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Platycodi Radix suppresses development of atopic dermatitis-like skin lesions

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ABSTRACT

Platycodi Radix has been used to treat chronic diseases, such as bronchitis, asthma, and hyperlipidemia. In this study, we examined the effect of an aqueous extract, Changkil (CK), from the root of Platycodi Radix on 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD)-like skin lesions. Administration of CK onto DNCB-induced AD-like skin lesions in NC/Nga mice ameliorated lesion intensity scores, levels of IgE, thymus and activation-regulated chemokine (TARC), TNF-α, and IL-4 in serum and ears. In contrast, CK increased level of the immunosuppressive cytokine IL-10. Histopathological examination showed reduced thickness of the epidermis/dermis and dermal infiltration of inflammatory cells in the ears. CK also suppressed TNF-α/IFN-γ-induced mRNA expression and production of TARC in HaCaT cells. CK exerts beneficial effects on AD symptoms, suggesting that CK is an effective potential therapeutic agent for AD.

1. Introduction

Atopic dermatitis (AD) is a chronic, relapsing, and inflammatory skin disease associated with eczematous symptoms and IgE hyperproduction (Gao et al., 2004). Itching is a serious problem in AD patients because scratching worsens the dermatitis (Jiang et al., 2009). Reduction of itching-associated scratching is the most effective therapeutic strategy for improving the quality of life for AD patients. Various allergens including the immunological and non-immunological abnormalities contribute to the pathogenesis and development of AD. AD incidence is increasing throughout the world, and its onset typically occurs during early infancy or childhood, but can also occur in adulthood. For the last four decades, topical steroids and immunosuppressive agents have been standard treatments for severe cases of AD. However, these treatments are often associated with severe adverse effects and are not sufficiently effective in a substantial number of patients with AD (Ingber, 2002). Therefore, there is a great need for the development of new and effective therapies for AD.

T helper (Th) cells are classified into two types, types 1 and 2, depending on the profile of cytokines secreted by the cell. The Th1/Th2 balance is important for the response to a variety of immunological diseases. The pathogenesis of AD is known to be the result of local and systemic immunologic dysfunction that leads to a Th1/Th2 cell imbalance, as well as susceptibility genes and environmental factors (Leung et al., 2004; Beltrani, 2005). Many studies report that Th2 cells are

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predominantly acute phase reactors, whereas Th1 reactions are predominantly chronic in AD skin lesions (Di Cesare et al., 2008). IL-4 induces the differentiation of T lymphocytes into Th2 cells and the switching of B lymphocytes into IgE producers (Di Cesare et al., 2008; Kawakami et al., 2009). TNF-α is also reported to be a contributor to the initiation of AD (Homey et al., 2006).

Platycodi Radix, the aqueous extract from the root of *Platycodon grandiflorum* A.DC. (Campanulaceae family), has been used as a food and traditional oriental medicine to treat chronic adult diseases (e.g., bronchitis, asthma, pulmonary tuberculosis, hyperlipidemia, and hypercholesterolemia) and inflammatory diseases (Lee, 1973). Changkil (CK) is the aqueous extract from the root of Platycodi Radix cultivated for more than 21-year-old plants. Recent studies have shown that CK has antioxidant effects (Lee and Jeong, 2002), antimetastatic activities (Lee et al., 2006a,b), hepatoprotective and anti-fibrotic effects (Lee et al., 2001; Lee and Jeong, 2002; Lee et al., 2004), anti-inflammatory effect (Choi et al., 2009), and immune stimulation (Choi et al., 2001). Nonetheless, the effect of CK as an anti-atopic agent for AD remains unclear. In this study, we investigated the inhibitory effect of CK on the development of AD in NC/Nga mice and the human keratinocyte cell line HaCaT.

2. Materials and methods

2.1. Chemicals

DNCB (2,4-dinitrochlorobenzene), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide), TNF-α, and IFN-γ were all obtained from Sigma–Aldrich (Milwaukee, WI, USA). Enzyme-linked immunosorbent assay (ELISA) kits for IgE, TARC, and IL-10 were obtained from R&D Systems (Minneapolis, MN, USA) and BD Biosciences (San Diego, CA, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Life Technologies, Inc. (Carlsbad, CA, USA). Polymerase chain reaction (PCR) oligonucleotide primers were custom synthesized by Bioneer Co. (Daejeon, Korea). All chemicals and solvents were of the highest grade commercially available.

2.2. Preparation of CK

CK, the aqueous extract from a 21-year-old Platycodi Radix root, was supplied by Jangsaeng Doraji Co., Ltd. (Jinju, South Korea) and prepared as described previously (Lee and Jeong, 2002). Briefly, distilled water at 90 °C was added to powered root (5 mL/g) and the temperature was maintained for 10 h. The mixture was allowed to cool to room temperature, filtered, and lyophilized. The yield of lyophilized residue corresponded to 33.5% (33.5 g of residue for each 100 g of original dry roots). The pale-yellow extract was dissolved directly in sterilized saline. The composition of CK has been previously published (Kim et al., 1995) and CK consisted of saponin (~2.5%), inulin (~60%) and oligosaccharide (~25%).

2.3. Animals and treatment

Specific pathogen-free male 6-week-old NC/Nga mice were obtained from SLC, Inc. (Shizuoka, Japan). Animals were acclimatized to the temperature (22 ± 2 °C) and humidity (55 ± 5%) of controlled rooms with a 12 h light/dark cycle for at least two weeks prior to experiments. Animals were allowed free access to Purina rodent chow (Seoul, Korea) and tap water. All experimental protocols for animal care were performed according to the rules and regulations of the Animal Ethics Committee, Chungnam National University. The mice were divided into three groups ($n = 5$ per group). To induce AD-like immunologic and skin lesions, DNCB was applied onto the dorsal skin and ears. After complete removal of dorsal hairs within an area approximately 8 cm^2, 200 μL of 1% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied for three consecutive days for sensitization. Four days after sensitization, the dorsal skin and ears were challenged with 200 μL of 0.2% DNCB solution three times per week for eight weeks. As soon as the challenge was completed, 100 mg/kg CK, dissolved in saline solution, was administered orally six times per week for four weeks. Control and DNCB-treated mice were administered saline intragastrically. Animals were sacrificed 64 days after the first application of DNCB (Fig. 1). Blood was collected from the vena cava, and the right ear was removed and used for histopathological examination.

2.4. Measurement of ear thickness

Ear thickness was measured with a micrometer (Mitutoyo, Kawasaki, Japan) on the day of sacrifice.

2.5. Histopathological studies

The right ear was removed from each mouse and fixed in 10% formalin solution. It was subsequently embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin. Histological changes were examined by light microscopy.

2.6. Evaluation of skin dermatitis severity

The severity of dermatitis in the ear and back lesions was evaluated twice a week. The development of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema, and (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was defined as the dermatitis score (Matsuda et al., 1997).

2.7. Cell culture

The spontaneously immortalized human keratinocyte cell line HaCaT (a kind gift from N.E. Fusenig, German Cancer Research, Germany) (Boukamp et al., 1998) was grown in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Stock solutions of CK dissolved in dimethyl sulfoxide (DMSO) were directly applied to the culture medium 1 h before the addition of 10 ng/mL TNF-α and 10 ng/mL IFN-γ.