Effect of Chinese herbal medicine, Sho-fu-san, on IgE antibody-mediated biphasic cutaneous reaction in mice

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(Received November 30, 1995. Accepted January 17, 1996.)

Abstract

The effects of some traditional Chinese herbal medicines on IgE-mediated biphasic cutaneous reaction were studied in BALB/c mice. Mice were passively sensitized by an intravenous injection of monoclonal anti-dinitrophenyl IgE antibody. Biphasic cutaneous reaction with peak responses at 1 (early phase) and 24 hr (late phase) was elicited by epicutaneous challenge with an antigen in passively sensitized mice. Sho-fu-san clearly inhibited the IgE-mediated biphasic cutaneous reaction, whereas Jumi-haidoku-to and Oren-gedoku-to did not affect the reaction. In order to elucidate the inhibitory mechanism of Sho-fu-san, the effects of Sho-fu-san on histamine- and tumor necrosis factor-α (TNF-α)-induced cutaneous reactions were examined in mice. Sho-fu-san suppressed the edema induced by histamine and TNF-α in mouse ears. The expression of TNF-α and IL-1β mRNA caused by antigen challenge in passively sensitized mouse ears was not affected by Sho-fu-san. These results suggest that Sho-fu-san inhibited IgE antibody-mediated biphasic cutaneous reaction due to the suppression of histamine- and TNF-α-induced cutaneous reactions.

Key words Sho-fu-san (Xiao-Feng-San) 消風散, Jumi-haidoku-to (Shi-Wei-Bai-Du-Tang) 十味敗毒湯, Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) 黄連解毒湯, allergic cutaneous reaction, IgE, tumor necrosis factor-α, prednisolone.

Abbreviations IL-1β, interleukin-1β ; TNF-α, tumor necrosis factor-α ; DNP, dinitrophenyl ; DNFB, dinitrofluorobenzene ; EPR, early phase reaction ; LPR, late phase reaction ; RT-PCR, reverse transcriptase-polymerase chain reaction.

Introduction

An increasing number of patients with chronic, allergic and inflammatory diseases, such as bronchial asthma, allergic rhinitis and atopic dermatitis, has been recently reported.[1-5] Even though glucocorticoids seem to be the most effective drugs for treatment of chronic allergic diseases, they have shown to have some severe side effects.[6-9] For example, long term treatment with glucocorticoids can lead to skin-thinning and decreased resistance to infection. In order to counter such adverse effects of glucocorticoids, some Chinese herbal medicines have been used for long term treatment of chronic allergic inflammation because of their low toxicity and high effectiveness.[1,5,10-14] However, despite their valuable clinical effectiveness, precise pharmacological mechanisms of Chinese herbal medicines are still obscure.

Previously we reported on an IgE-mediated biphasic cutaneous dermatitis model and its pharmacological usefulness.[15,16] When mice were passively sensitized with anti-dinitrophenyl (DNP) IgE, a biphasic cutaneous reaction was elicited at the challenge site with an antigen, dinitrofluorobenzene (DNFB). This dual response was composed of an IgE-mediated cutaneous early phase reaction (EPR) and a late phase reaction (LPR), which peaked at 1 and 24 hr after the antigen challenge, respectively. Since the antigen-induced biphasic skin reaction, especially its

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late phase, is one of the promoting factors in severe or chronic allergic reactions,[17] investigating the effects of various agents on the late phase reaction is important for finding out the effective agent in chronic allergic reactions.

The present study was, therefore, conducted to examine the effects of some Chinese herbal medicines on the IgE antibody-mediated biphasic cutaneous reaction, especially on its late phase reaction, in mice.

Materials and Methods

Mice : BALB/c mice (female, 9-10-weeks-old), barrier-derived and specific pathogen-free, were purchased from Japan SLC (Hamamatsu, Japan).

Antigen : 2,4-Dinitrofluorobenzene (DNFB) was purchased from Nakalai Tesque (Kyoto, Japan) and dissolved in acetone-olive oil (3 : 1).

Monoclonal IgE preparation : Monoclonal anti-DNP IgE antibodies were prepared as previously described.[15,16] Briefly, the monoclonal antibody producing cell line, EC1, was cultured in a medium (composed of equal volumes of RPMI 1640 and DMEM) until a confluent state was reached. The supernatant was harvested, centrifuged at 400×g and stored at -80°C until use. PCA titer of the IgE preparation was 1 : 1024.

