression were determined using IMT i-Solution software (IMT i-Solution Inc.). Scale bar = 50 μ m. (A) Immunohistochemical staining for MMP-9 in mouse lung tissue is shown. (B) Quantitative analysis of MMP-9 staining is shown. NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN-20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD (n = 7). ^{##}Significantly different from NC, $p < 0.01$; ^{**}significantly different from OVA, $p < 0.01$.

[15]. These phenomena are related to various factors, particularly Th2 cytokines, and IgE production, which are involved in eosinophil recruitment, maturation, and activation [16]. Additionally, Th2 cytokines aggravate the inflammatory response, mucus hypersecretion, and smooth muscle contraction, attributable to the production of other proinflammatory mediators such as chemokines, inflammatory proteins, and proteolytic enzymes [17]. Therefore, modulation of Th2 cytokine production is considered as an important factor for the treatment of asthma [7,18]. In this study, genipin significantly decreased the number of inflammatory cells, particularly eosinophils in BALF from OVA-induced asthmatic mice. Consistent with the results from the inflammatory cell count, IL-5 and IL-13 in BALF and OVA-specific IgE in serum from OVA-induced asthmatic mice were markedly suppressed by

treatment with genipin. Furthermore, genipin attenuated the accumulation of inflammatory cells and mucus overproduction in lung tissue from OVA-induced asthmatic mice. These data suggest that genipin is a potential therapeutic material for the treatment of asthma via Th2 cytokine suppression.

Airway inflammation is induced by various inflammatory proteins, including iNOS and COX-2. These proteins are activated by several stimuli and aid in the progression of inflammatory responses via their specific signaling [19]. The iNOS enzyme synthesizes nitric oxide (NO) from L-arginine. NO produced by iNOS contributes to the inflammatory response, cell injury, and tissue damage [20]. In particular, NO is considered an important factor in the development of airway inflammation in asthmatic patients [7]. On the other hands, iNOS

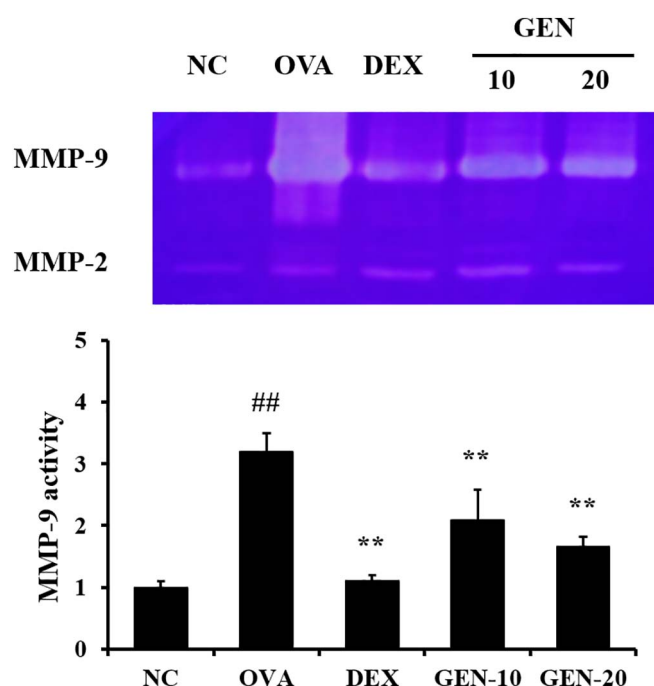


Fig. 7. Genipin reduces matrix metalloproteinase-9 (MMP-9) activity in lung tissue from ovalbumin (OVA)-induced asthmatic mice. MMP-9 activity was determined via gelatin zymography. Quantitative analysis of MMP-9 activity was performed with a ChemiDoc (Bio-Rad Laboratories). NC, normal control mice treated with phosphate buffered saline (PBS) only; OVA, OVA-induced asthmatic mice; DEX, OVA-induced asthmatic mice treated with dexamethasone (10 mg/kg); GEN-10 and GEN 20, OVA-induced asthmatic mice treated with genipin (10 and 20 mg/kg, respectively). Values are expressed as means $\pm$ SD ($n = 7$). ##Significantly different from NC, $p < 0.01$; **significantly different from OVA, $p < 0.01$.

expression is closely involved in the production of Th2 cytokines, which eventually results in asthmatic signs, such as eosinophilia, bronchial constriction, mucus overproduction, and airway remodeling [21]. Elevated COX-2 expression has been observed in asthma [6], and inflammatory cell recruitment increases COX-2 expression by stimulating toxic materials such as lipopolysaccharide (LPS), allergens, and chemical, via stimulation by toxic materials such as LPS, allergens, and chemicals, resulting in increased expression of prostaglandins and exacerbation of the inflammatory response [22–24]. In this study, genipin markedly decreased the expression of iNOS and COX-2 in lung tissues from OVA-induced asthmatic mice. These results indicate that the anti-inflammatory functions of genipin may be related to suppressed iNOS and COX-2 expression. Further, these properties of genipin are supported by previous studies. Specifically genipin reportedly inhibits LPS and interferon- γ -induced NO production and iNOS expression in RAW 264.7 cells [25] and exhibits protective effects against acute lung injury [26] and carrageenan-induced paw edema [27] by inhibiting iNOS and COX-2 expression. Based on these studies, the protective effects of genipin on asthmatic responses could be attributable to genipin-induced suppression of iNOS and COX-2 expression.

MMP-9 is a proteolytic enzyme involved in the extracellular matrix. During the development of asthma, MMP-9 is associated with the infiltration of inflammatory cells into the lung tissue and airway remodeling attributable to degradation of extracellular matrix [14,28]. Indeed, MMP-9 expression markedly increases in the BALF, blood, and sputum of patients with asthma [29], and treatment with an MMP-9 inhibitor suppresses the asthmatic response [30]. Furthermore, MMP-9 is modulated by iNOS expression [31] and aggravates the asthmatic response by upregulating Th2 cytokine production [14]. In the present study, genipin markedly reduced the activity and expression of MMP-9 in lung tissue from OVA-induced asthmatic mice. These results indicate that genipin effectively suppresses the MMP-9 expression, thereby

inducing a release in Th2 cytokines and consequent inflammatory response, leading to allergic asthma.

In conclusion, genipin effectively reduced the inflammatory response observed in OVA-induced asthmatic mice, including eosinophilia, mucus overproduction, and the production of Th2 cytokine and OVA-specific IgE. These properties were closely related to suppressed iNOS, COX-2, and MMP-9 expression. Overall, our results suggest that genipin is a potent therapeutic agent for the treatment of asthma.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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