Effect of Chunghyuldan in Chronic Oxazolone-Induced Mouse Dermatitis

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To evaluate the antipsoriatic effect of Chunghyuldan (CHD, Daio-Orengedokuto in Japanese), which exhibited anti-inflammatory and anti-ischemic actions, the inhibitory activity of CHD metabolized with and without human intestinal microflora was investigated in oxazolone-induced mouse ear dermatitis. The CHD and metabolized CHD (MCHD) at concentrations of 0.1% also potently suppressed mouse ear swelling by 52.7% and 63.2% at 16 d, respectively. The antipsoriatic effect between CHD and MCHD was not significantly different, although that of CHD weakly increased by the metabolism of human intestinal microflora. Both CHD and MCHD also potently reduced the mRNA levels of cyclooxygenase (COX)-2, interferon (IFN)-γ and IL-4 increased in oxazolone-applied mouse ears, but weakly inhibited that of IL-1β and TNF-α. Based on these findings, CHD may improve contact dermatitis or psoriasis by the regulation of COX-2 produced by macrophage cells and IFN-γ and IL-4 produced by Th cells.

Key words Chunghyuldan; Daio-Orengedokuto; psoriasis; contact dermatitis; COX-2; IFN-γ

Psoriasis is a chronic and inflammatory skin disorder. Psoriasis patients have been shown to have the bias of interferon (IFN)-γ producing Th1 and cyclooxygenase (COX)-induced macrophage in lesion skin and peripheral blood.

Cyclooxygenase (COX)-2 inhibitors non-steroidal anti-inflammatory drugs and corticosteroids and immunosuppressants FK-506 and cyclosporine A for Th1 cells have been used clinically for psoriasis.5—7) 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 uses for chronic skin diseases.5,7) Repeated application of corticosteroids on dorsal skin of rats also causes dramatic skin atrophy. FK-506 and cyclosporine A also exhibited side effects, such as severe nephrotoxicity and neurotoxicity.6) Therefore, herbal medicines for clinical use, such as Centella asiatica extract, Orengedokto and Sosusan, should be developed.10,11) Nevertheless, antipsoriatic effects of these herbal medicines have not been thoroughly studied.

During the screening program to discover such agents from herbal medicines, Chunghyuldan (CHD, Daio-Orengedokuto in Japanese) exhibited the anti-inflammatory effect.12) Therefore, we evaluated the antipsoriatic effect of CHD in the oxazolone-induced mouse contact dermatitis model by topical administration and measured mRNA levels of COX-2 and some cytokines.

MATERIALS AND METHODS

Materials Oxazolone, lipopolysaccharide (LPS), dexamethasone and RNase-free DNase were purchased from Sigma Co. (St. Louis, MO, U.S.A.). TRI reagent was purchased from Molecular Research Center Inc. (Cincinnati, Ohio, U.S.A.).

CHD and metabolized CHD were prepared according to the previously reported methods of Cho et al.12) Animals The female BALB/c mice (20—25 g) were supplied from Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20—22 °C and 50 ± 10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center, 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).

Contact Hypersensitivity An oxazolone-induced dermatitis was measured according to the previous method of Fuji et al.9) BALB/c mice were sensitized by application of 100 μl of 1.5% oxazolone in ethanol to the abdomen. 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 mouse ear every 3 d starting from 7 d after sensitization. Ear thickness was measured using a Digimatic Micrometer (Mitsutoyo Co., Tokyo, 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.

RT-PCR Analysis Ear tissue extract for RT-PCR analysis were performed by the modified method of Chi et al.13). Briefly, ears were excised 6 h after the last application of oxazolone, frozen in liquid nitrogen and homogenized by a mortar and pestle prechilled in liquid nitrogen. Total RNA was extracted by using TRI reagent according to the manufacturer's instructions, and treated with RNase-free DNase. The concentration of RNA content was determined by measuring the absorbance at 260 and 280 nm and stored at −70 °C until RT-PCR analysis. The RT-PCR was performed with AccPower® RT/PCR Premix (Bioneer, Seoul, Korea). The primers were designed as described by UniSTS database: COX-1, forward primer 5′-CTTTATCTCTCCACAG-GATTGTTG-3 and reverse primer 5′-GCTTGTCTCTCCTCCTCCAG-3′ (product size 231 bp); COX-2 (UniSTS 254306), forward primer 5′-TGTATCCCCACCAGTCAAGACAC-3′ and reverse primer 5′-GTGCTTCAGAGCCAGTGG-3′ (product size 146 bp); IL-1β, forward primer 5′-ATGGC-AACTGTCCCTGAACAC-3′ and reverse primer 5′-GCTGTTGCTTTGCTCTCCC-3′ (product size 508 bp); IFN-γ (UniSTS 160031), forward primer 5′-CTTTACCAAGCTTGAACCTAGCAG-3′ and reverse primer 5′-GCGGAGTATTGTGT-CATTGGG-3′ (product size 144 bp); IL-4 (UniSTS 143568), forward primer 5′-CCAGATTATGGTGTAATTTCTTATGCTG-3′ and reverse primer 5′-GGCCAATCAGACCTCTCTCTC-3′.
RESULTS AND DISCUSSION