Reagents : Recombinant murine tumor necrosis factor-α (TNF-α) and polyclonal rabbit anti-human TNF-α were purchased from Genzyme Corporation (Boston, USA).

Sho-fu-san (Xiao Feng-San, TJ-22), Jumi-haidoku-to (Shi-Wei-Bai-Du-Tang, TJ-6) and Oren-gedoku-to (Huang-Lian-Jie-Du-Tang, TJ-80) were kindly donated from Tsumura & Co. LTD (Tokyo, Japan). Each medicine was administered orally 1 hr prior to antigen challenge. Prednisolone acetate (prednisolone, Shionogi, Osaka, Japan) was obtained commercially. Prednisolone was injected intraperitoneally 2 hr before each of the skin tests.

IgE-mediated cutaneous reaction : The cutaneous reaction was elicited by the method previously described.[15,16] Briefly, BALB/c mice were passively sensitized by an intravenous injection of anti-DNP monoclonal IgE. Cutaneous reaction was elicited by painting 25 μl of 0.15 % DNFB acetone-olive oil solution on both sides of each ear 24 hr after the sensitization. The ear thickness was measured by a micrometer, Upright Dial Gauge (Peacock, Ozaki, Tokyo, Japan) before and after challenge.

Cytokine- and histamine-induced mouse ear edema : Mice received an injection of 10 μl of TNF-α at a concentration of 10^5 U/ml or histamine at a concentration of 10^{-4} g/ml in both sides of each ear. Ear thickness was measured by the same method as described above.

Cytokine mRNA expression : Cytokine mRNA levels of the mouse ears were assessed by reverse transcriptase-polymerase chain reaction (RT-PCR). Mice were sacrificed before and after the skin testing with DNFB. Immediately after sacrificing the mice, the ears were cut and total ear RNA was extracted by using ISOGEN (Nippon Gene, Co. Ltd., Japan). The amount of each RNA was measured using a ratio of 260/280 nm. cDNA was synthesized from 1 μg of RNA using a cDNA synthesis kit (Clontech Lab. Inc., USA). RNA was reverse-transcribed for 1 hr at 42°C. The cDNA so obtained was amplified by using a GeneAmp RNA PCR kit (Perkin Elmer Co. Ltd., USA) with an appropriate specific primer shown in Table I. For the polymerase chain reaction, temperature cycling was done using a thermal cycler. Conditions were set as 5 min at 94°C for one cycle, 5 min at 62°C for one cycle, 1.5 min at 94°C, 1.5 min at 62°C and 1.5 min at 72°C for 30 cycles, and 10 min at 72°C for one cycle. Gel electrophoresis was done to detect each product. To visualize the DNA, ethidium bromide was used.

Statistics : Results were expressed as the mean± S.E.M. Either Student's or Welch's t-test was used to evaluate the data, after the variances of data were examined.

Table I Primers used for detecting IL-1β, TNF α and β-actin mRNA in RT-PCR.

IL-1β	330	CAGGATGAGGACATGAGCACC 350
	756	CTCTGCAGACTCAAACTCCAC 776
TNF α	157	ATGAGCACAGAAAGCATGATC 177
	412	TACAGGCTTGTCACTCGAATT 432
β Actin	105	GTGGGCCGCTCTAGGCACCA 124
	325	CGGTTGGCCTTAGGGTTCAGGGGGG 349

Results

IgE-mediated biphasic cutaneous reaction

Epicutaneous antigen challenge resulted in biphasic cutaneous edema in BALB/c mice which were passively sensitized with anti-DNP IgE antibodies. As shown in Fig. 1, Sho-fu-san clearly inhibited both EPR and LPR at doses of 100, 300 and 1,000 mg/kg, whereas Jumi-haidoku-to and Oren-gedoku-to did not affect the IgE-mediated biphasic cutaneous reaction (Fig. 2). Prednisolone almost completely inhibited EPR and LPR at a dose of 10 mg/kg (Fig. 1). In order to confirm the participation of histamine and TNF-α, the effects of diphenhydramine, cetirizine and anti-TNF-α monoclonal antibodies on the reaction were examined. As shown in Figs. 3 and 4, the two antihistamines clearly inhibited the EPR but not the LPR. On the other hand, anti-TNF-α inhibited both EPR and LPR significantly.

