Journal of Ethnopharmacology 145 (2013) 294–302

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Effects of Korean red ginseng extract on the prevention of atopic dermatitis and its mechanism on early lesions in a murine model

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ARTICLE INFO

Article history:
Received 21 July 2012
Received in revised form
2 November 2012
Accepted 4 November 2012
Available online 10 November 2012

Keywords:
Allergy
Atopic dermatitis
Dermatosis
Inflammation
Korean red ginseng

ABSTRACT

Ethnopharmacological relevance: Korean red ginseng (KRG) has been shown to possess various biological activities including anti-inflammatory properties.
Aim of the study: We aimed to investigate the effects and mechanism of KRG on the prevention of atopic dermatitis (AD) using a mouse model.
Materials and methods: The effect of KRG in trinitrochlorobenzene (TNCB)-treated NC/Nga mice was assessed by measuring ear thickness, transepidermal water loss (TEWL), total serum IgE, histologic changes of lesional skin, mRNA and protein expression of thymic stromal lymphopoietin (TSLP) and tumor necrosis factor (TNF)-α, immunohistochemistry for tissue interleukin (IL)-4, IL-17, and interferon (IFN)-γ.
Results: KRG significantly reduced ear thickness. Oral administration of KRG significantly prevented the increase in TEWL induced by TNCB. The serum IgE level was significantly lower in the KRG group. Histologically, lymphocyte infiltration was markedly decreased by KRG. CD1a positive (CD1a+) cells were diminished by KRG. Immunohistochemically, KRG significantly suppressed the protein expression of TSLP and TNF-α. The mRNA expression of TSLP in the lesions was significantly reduced by KRG. These results demonstrate that oral administration of KRG may inhibit the development of AD-like skin lesions in NC/Nga mice by modifying TSLP, DCs, and at least in part, the Th2 response.
Conclusion: KRG may be a potential therapeutic modality for the prevention of AD.
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1. Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disorder (Novak and Leung, 2011). Disturbance of epidermal-barrier function and correlation to local and systemic immunologic factors in the development and severity of AD has been demonstrated by recent studies (Boguniewicz and Leung, 2011). Whether epidermal-barrier abnormalities precede immune dysregulation ('outside-in' hypothesis) (Elias et al., 2008) or immune dysregulation precedes changes in barrier function ('inside-out' hypothesis) continues to be an unsolved issue with data supporting both hypotheses (Boguniewicz and Leung, 2011).

In the acute phase of AD, the IL-7-like cytokine, thymic stromal lymphopoietin (TSLP), triggers dendritic cell (DC)–mediated type 2 helper T (Th2) inflammatory responses. Langerhans cells (LCs) play an important role in cutaneous antigen presentation and contribute to Th2 polarization (Novak and Bieber, 2005). Inflammatory dendritic epidermal cells (IDEC) produce proinflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α) (Bieber, 2008). Antigen-specific Th2 cells and their cytokines such as IL-4, and IL-13 orchestrate the acute skin lesions in AD. IL-17 expression is enhanced in acute lesions. During the chronic phase, a switch to the type 1 helper T (Th1) cell-mediated responses occurs. Th1 cells mainly produce interferon (IFN)-γ, and IL-17 shows a diminished presence.

Transepidermal water loss (TEWL) is linked to the damage of the stratum corneum lipid barrier and a consecutive loss of corneocyte adhesion. Skin barrier abnormalities, including increased TEWL and decreased skin hydration, are biomarkers for the severity and itch intensity in AD (Lee and Yu, 2011).

For the management of AD, the prevention of recurrence and early treatment is important. Use of topical calcineurin inhibitors and intermittent use of topical glucocorticoids is beneficial for the

Abbreviations: KRG, Korean red ginseng; AD, atopic dermatitis; TNCB, trinitrochlorobenzene; TEWL, transepidermal water loss; TSLP, thymic stromal lymphopoietin; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; CD1a+, CD1a positive; DC, dendritic cell; Th2, type 2 helper T; LC, Langerhans cell; Th1, type 1 helper T; EPO, evening primrose oil; PFA, paraformaldehyde; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription–polymerase chain reaction; S.E.M, standard error of means; H&E, hematoxylin and eosin

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http://dx.doi.org/10.1016/j.jep.2012.11.006

management of AD (Abramovits, 2005b). Results from several studies show the possibility that patients with AD may benefit from the traditional Oriental medicine therapy (Koo and Arain, 1999).

Korean red ginseng (KRG, the steamed root of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently taken orally as a remedy, especially in Asian countries. The major components of raw ginseng have been recently known to be ginsenosides, which contain an aglycone with dammarane skeleton (Shibata et al., 1963). KRG was further studied to have anti-allergic effects. (Bae et al., 2006; Matsuda et al., 1990; Park et al., 2003) Recently, we have demonstrated that oral administration of KRG could inhibit the inflammation of AD-like skin lesions in NC/Nga mice by suppressing LCs, Th1 related cytokines, and regulatory T (Treg) cells (Lee and Cho, 2011). To date, there have been no studies to assess the preventive effects of oral KRG administration and its mechanism on early lesions in the AD murine model. We further studied to investigate the effects and mechanism of KRG on the prevention of AD.

The aim of this study was to assess the anti-inflammatory effects of oral administration of KRG on the prevention and early events of AD-like skin lesions that were provoked by trinitrochlorobenzene (TNCB) in NC/Nga mice. In order to investigate the preventive effects of KRG, the oral administration of KRG was initiated and completed before the TNCB challenge. We aimed to determine whether KRG exerts anti-inflammatory effects on cytokines, T cells and DCs that are related to immune dysregulation in the early pathogenesis of AD. In this study, we measured ear thickness, TEWL, serum IgE levels, histologic changes, immunohistochemistry of TSLP, TNF-α, IL-4, IL-17, IFN-γ, and mRNA expression of TSLP and (TNF)-α in the lesional skin and to examine the preventive effects of KRG on the development of AD-like skin lesions in NC/Nga mice.

