Research Article

Ethanol Extract of Sanguisorbae Radix Inhibits Mast Cell Degranulation and Suppresses 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis-Like Skin Lesions

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Sanguisorbae Radix (SR) is well known as herbal medicine named “Zi-Yu” in Korea, which is the dried roots of Sanguisorba officinalis L. (Rosaceae). We investigated the underlying mechanism on the inhibition of atopic dermatitis (AD) of an ethanol extract of SR (ESR) using 2,4-dinitrochlorobenzene- (DNCB-) induced AD mice model. Oral administration of ESR significantly suppressed DNCB-induced AD-like symptoms such as scratching behavior, ear thickness, epidermal thickness, and IgE levels. To investigate the effects of ESR treatment on degranulation of IgE/Ag-activated mouse bone marrow-derived mast cells (BMMCs), we measured the release of β-hexosaminidase (β-HEX, degranulation marker). ESR decreased the infiltration of eosinophils and mast cells into the AD skin lesions. Furthermore, ESR significantly inhibited degranulation of IgE/Ag-activated BMMCs. We have demonstrated that ESR decreased AD symptoms in mice and inhibits degranulation of IgE/Ag-activated mast cells. Our study suggests that ESR may serve as a potential therapeutic candidate for the treatment of AD symptoms.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease. AD causes epidermal thickness with cutaneous hypersensitivity associated with increased serum immunoglobulin E (IgE) levels and infiltration of inflammatory cell types including mast cells and eosinophils [1, 2]. AD is a complex interaction of innate and adaptive immune responses based on an individual’s genetic, environmental, pharmacological, and psychological conditions.

IgE secretion is an important characteristic of AD, with elevated levels related to disease severity in AD patients. Previous studies report that high serum IgE levels induce activation of mast cells and cause an allergic reaction [2, 3]. Because mast cells aggregate high-affinity IgE receptors (FcεRI) on their surfaces, which is important in the proinflammatory/allergic response, mast cells are believed to play an important role in the induction of AD [4, 5]. An association between mast cell activation and AD is suggested by the increase in mast cell counts and activation in AD lesions [6]. In addition, mast cells produce inflammatory mediators such as prostaglandin D$_2$ (PGD$_2$) and induce eosinophil chemotaxis to inflammatory sites in the skin of AD patients [7].

Sanguisorbae Radix (SR) is well known as herbal medicine named “Zi-Yu” in Korea, which is the dried roots of Sanguisorba officinalis L. (Rosaceae) [8]. SR has been used as a traditional herbal medicine to treat diarrhea, chronic intestinal infections, duodenal ulcers, internal hemorrhage, and burns [8–10]. SR includes saponin glycosides and ellagitannins (i.e., pomolic acid, sanguisorbic acid dilactone, and ziyuglycoside I). SR and its active components have been demonstrated to have biological activity in vivo and in vitro. SR inhibits the renal dysfunction induced by lipopolysaccharide (LPS) endotoxin in vivo by suppressing the serum nitrite/nitrate levels and the activity of inducible nitric oxide.
2 Mediators of Inflammation

By suppressing the expression of TNF-α/IFN-γ-stimulated chemokines and proinflammatory molecules via blockade of NF-κB, STAT-1, and ERK activation. However, the biological and pharmacological actions of SR are not fully understood in atopic dermatitis.

In this study, we examined the effects of ESR on 24-dinitrochlorobenzene- (DNCB-) induced AD mouse skin lesions and mouse bone marrow-derived mast cells (BMMCs) to determine its therapeutic potential for the treatment of AD.

2. Materials and Methods

2.1. Preparation of ESR. SOL roots were obtained from Yeongcheon Oriental Herbal Market (Yeongcheon, Korea). All samples were deposited in the herbal bank of KM Application Center, Korea Institute of Oriental Medicine (KIOM; Daejeon, Republic of Korea). To prepare the ESR, dried SR pieces (50.0 g) were extracted using 390 mL 70% (KIOM; Daejeon, Republic of Korea). All samples were deposited in the herbal bank of KM Yeongcheon Oriental Herbal Market (Yeongcheon, Korea).

