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Effect of Hwangryunhaedoktang on Contact Hypersensitivity and Passive Cutaneous Hypersensitivity in Mice

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Abstract – During the screening program to discover antiatopic agents from herbal formulas, we investigated the inhibitory effect of Hwangryunhaedoktang (HT) on passive cutaneous anaphylaxis and oxazolone-induced mouse ear dermatitis. HT significantly inhibited PCA reaction in mice at doses of 50 and 200 mg/kg with inhibitory activity of 31 and 53%, respectively. HT at concentration of 0.05 and 0.1% inhibited ear swelling by 23 and 46% at 16 days in oxazolone-induced mouse ear dermatitis. Both HT with and without human intestinal microflora showed potent inhibitory activity on the β-hexosaminidase release induced by IgE. Based on these findings, HT may be a usable agent for skin disorder contact dermatitis.

Keywords □ hwangryunhaedoktang, psoriasis, passive cutaneous anaphylaxis, oxazolone-induced dermatitis

INTRODUCTION

Contact hypersensitivity, such as psoriasis, is a chronic and inflammatory skin disorder. Psoriasis patients have been shown to have interferon-γ producing Th1 bias in lesion skin and peripheral blood and are thought to develop cytokine net works of Th1 cell, resulting in keratinocyte hyperplasia and angiogenesis (Austin *et al.*, 1999; Nicoloff, 1991; Ovigne *et al.*, 2001). Fujii *et al.* (2002) developed oxazolone-induced animal model for chronic psoriatic dermatitis featuring epidermal hyperplasia in which interferon-g plays a crucial role. This dermatitis was accompanied by sustained swelling, predominant epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages but not eosionphils. Antipsoriatic agents nonsteroidal anti-inflammatory drugs (NASIDs) and betamethasone and immunosuppressants FK-506 and cyclosporin A for Th1 cells have been used clinically for psoriasis (Hernandez *et al.*, 2001; Schafer-Korting *et al.*, 1996; Sakuma *et al.*, 2001; Friedman *et al.*, 2002). Betametasone valerate is a potent corticosteroid used clinically in the treatment of psoriasis and other skin disorders. Corticosteroids are well known to have potent anti-inflammatory effects, but topical use can cause intense skin atrophy, one of the serious side effects limiting their use for chronic skin diseases (Schafer-Korting *et al.*, 1996). Repeated application of corticosteroids on dorsal skin of rats also causes dramatic skin atrophy. FK-506 and cyclosporin A are a potent immunosuppressant currently used for preventing allograft rejection. FK-506 also suppressed the increase in ear thickness and epidermal thickness (Fujii *et al.*, 2002). However, it also exhibited side effects, such as severe nephrotoxicity and neurotoxicity. Therefore, new agents for clinical uses should be developed.

To discover antipsoriatic herbal medicines from antiallergic herbal medicinal formulas, we screened the inhibitory effects of herbal medicinal formulas against passive cutaneous anaphylaxis (PCA) reaction. In the preliminary study, Hwangryunhaedoktang (HT) showed the potent inhibition against PCA reaction. Therefore, we investigated inhibitory effects of HT against contact hypersensitivity.

MATERIALS AND METHODS

Materials

Oxazolone, p-nitrophenyl-N-acetyl-β-D-glucosaminide, Freund's complete adjuvant, anti-dinitrophenol (DNP)-IgE, DNP-human serum albumin (HSA), Evans blue, disodium cromoglycate (DSCG), azelastine, and betametasone were all purchased from Sigma Chemical Co. (St Louis, MO, U.S.A).

HT consists of water extract of Coptidis Rhizoma 4 g, Phello-

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dendri Cortex 4 g, Scutellariae Radix 4 g, and Gardeniae Fructus 4 g. Metabolized HT (MHT) was prepared by incubating HT with human intestinal microflora as follows. HT (2 g) was incubated with human intestinal bacteria at 37°C for 24 h, extracted with ethyl acetate and concentrated. To use HT as a test sample in the present study, it also extracted with ethyl acetate and concentrated. Each concentrate was used as MHT or HT sample.