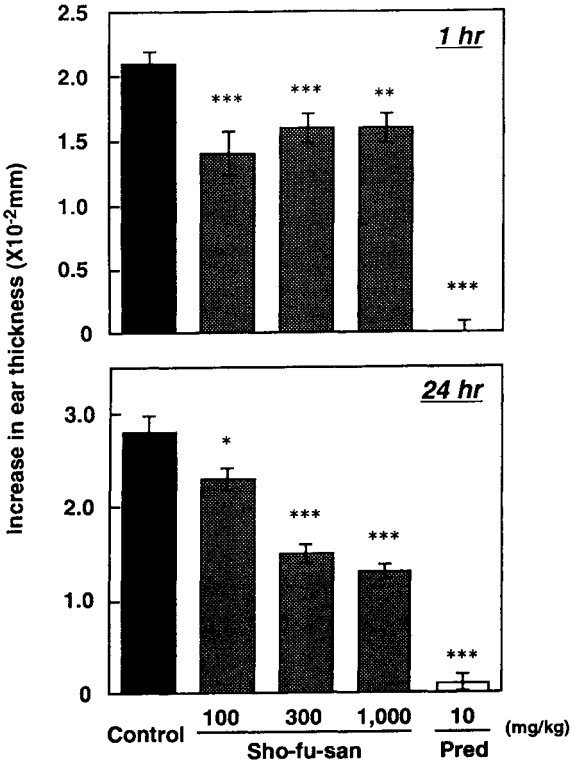

Fig. 1 The effects of Sho-fu-san and prednisolone on IgE-mediated biphasic cutaneous reaction in mice. Sho-fu-san was administered orally 1 hr before challenge. Prednisolone was given intraperitoneally 2 hr before challenge. Each value represents the mean±S.E.M. of 8 mice. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

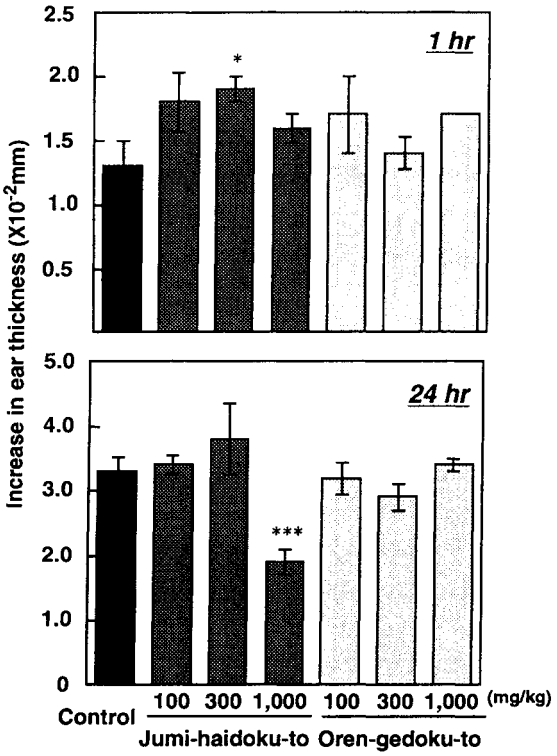

Fig. 2 The effects of Jumi-haidoku-to and Oren-gedoku-to on IgE-mediated biphasic cutaneous reaction in mice. Jumi-haidoku-to and Oren-gedoku-to were administered orally 1 hr before challenge. Each value represents the mean±S.E.M. of 8 mice. $^{*}p<0.05$, $^{***}p<0.001$

Since, Sho-fu-san showed clear inhibitory action on the biphasic cutaneous reaction, the following experiments were carried out to investigate its mechanism.

Histamine-induced ear edema

In order to estimate the inhibitory mechanism of Sho-fu-san on EPR, a histamine-induced cutaneous reaction was examined. As shown in Fig. 5, ear thickness reached its maximum 10 min after the injection of histamine into the skin. Sho-fu-san suppressed the histamine-induced edema at doses of 300 and 1,000 mg/kg.

Cytokine mRNA expression

The effect of Sho-fu-san on the expression of mRNA in the ear was examined. Cytokine mRNA