2.2. Animals. Male BALB/c mice (5 weeks old) were purchased from Samtako Bio Korea (Osan, Korea). Mice were observed every day for one week during quarantine and acclimation. Mice were divided into six groups (n = 5 per group): (1) negative control (vehicle), (2) DNCB + vehicle (control), (3) DNCB + 50 mg/kg ESR, (4) DNCB + 100 mg/kg ESR, (5) DNCB + 200 mg/kg ESR, and (6) and 1 mg/kg dexamethasone (Dexa.). All groups were maintained under standard conditions of temperature (22.5 ± 0.5 °C), humidity (42.6 ± 1.7%), 12 h lighting (8:00 AM–8:00 PM, 290 lx), ventilation (10–15 times per hour), and diet (Teklad Global Diets, Harlan Laboratories Inc., USA). Inhibitory effects of ESR were determined based on absorbance at 450 nm measured using a multilabel microplate reader (SpectraMax i3, Molecular Devices, Silicon Valley, CA, USA).

2.3. Induction of AD and Drug Treatment. After 1 week of acclimation, DNCB was applied to the dorsal skin and both ears of BALB/c mice to induce AD-like symptoms and skin lesions. One day after complete dorsal hair removal, 200 μL 1% DNCB dissolved in an acetone: olive oil mixture (3:1 vol/vol) was applied to the dorsal skin and 20 μL was applied to both ears (days 2–4). Five days after dorsal hair removal, 0.2–0.8% DNCB dissolved in an acetone: olive oil mixture (3:1 vol/vol) was applied to challenge the dorsal skin (200 μL) and both ears (20 μL each) two times a week for 2 weeks. Similarly, 1% DNCB solution was applied one day prior to sacrifice. ESR dissolved in saline (10 mL/kg body weight) was orally administered by gavage at 50, 100, and 200 mg/kg or using dexamethasone (Sigma-Aldrich, St. Louis, MO, USA; 1 mg/kg) three times a week for 4 weeks (days 0–24). The experimental scheme is summarized in Figure I(a).

2.4. Scratching Behavior and Ear Thickness Measurements. Scratching behavior was measured by placing each mouse into a cage once a week for 10 min and observing and recording behavior [20]. For each mouse, ear thickness was measured and recorded with a micrometer (Mitutoyo, Kawasaki, Japan). To minimize variation, a single investigator performed all measurements [21].

2.5. Histopathological Analysis. At the end of the study period, the dorsal skin lesions of each mouse were removed, fixed with 10% neutral-buffered formalin, and embedded in paraffin. 4 μm thick sections were stained with hematoxylin and eosin (H&E) and toluidine blue to detect epidermal thickness and inflammatory cells (i.e., eosinophils and mast cells), respectively. Histopathological evaluation of all skin sections occurred in a blind fashion [1]. All samples were observed using an inverted microscope and data are representative of five observations (Nikon Eclipse Ti, Nikon, Tokyo, Japan).

2.6. Serum IgE Measurements. At the end of the study period, mice were sacrificed and whole blood was collected. Blood samples were centrifuged at 2,000 xg for 20 min at 4 °C. Serum obtained from whole blood was stored at −80 °C until use. Serum IgE levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (BioLegend, San Diego, CA, USA). Inhibitory effects of ESR were determined based on absorbance at 450 nm measured using a multilabel microplate reader (SpectraMax i3, Molecular Devices, Silicon Valley, CA, USA).

2.7. BMMCs. BMMCs isolated from male BALB/c mice were cultured for up to 10 weeks in RPMI-1640 media containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics, and 10% fetal bovine serum (FBS) with 20% pokeweed mitogen-stimulated spleen condition medium (PWM-SCM) as a source of interleukin-3 (IL-3). After 3 weeks, more than 98% of the cells were verified as BMMCs according to a previously described procedure [22].