During the screening program to discover antipsoriatic agents from herbal medicines, the effect of CHD was measured in an oxazolone-induced dermatitis mouse model by topical administration (Fig. 1). The ear applied with oxazolone to sensitized mice caused erythema (reddening of the skin), edema and/or induration, and sometimes abrasion. When ear thickness was measured as an index of skin inflammation, it increased as application was repeated, and reached its maximum 16 d after sensitization. The ear applied with oxazolone dramatically swelled. Betamethasone used as a positive agent at a concentration of 0.1% potently suppressed oxazolone-induced ear swelling with a suppressive rate of 83.1% at 16 d. CHD also potently suppressed ear swelling at each time-point. The suppressive rates of CHD at concentrations of 0.05% and 0.1% were 50.1% and 52.7% at 16 d, respectively. MCHD also inhibited the increment of epidermal thickness of ear treated by oxazolone. For histopathological analysis, we excised the ear at 16 d and stained it with hematoxylin–eosin (Fig. 2). The ear applied with oxazolone swelled so dramatically that the entire section could not be shown. Epidermal hyperplasia and intra-epidermal inflammatory cell infiltration in the superficial dermis was also observed. Both CHD and MCHD improved the ear that the entire section could not be shown by the application with oxazolone, and epidermal hyperplasia in its superficial dermis.

The effects of CHD and MCHD in mRNA levels of COX-1 and COX-2 of mouse ear dermatitis induced by oxazolone were investigated by using RT-PCR analysis (Fig. 3). Oxazolone significantly induced mRNA levels of COX-2, however, did not induce that of COX-1. When CHD and MCHD were treated in oxazolone-stimulated mouse ears, the CHDs did not affect the COX-1 mRNA level. However, CHD at concentrations of 0.05% and 0.1% inhibited mRNA levels of COX-2 by 29.3% and 41.0%, respectively. When CHD was treated with human intestinal microflora, its inhibitory activity was slightly increased, compared to that of untreated CHD. The effects of CHD and MCHD in mRNA levels of TNF-α and IL-1β, which are produced by macrophage or monocyte, and IFN-γ and IL-4, which are by Th1 and Th2 cells, respectively, were investigated by using RT-PCR analysis (Fig. 3). Oxazolone significantly induced their mRNA levels. CHD and MCHD similarly inhibited IFN-γ and IL-4 mRNA levels. However, they weakly inhibited mRNA levels of IL-1β and TNF-α.

Fujii et al. developed oxazolone-induced animal model for chronic psoriatic dermatitis featuring epidermal hyperplasia in which IFN-γ 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 eosinophils. In the present study, the oxazolone-induced dermatitis was also accompanied by sustained swelling and predominant epidermal hyperplasia as reported by Fujii et al. IFN-γ and TNF-α, which are cytokines involved in
chronic skin inflammatory disease, and COX-2, which is an acute marker of acute inflammatory disease were induced. CHD, which is a potent inhibitor against NO and PGE2 productions in LPS-induced RAW264.7 cells, significantly inhibited sustained swelling (thickness) of mice ear induced by oxazolone as well as mRNA levels of COX-2. Furthermore, CHD potently inhibited that of IL-1β produced by macrophages, however, weakly reduced the mRNA levels of IL-1β and TNF-α. The CHD potently inhibited the mRNA increase of oxazolone-induced interferon-γ, which is produced by Th1 cells, and IL-4, which is produced by Th2 cells. Cho et al. reported that CHD inhibited COX-2 level in LPS-induced RAW264.7 cells. These results suggest that CHD may differently affect the expression of macrophage-associated cytokine genes in vivo, although the possibility of CHD to alter mRNA stability is not totally excluded. When CHD was metabolized by human intestinal microflora, its antipsoriatic activity was slightly increased, compared to that of CHD. Nevertheless, the antipsoriatic activity between CHD and MCHD was not significant. These results suggest that the antipsoriatic effect of CHD may not be affected by intestinal microflora. Based on these findings, the CHD can improve chronic and inflammatory skin disorders contact dermatitis or psoriasis by the regulation of COX-2 and IL-1β produced by macrophage cells and interferon-γ and IL-4 produced by Th cells.

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