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Research article

Korean Red Ginseng improves atopic dermatitis-like skin lesions by suppressing expression of proinflammatory cytokines and chemokines *in vivo* and *in vitro*

Ji-Ye Kee [1,☆], Yong-Deok Jeon [1,2,☆], Dae-Seung Kim [1], Yo-Han Han [1], Jinbong Park [3], Dong-Hyun Youn [3], Su-Jin Kim [4], Kwang Seok Ahn [3], Jae-Young Um [3,*], Seung-Heon Hong [1,*]

[1] *Department of Oriental Pharmacy, College of Pharmacy, Wonkwang-Oriental Medicines Research Institute, Wonkwang University, Iksan, Republic of Korea*
[2] *Department of Oriental Medicine Resources, College of Environmental and Bioresources Sciences, Chonbuk National University, Iksan-si, Jeollabuk-do, Republic of Korea*
[3] *College of Korean Medicine, Institute of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea*
[4] *Department of Cosmeceutical Science, Daegu Hanny University, Gyeongsan-si, Republic of Korea*

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ABSTRACT

Background: The prevalence of allergic inflammatory diseases such as atopic dermatitis (AD), asthma, and allergic rhinitis worldwide has increased and complete recovery is difficult. Korean Red Ginseng, which is the heat-processed root of *Panax ginseng* Meyer, is widely and frequently used as a traditional medicine in East Asia. In this study, we investigated whether Korean Red Ginseng water extract (RGE) regulates the expression of proinflammatory cytokines and chemokines via the mitogen-activated protein kinases (MAPKs)/nuclear factor kappa B (NF-κB) pathway in allergic inflammation.

Methods: Compound 48/80-induced anaphylactic shock and 1-fluoro-2,4-dinitrobenzene (DNFB)-induced AD-like skin lesion mice models were used to investigate the antiallergic effects of RGE. Human keratinocytes (HaCaT cells) and human mast cells (HMC-1) were also used to clarify the effects of RGE on the expression of proinflammatory cytokines and chemokines.

Results: Anaphylactic shock and DNFB-induced AD-like skin lesions were attenuated by RGE administration through reduction of serum immunoglobulin E (IgE) and interleukin (IL)-6 levels in mouse models. RGE also reduced the production of proinflammatory cytokines including IL-1β, IL-6, and IL-8, and expression of chemokines such as IL-8, thymus and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC) in HaCaT cells. Additionally, RGE decreased the release of tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and IL-8 as well as expressions of chemokines including macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation, normal T cell expressed and secreted (RANTES), monocyte chemoattractant protein (MCP)-1, and IL-8 in HMC-1 cells. Furthermore, our data demonstrated that these inhibitory effects occurred through blockage of the MAPK and NF-κB pathway.

Conclusion: RGE may be a useful therapeutic agent for the treatment of allergic inflammatory diseases such as AD-like dermatitis.

* Corresponding authors. Jae-Young Um, College of Korean Medicine, Institute of Korean Medicine, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea. Seung-Heon Hong, Department of Oriental Pharmacy, College of Pharmacy, Wonkwang-Oriental Medicines Research Institute, Wonkwang University, 460 Iksandae-ro, Iksan, Jeonbuk 54538, Iksan, Republic of Korea.
E-mail addresses: jyum@khu.ac.kr (J.-Y. Um), jooklim@wku.ac.kr (S.-H. Hong).
☆ These two authors contributed equally.

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1. Introduction

Allergic inflammation is a hypersensitivity disorder of the immune system and occurs with several important medical conditions, such as atopic dermatitis (AD), allergic eczema, allergic rhinitis, and other allergic diseases. Most of the allergic diseases have increased in prevalence in the past few decades. They represent an important public health problem due to their impact on quality of life and medical expenditure burdens [1]. Among allergic diseases, AD correlates with the expression of proinflammatory cytokines and chemokines from keratinocytes, mast cells, and other immune cells [2,3].

Keratinocytes are the major cell types in the epidermis, and they maintain the biochemical and physical condition of the skin. They are also involved in the progression of various inflammatory skin diseases [4]. Epidermal keratinocytes release inflammatory mediators such as proinflammatory cytokines and chemokines in response to immune triggers including UV light, allergens, and microbiological agents [5]. Activated keratinocytes are capable of producing biologically active interleukin (IL)-8, which mediates the influx of T cells and neutrophils into the epidermis [6]. Thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), which are secreted from keratinocytes, play important roles in the infiltration of Th2 cells into inflammatory tissues [7].

Mast cells are also the major effector cells related to the allergic inflammatory reaction and are considered to be involved in the progression of AD because they show increases in the majority of AD patients [8,9]. In response to several antigens, mast cell degranulation is initiated and these cells release a variety of bioactive substances such as histamine. Activated mast cells also produce various proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1β, and IL-6 as well as chemokines [10]. These mediators contribute to inflammation through the recruitment and activation of immune cells [11].