The RBL 2H3 cells were obtained from the Korean Cell line Bank (Seoul, Korea).

Herbal medicines were purchased form Kyung-Dong herbal medicine market and identified by Dr. N.J. Kim (East-West Medicinal Research Institute, Kyung Hee University).

Animals

The male or female ICR mice (20-25 g) were supplied from Orient Experimental Animal Breeding Center (Kapyung, Korea). All animals were housed in wire cages at 20-22°C and 50 ± 10% humidity, fed standard laboratory chow (Samyang feed production Co., Seoul, Korea) and allowed water *ad libitum*. All procedures relating to animals and their care conformed to the international guidelines Principles of Laboratory Animals Care (NIH publication no. 85-23, revised 1985).

Assay of Antiallergic activity in RBL-2H3 cell line

The inhibitory activity of ginsenosides against the release of β-hexosaminidase form RBL-2H3 cells was evaluated according to Choi *et al.* (1996). RBL-2H3 cells were grown in DMEM supplemented with 10% fetal bovine serum and L-glutamine. Before experiment, cells were dispensed into 24 well plates at the concentration of 5×10^5 cells per well, using the medium containing 0.5 μg/ml of mouse monoclonal IgE and were incubated overnight at 37°C in 5% CO_2 for sensitization of cells. The cells were washed with 500 μl of siraganian buffer (pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, 40 mM NaOH) and incubated in 160 μl of siraganian buffer containing 5.6 mM glucose, 1 mM $CaCl_2$ and 0.1% BSA for additional 10 min at 37°C. Then cells were exposed to 40 μl of test agents for 20 min, followed by the treatment with 20 μl of antigen (DNP-HSA, 1 μg/ml) for 10 min at 37°C to activate cells to evoke allergic reactions (degranulations). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 2000 rpm for 10 min and 25 μl aliquots of supernatant were transferred to 96 well plates and incubated with 25 μl of substrate (1mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1 h at 37°C. The reaction was stopped by adding 200 μl of 0.1 M Na_2CO_3/$NaHCO_3$. The absorbance was measured by ELISA reader at 405 nm.

Passive cutaneous anaphylaxis (PCA) reactions

An IgE-dependent cutaneous reaction was measured according to the previous method of Katayama *et al.* (1978). The male ICR mice (25-30 g) were injected intradermally with 10 g of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each mouse received an injection of 200 μl of 3% Evans blue PBS containing 200 g of DNP-HSA *via* the tail vein. The test agents were orally administered 1 h prior to DNP-HSA injection. Thirty min after DNP-HSA injection, the mice were sacrificed and their dorsal skins were removed for measurement of the pigment area. After extraction with 1 ml of 1.0 N KOH and 4 ml of a mixture of acetone and 0.6 N phosphoric acid (13:5), the amount of dye was determined colorimetrically (the absorbance at 620 nm).

Contact hypersensitivity

An oxazolone-induced dermatitis was measured according to the previous method of Fujii *et al.* (2002). ICR mouse (female, 25-28 g) were sensitized by application of 100 ìl of 1.5% oxazolone in ethanol to the abdomen and then a total of 20 μl of 1% oxazolone in a mixture of acetone and olive oil (4:1) was applied to both sides of the ear every 3 days starting from 7 days after sensitization. Ear thickness was measured using a Digimatic Micrometer (Mitsutoyo, Japan) 72 h after each application of the oxazolone, test agents were applied in a total volume of 20 μl to both sides of the ear 30 min before and 3 h after each application of oxazolone

Statistical analysis

All the data from the experiment were expressed as mean ± standard deviation and the statistical significance was determined using ANOVA.

RESULTS

Inhibition of HT and MHT on PCA reaction

To investigate whether HT possess antiallergic activity, HT was orally administered 60 min prior to challenge with antigen and determined the inhibitory effect of HT on PCA reaction in mice (Fig. 1). HT dose-dependently inhibited PCA reaction on mice. HT significantly inhibited PCA at doses of 50 and 200 mg/kg with inhibitory activity of 31 and 53%, respectively.