2. Material and methods

2.1. Animals

NC/Nga female mice (5-weeks old) were purchased from Charles River Laboratories (Kanagawa, Japan).

2.2. Test drugs

TNCB was purchased from Sigma (St. Louis, MO, USA) to induce AD-like skin lesions. KRG extract was manufactured from roots of a 6-year-old fresh ginseng, *Panax ginseng* Meyer, harvested in Republic of Korea by Korea Ginseng Corporation, Seoul, Korea. Red ginseng was made by steaming fresh ginseng at 90–100 °C for 3 h and then drying at 50–80 °C. Red ginseng extract was prepared from red ginseng water extract, which was extracted at 85–90 °C for 8 h of circulating hot water three times. The water content of the pooled extract was 36% of total weight. KRG was analyzed by high-performance liquid chromatography. KRG extract contained major ginsenoside-Rb1: 8.27 mg/g, -Rb2: 3.22 mg/g, -Rc: 3.90 mg/g, -Rd: 1.09 mg/g, -Re: 2.58 mg/g, -Rf: 1.61 mg/g, -Rg1: 2.01 mg/g, -Rg2: 1.35 mg/g, -Rg3: 1.04 mg/g, and other minor ginsenosides. Cyclosporine (Cipol-N®) was kindly donated by Chong Kun Dang (Seoul, Korea). Evening primrose oil (EPO) (Evoprim®) was purchased from Dalim Biotech (Seoul, Korea). Antibodies used for immunohistochemical markers are as follows; CD1a (1:50, Thermo Scientific, Rockford, IL, USA), TSLP (Abnova, Taipei city, Taiwan), TNF-α (1:200; Abcam, Cambridge, UK), IL-4 (1:50, Biolegend), IL-17 (1:100; Abcam, Cambridge, UK), and IFN-γ (1:100; Biolegend, San Diego, CA, USA).

2.3. TNCB induced AD-like skin lesions and oral administration of drugs

Twenty-five SPF NC/Nga female mice (20 ± 5 g each) were housed at 22 °C with a 12 h light–dark cycle. The mice were placed in separate cages and assigned individual groups with ad libitum access to food and water. Mice were anesthetized with isoflurane and the hair on their back and abdomen was shaved using an electric shaver. The animal care, handling and experimental procedures were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee. The study was approved by the Institutional Review Board.

The NC/Nga mice were allocated into five groups (AD control, KRG, cyclosporine, EPO, and sham groups). TNCB was used to induce AD-like skin lesions in NC/Nga mice as previously described (Lee and Cho, 2011). The abdomens were sensitized epicutaneously by applying 150 μl of 5% TNCB dissolved in ethanol/acetone mixture (4:1). Seven days after sensitization, the backs and dorsal surface of the ears were challenged with 190 μl of 1% TNCB dissolved in acetone/olive oil (4:1). The KRG, cyclosporine, and EPO groups were treated with 200 mg/kg KRG, 2.5 mg/kg cyclosporine, and 50 mg/kg EPO for 5 days, respectively. The AD control group received phosphate-buffed saline (PBS) 0.2 mL, only. KRG, cyclosporine, EPO, and PBS were orally administered by gastric intubation with an animal-feeding needle. The sham group was untreated. The design of the study is summarized in Fig. 1.

2.4. Measurement of ear thickness and total clinical severity score

The severity of AD-like lesions was evaluated by scoring the clinical signs using digital photographs as in our previous study (Lee and Cho, 2011). The total clinical severity score was defined as the sum of each score grades: 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for each of the four manifestations: (1) erythema/hemorrhage, (2) edema, (3) excoriation/erosion, and (4) scaling/dryness. The ear thickness was measured using a dial caliper (Kori Seiki MFG, Tokyo, Japan) at the time of 1% TNCB challenge (baseline) and 1, 3, 6, 12, 24, 48, and 72 h after the baseline. Clinical photographs of the ears and abdomens were taken with a digital camera (Canon 50D, Canon Inc., Tokyo, Japan).

2.5. Measurement of transepidermal water loss

TEWL was assessed on the dorsal skin of the NC/Nga mice using VapoMeter (Delfin Technologies, Kuopio, Finland). TEWL was measured at baseline, and 1, 3, 6, 12, 24, 48, and 72 h thereafter.

2.6. ELISA

Blood samples were collected from the retro-orbital plexus of anesthetized mice at the end of the experiment and added to tubes (Scientific Glass, Inc., Rockwood, TN, USA). Samples obtained by centrifugation were stored at −20 °C until use. The

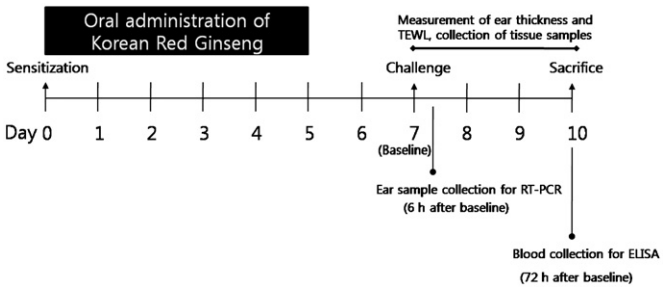

Fig. 1. Summary of the design of study.

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