Mitogen-activated protein kinases (MAPKs) including the extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 MAPK regulate diverse cellular functions such as proliferation, activation, and degranulation [12]. The activation of MAPKs is associated with the allergic inflammatory response via the translocation of nuclear factor kappa B (NF-κB), which causes the production of proinflammatory cytokines and chemokines [13–15]. After phosphorylation of MAPKs, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) proteins are degraded and NF-κB is translocated into the nucleus and initiates the transcription of target genes. Therefore, as the inhibition of MAPKs and NF-κB activation subsequently decrease secretion of allergic mediators, MAPKs are important in the prevention of allergic inflammation [16–18].

Ginseng, the root of *Panax ginseng* Meyer, has been used for the treatment of many diseases and for enhancing physical strength and immunity. Korean Red Ginseng, which is heat-processed ginseng, is a popular traditional medicine in East Asia and has been widely used to treat a number of diseases such as cancer, Alzheimer's disease, and vascular diseases [19–21]. Several studies have reported that Korean Red Ginseng has antiallergic effects in *in vitro* and *in vivo* models [22,23]. Although the antiallergic effects of Korean Red Ginseng have already been reported, mechanistic understanding of its immunopharmacological roles is poorly elucidated.

In this study, we investigated the effects of Korean Red Ginseng water extract (RGE) on allergic inflammation and mechanisms. We evaluated the effects of RGE on systemic and local allergic reactions to assess its antiallergic effect in compound 48/80-induced anaphylactic shock and 1-fluoro-2,4-dinitrobenzene (DNFB)-induced dermatitis mice models. Moreover, the effects of RGE on the expression of proinflammatory cytokines and chemokines, as well as its mechanisms, were investigated in HaCaT cells and HMC-1 cells.

2. Materials and methods

2.1. Reagents

Compound 48/80, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), phorbol 12-myristate 13-acetate (PMA), and calcium ionophore A23187 (A23187) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) was purchased from Promega Corporation (Madison, WI, USA). Avidin peroxidase (AP), fetal bovine serum (FBS), RPMI 1640, and Iscove's Modified Dulbecco's Medium (IMDM) were purchased from Gibco BRL (Grand Island, NY, USA). Anti-human TNF-α/IL-6/IL-8, anti-mouse immunoglobulin E (IgE)/IL-6, recombinant TNF-α/IL-6/IL-8, and biotinylated TNF-α/IL-6/IL-8 were purchased from BD Pharmingen (San Diego, CA, USA). Anti-phospho-p38, -ERK, -JNK, -Akt, -IκBα, anti-GAPDH, IκBα, and NF-κB antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Anti-p38, JNK, and histone antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-Akt and ERK antibodies were purchased from Bioworld Technology (Minneapolis, MN, USA).

2.2. Preparation of RGE

RGE was kindly provided by the Korea Ginseng Corporation (Daejeon, Korea). It was manufactured by Korea Ginseng Corporation, Seoul, Korea from roots of a 6-yr-old red ginseng, *Panax ginseng* Meyer, harvested in the Republic of Korea. Red ginseng was made by steaming fresh ginseng at 90–100°C for 3 h and then drying at 50–80°C. RGE was prepared from red ginseng water extract, which was extracted by circulating 85–90°C water for 8 h three times. The water content of the pooled extract was 36% of the total weight. RGE was analyzed by high-performance liquid chromatography. RGE contained major ginsenoside-Rb1: 7.44 mg/g, -Rb2: 2.59 mg/g, -Rc: 3.04 mg/g, -Rd: 0.91 mg/g, -Re: 1.86 mg/g, -Rf: 1.24 mg/g, -Rg1: 1.79 mg/g, -Rg2: 1.24 mg/g, -Rg3: 1.39 mg/g, and other minor ginsenosides.

2.3. Animals

BALB/c mice (5 wk, male, 19–20 g) were purchased from DaMool Science (Daejeon, Republic of Korea). The animals were housed in a laminar air-flow room maintained at a temperature of 22 ± 1°C and a humidity level of 55 ± 1% throughout the study. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as stated in the Wonkwang University (Iksan, Republic of Korea) guidelines (WKU-14-35).

2.4. Cell culture

Human keratinocytes HaCaT cells were obtained from CLS Cell Lines Service (Eppelheim, Baden-Württemberg, Germany) and the human mast-cell line (HMC-1) was generously provided by Eichi Morri (Osaka University, Osaka, Japan). HaCaT cells were cultured in RPMI 1640 and HMC-1 cells were cultured in IMDM supplemented with 10% FBS, 100 units/mL of penicillin, and 100 μg/mL of streptomycin in a humidified atmosphere of 5% CO_2 at 37°C.

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