2.8. Cell Viability Assay. Cell cytotoxicity was analyzed using a cell counting kit (CCK) (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). BMMCs (2 × 10⁵ cells/well) were seeded into 96-well plates. After 24 h, ESR was added at concentrations of 10, 50, 100, and 200 μg/mL, and plates were incubated for 24 h at 37 °C in a 5% CO₂ incubator. CCK solutions were added to each well and cells were incubated for 1 h. Optical density was measured at 570 nm using
2.9. β-HEX Release Assay. β-hexosaminidase (β-HEX), a marker of mast cell degranulation, was quantified by spectrophotometric analysis of the hydrolysis of p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (4-Nitrophenyl N-acetyl-β-D-glucosaminide (p-NAG), Sigma-Aldrich, St. Louis, MO, USA).

For cell stimulation, BMMCs (5 × 10^5 cells/mL) were sensitized overnight with 100 ng/mL anti-dinitrophenyl (DNP) antibody and then stimulated for 15 min with 25 ng/mL DNP-human serum albumin (HSA). To investigate the effects of ESR, ESR of varying concentrations was added 1 h prior to the addition of DNP-HSA. After harvesting supernatants according to a previously described procedure [23], the percentage of β-HEX released into the supernatant was calculated using the following formula: [supernatant/(supernatant + pellet)] × 100.

2.10. Statistical Analysis. Data were analyzed using GraphPad Prism software (ver. 5.0 GraphPad Software, San Diego, CA, USA). Results are expressed as the mean ± standard error of the mean (SEM) and were evaluated using Student’s t-test or analysis of variance (ANOVA). A p value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of ESR on the Development of DNCB-Induced AD Mouse Skin Lesions. To investigate the therapeutic effects of ESR on AD mouse lesions, we administered ESR following the induction of AD mouse skin lesions using DNCB. Topical application of DNCB-induced crusting, epidermal thickness, redness, and dryness of skin are shown in Figure 1(b). However, skin conditions significantly improved in ESR-administered groups compared to the control group.

3.2. Effects of ESR on Scratching Behavior and Ear Thickness in AD Mice. Scratching behavior of the control group was rapidly increased and became significantly different from that observed in the vehicle group at day 14 after DNCB application. In AD mice treated with 200 mg/kg ESR, scratching was inhibited strongly compared to the vehicle group (Figure 2(a)). In addition, ESR significantly reduced DNCB-induced ear thickness in a dose-dependent manner. These results suggest that ESR has a therapeutic effect that can reduce AD symptoms in mice.

3.3. Effects of ESR on Dorsal Skin Thickness and Eosinophil Accumulation in AD Mouse Skin Lesions. Multiple applications of DNCB-induced infiltration of high levels of inflammatory cells into the skin result in increased dermal thickness [24]. To determine whether ESR treatment...
Figure 2: Effects of ESR on scratching behavior and ear thickness in AD mouse skin lesions. (a) Experimental induction of AD mouse skin lesions. (b) Ear thickness was measured with a dial thickness gauge. Results are expressed as the mean ± standard error of the mean (SEM) (n = 5). Vehicle, negative control; control, DNBC + vehicle; ESR, DNBC + 200 mg/kg ESR; Dexa., DNBC + 1 mg/kg dexamethasone treated group. *P < 0.05, **P < 0.01, and ***P < 0.001 versus vehicle; **P < 0.01 and ***P < 0.001 versus control.

3.4. Effects of ESR on Mast Cell Infiltration and Serum IgE Levels in AD Mice. Elevated serum IgE levels and mast cell infiltration are major characteristics of AD and related to disease severity [24]. To assess the effects of ESR on mast cell infiltration, we stained sliced cross sections of skin lesions with toluidine blue. As shown in Figure 4, we found an increase in mast cell infiltration (Figures 4(a) and 4(b)) and IgE levels in DNBC-treated mice compared to the vehicle group (Figure 4(c)). However, ESR significantly decreased the level of IgE elevated by DNBC induction and inhibited mast cell infiltration, in a dose-dependent manner (Figures 4(b) and 4(c)).

3.5. Effects of ESR on BMMC Cytotoxicity and Mast Cell Degranulation. Degranulation of mast cells is correlated with AD severity and the recruitment of immune cells to inflammatory sites in AD patients [2]. To investigate the effect of ESR treatment on degranulation of IgE/Ag-activated BMMCs, we measured the release of β-HEX in the presence or absence of ESR. As shown in Figure 5(b), ESR potently reduced β-HEX release in a dose-dependent manner.