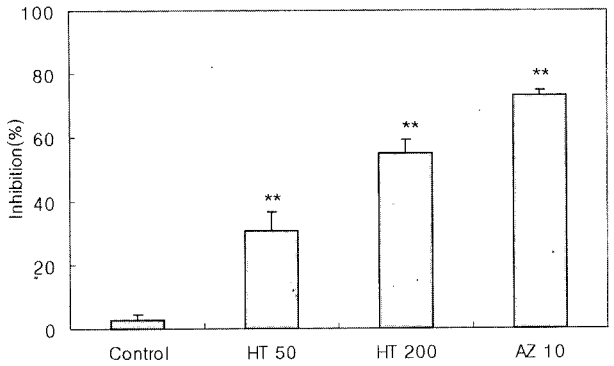

Fig. 1. Inhibitory effects of HT on passive cutaneous anaphylaxis reaction induced IgE in mice. HT was orally administered into mice at doses of 50 (HT 50) and 200 mg/kg (HT 200), and azelastine (AZ 10) 10 mg/kg was orally administered. *Significantly different from control group(**P<0.01).

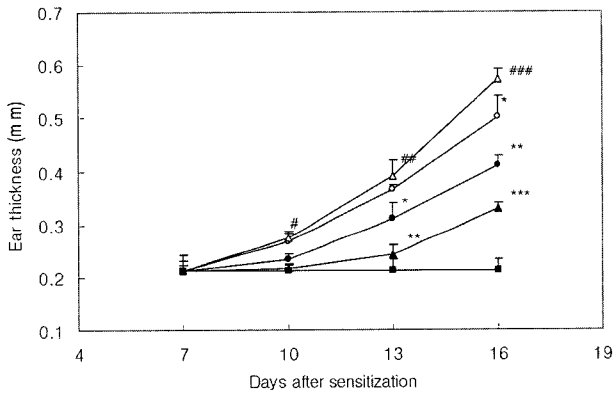

Fig. 2. Effects of HT on the ear thickness of mice induced by repeated application of oxazolone. ■, vehicle alone (normal) treated control; △, oxazolone alone treated control; ○, oxazolone and 0.05% HT treated group; ●, oxazolone and 0.1% HT treated group; ▲, oxazolone and 0.1% betamethasone treated group. Values represent means ± S.D. for five mice. #Significantly different from normal control group (#p<0.05, ##*P* <0.01). *Significantly different from the oxazone alone treated group (*P<0.05, **P<0.01,***p<0.001).

Inhibition of HT on ear thickness of oxazolone-induced mouse ear dermatitis

To evaluate inhibitory effect of contact hypersensitivity, We investigated its inhibitory activity in oxazolone-induced mouse ear dermatitis (Fig. 2). Betamethasone used as a positive agent at concentration of 0.1% potently suppressed ear swelling with a suppressive rate of 84% at 16 days. HT also potently suppressed ear swelling at each time-point. The suppressive rate of HT at concentration of 0.05 and 0.1 was 23 and 46% at 16 days.

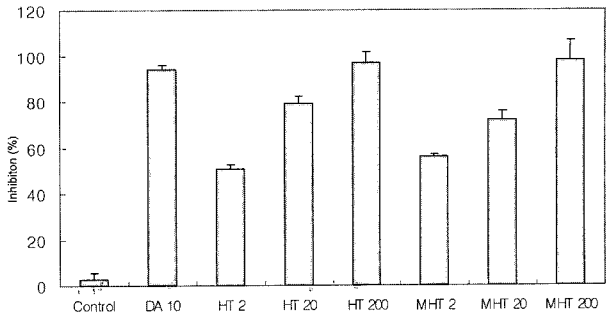

Fig. 3. Inhibitory effect of HT and MHT on b-hexosaminidase release of RBL-2H3 cells induced by IgE. HT, Hwangryundaedoktang; MHT, HT metabolized by human intestinal bacteria. HT and MHT were treated at doses of 2 (HT2 and MHT2), 20 (HT 20 and MHT 20) and 200 μg/ml (HT 200 and MHT 200). Dexamethasone (DA10, 10 μg/ml) was treated.