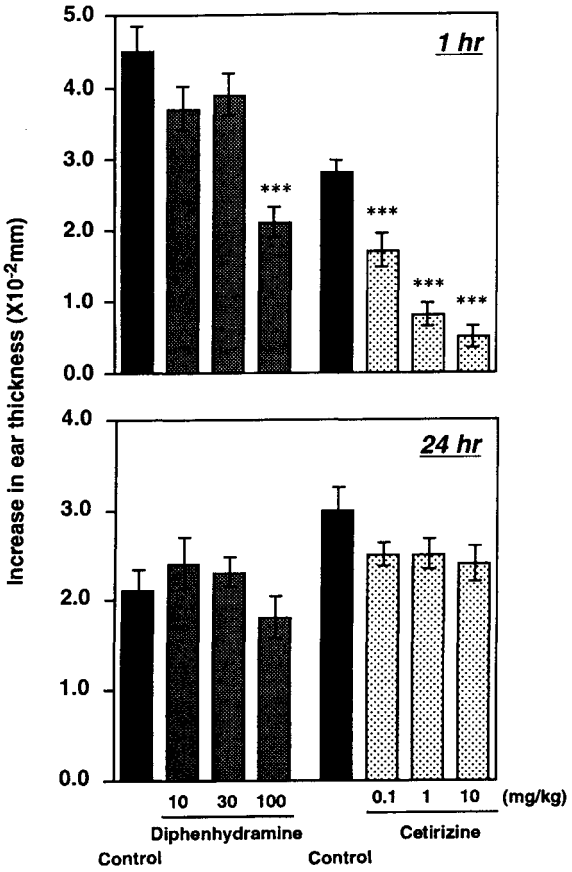

Fig. 3 The effects of diphenhydramine and cetirizine on IgE-mediated biphasic cutaneous reaction in mice. Diphenhydramine and cetirizine were administered orally 1 hr before challenge. Each value represents the mean±S.E.M. of 8 mice. $^{***}p<0.001$

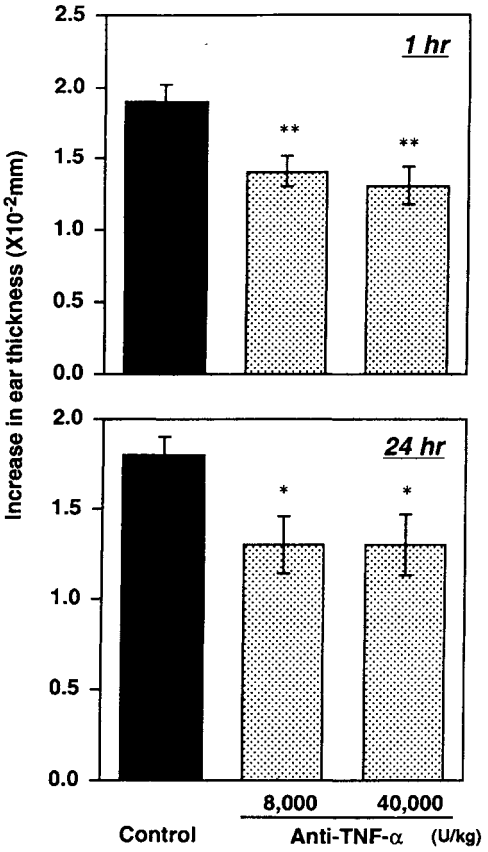

Fig. 4 The effects of anti-TNF-α on IgE mediated biphasic cutaneous reaction in mice. Anti TNF-α was administered intravenously 10 min before challenge. Each value represents the mean±S.E.M. of 8 mice.
$^{*}p<0.05$, $^{**}p<0.01$

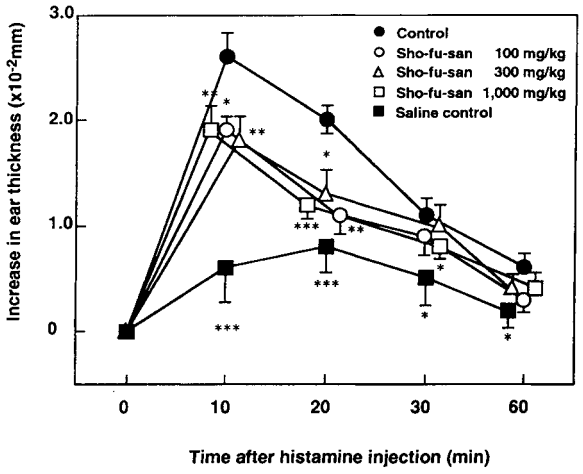

Fig. 5 The effect of Sho-fu-san on histamine-induced skin reaction in mice. Sho-fu-san was given orally 1 hr before histamine injection. Each value represents the mean ± S.E.M. of 6 or 7 mice. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