To check cytotoxicity of ESR on BMMCs, the cells were incubated with different ESR concentrations (10, 50, 100, and 200 μg/mL) for 18 h and subjected for CCK-8 assay. ESR at 200 μg/mL had no significant cytotoxic effect on BMMCs after 24 h (Figure 5(a)).

4. Discussion

AD is an inflammatory disease characterized by an increase of cells associated with a Th2 response, including monocytes, macrophages, eosinophils, and mast cells. The pathogenesis of AD is primarily driven by Th2 immune responses and increased IgE production [5]. Proliferation, migration, and local activation of eosinophils are common in AD. Eosinophils act as immunoregulatory factors by secreting a variety of cytokines and chemokines attracting more eosinophils to the site of inflammation. Furthermore, eosinophils promote a switch from acute to chronic responses in AD [2]. ESR treatment reduces eosinophil accumulation in AD mouse skin lesions (Figures 3(a) and 3(c)).

High levels of serum IgE represent another characteristic of AD; thus, it is likely that targeting IgE may impact AD disease outcome. Specifically, IgE binding to mast cells affects the development and severity of AD [21]. Mast cells play a key role in allergic reactions via the production and secretion of proinflammatory mediators such as histamine, chemokines, cytokines, and growth factors. On the surface of mast cells, Th2 cells produce IgE, which binds to the FcεRI on the mast cell surface [25]. FcεRI-mediated mast cell activation is triggered by antigen IgE cross-linking and leads to the degranulation and expression of proinflammatory mediators. FcεRI-activated mast cells induce IgE elevation and increase mast cells in a majority of AD patients; therefore, mast cells are hypothesized to contribute to the pathogenesis of AD [3]. Our findings show that ESR treatment suppresses serum IgE levels and mast cell infiltration in a DNBC-induced AD mouse model.
β-HEX, a degranulation marker, is released along with other proinflammatory mediators when mast cells are activated [23, 26]. Therefore, we examined the degranulation by measuring β-HEX release in antigen IgE-activated BMMCs and confirmed that ESR treatment inhibited β-HEX release in a dose-dependent manner.

Active components of SR include phenolic compounds including tannins and flavonoids, saponin glycosides, and ellagitannins (i.e., pomolic acid, sanguisorbic acid dilactone, and ziyuglycoside I) [27]. Ziyuglycoside I and ziyuglycoside II are the major effective ingredients of triterpenoid saponins extracted from Sanguisorba officinalis L., and many studies
have focused on their pharmacological activities. In addition, ziyuglycoside I is a major marker of SR to confirm the origin of the medicinal plant in Korean Pharmacopoeia. Thus, we used ziyuglycoside I as a phytochemical marker of SR in the experiment (data not shown).

In this study, we investigated the therapeutic effects of ESR in the treatment of AD using a DNBC-induced AD mouse model and BMMCs. ESR significantly improved scratching behavior, ear thickness, epidermal thickness, and serum IgE levels in DNBC-induced AD mice. We observed...
a reduction in the infiltration of eosinophils and mast cells into the AD skin lesions following ESR treatment. In addition, ESR significantly inhibited degranulation of IgE/Ag-activated BMMCs. The therapeutic effects of ESR treatment on AD are supported by the beneficial effect of anti-IgE therapy in a number of clinical studies [2, 3].

In conclusion, these results demonstrate that ESR decreases AD symptoms in mice and inhibits degranulation of IgE/Ag-activated mast cells. Our study suggests that ESR may serve as a potential therapeutic candidate for the treatment of AD.

Abbreviations
ESR: Ethanol extract of Sanguisorbae Radix
AD: Atopic dermatitis
DNCCB: 2,4-Dinitrochlorobenzene
H&E: Hematoxylin and eosin
FceRI: High-affinity immunoglobulin E receptor
BMMCs: Mouse bone marrow-derived mast cells
β-HEX: β-Hexosaminidase.

Competing Interests
The authors declare that there are no competing interests.

Authors’ Contributions
Ju-Hye Yang designed the study, performed the research, and wrote the paper; Jae-Myung Yoo was involved in experiments; Won-Kyung Cho and Jin Yeul Ma supervised the study.

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