Inhibition of HT and MHT on β-hexosaminidase release from RBL-2H3 Cells

To investigate whether HT and MHT possess antiallergic activity, we examined the inhibitory effects of HT and MHT metabolized by human intestinal bacteria on β-hexosaminidase release from RBL-2H3 cells induced by IgE (Fig. 3). Both HT and MHT had the strongest inhibitory activity on the β-hexosaminidase release induced by IgE. β-Hexosaminidase release induced by antigen (DNP-HSA) was 45.2% for the cell content of 5×10^5 cells/ well. The spontaneous β-hexosaminidase release was 3.2% of the cell contents. HT and MHT did not show cytotoxicity in a concentration of less than 0.1 mg/ml (Data not shown).

DISCUSSIONS

Allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and food allergy afflict up to 20% of the human population in most countries (Wuthrich, 1989). The etiology of allergy reactivity is based on IgE-medicated pharmacological processes of a variety of cell populations such as mast cell and basophils (Stevens and Austen, 1989). Degradation of mast cells and basophils with antigen-crosslinked IgE releases histamine, prostaglandins, leukotrines and cytokines. These cytokines activates chemotaxis and phagocytosis of neutrophils and macrophages. Finally cytokine-induced reaction causes tissue inflammation. Antiallergic psoriasis is a chronic and inflammatory skin disorder. To evaluate antipsoriastic agents, Fujii *et al.* (2002) developed oxazolone-induced animal model for chronic psoriatic dermatitis featuring epidermal hyperplasia. The dermatitis induced by oxazolone was accompanied by sustained swelling, predominant epidermal hyperplasia and marked infiltration of inflammatory

cells consisting of monocytes, granulocytes and macrophages but not eosionphils. Therefore, antipsoriastic agents with anti-allergic action may be beneficial for psoriastic patients.

Although DSCG is a representative antiallergic drug (Cox, 1967), it did not show antipsoriastic activity. Although NASIDs and betamethasone and immunosuppressants FK-506 and cyclosporin A for Th1 cells have been used clinically for psoriasis, these exhibit side effects. Therefore, the antiallergic and activities of herbal formulas were evaluated. HT showed the potent inhibitory activity against PCA reaction and oxazolone-induced contact dermatitis. The HT inhibited the release of β-hexosamindase on RBL 2H3 cells induced by IgE. DSCG and azelastine are mainly known to inhibit the release of chemical mediators from mast cells induced by the anigen-IgE antibody reaction, but weakly inhibit the chemical mediator from RBL-2H3 cells (Hernandez *et al.*, 2001; Schafer-Korting *et al.*, 1996; Sakuma *et al.*, 2001; Friedman *et al.*, 2002).

In addition, most herbal medicines are orally administered. Their constituents are inevitably brought into contact with intestinal microflora in the alimentary tract (Kobashi and Akao, 1997; Kim, 2002). Some could be transformed by the intestinal bacteria before absorption from the gastrointestinal tract. Studies on the metabolism of the components of these drugs by human intestinal microflora are of a great importance to an understanding of their biological effects. Therefore, we investigated antidegranulation activity of HT and MHT against IgE-induced RBL-2H3 cells. However, HT and its metabolite MHT both potently inhibit the release of β-hexosamindase from RBL-2H3 cells. The difference between Ht and MHT was not significant. When the constituents of HT and MHT were analyzed, baicalin in HT was decreased by the treatment of intestinal microflora, but baicalein was increased (Data not shown). Nevertheless, inhibitory activity of MHT against degranulation of RBL-2H3 cells induced by IgE was not increased compared to that of HT. And HT and MHT both scavenged the superoxide anion (Data not shown). However, their antioxidant activities were not different. These results suggest that HT may be effective for PCA reaction and inflammation such as atopic and psoriastic diseases and HT may be a usable agent for skin disorder contact dermatitis.

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