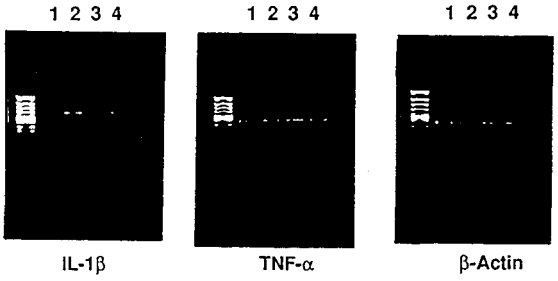

Fig. 6 The effect of Sho-fu-san on the expression of IL-1β, TNF-α, and β-actin mRNA in mouse ears. 300 mg/kg of Sho-fu-san was administered 1 hr before skin testing with DNFB. mRNA was extracted from the ears of mice 4 hr after challenge. Lane 1 : non-treated (without challenge), lane 2 : control (challenged), lane 3 : Sho-fu-san (without challenged), lane 4 : Sho-fu-san (challenged)

was extracted from ears 4 hr after antigen challenge. Sho-fu-san at a dose of 300 mg/kg was given orally 1 hr before the skin testing with DNFB. As shown in Fig. 6, the degree of the expression of interleukin 1 β (IL 1 β) and TNF α mRNA was not affected by Sho-fu-san.

TNF-α-induced ear edema

In order to determine the role of TNF-α in the cutaneous reaction, TNF-α induced ear edema was examined. Increased ear thickness, which peaked at 24 hr, was observed after the injection of recombinant murine TNF-α at a dose of 1,000 U/ear into the ear. As shown in Fig. 7, Sho-fu-san inhibited TNF-α-induced ear edema. Prednisolone clearly suppressed the TNF-α-induced edema at doses of 3 and 10 mg/kg.

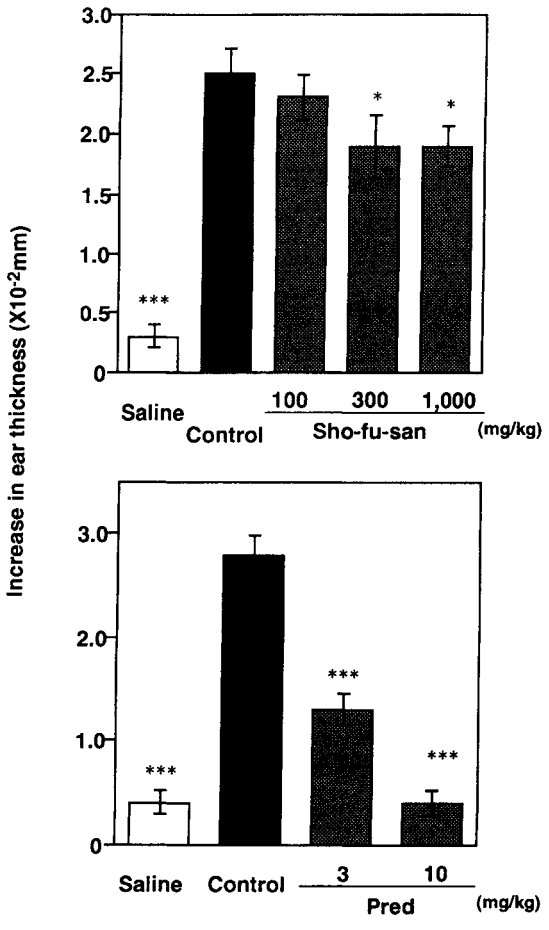

Fig. 7 The effects of Sho-fu-san and prednisolone on TNF-α-induced skin reaction in mice. Skin reaction was caused by injecting 1,000 U TNF α into the ear. Sho-fu-san was administered orally 1 hr before TNF α injection. Prednisolone was given intraperitoneally 2 hr before TNF-α injection. Each value represents the mean ± S.E.M. of 6 to 8 mice. $^{*}p<0.05$, $^{***}p<0.001$

Discussion

Many Chinese herbal medicines have been used in the treatment of patients with chronic allergic inflammatory diseases such as bronchial asthma and atopic dermatitis.[1-5] Recently some reports have indicated that many Chinese plants and Chinese herbal medicines have shown anti-allergic and anti-inflammatory effects[10, 14, 18, 19] in experimental animals and clinical patients. Sho-fu-san, Jumi-haidoku-to and Oren-gedoku-to, three Chinese blended medicines, seem to be effective drugs for the treatment of allergic cutaneous diseases. In the present study, we examined the effects of Sho-fu-san, Jumi-haidoku-to and Oren-gedoku-to on IgE-mediated biphasic cutaneous reaction in mice.

As indicated in the results, a single administration of Sho-fu-san inhibited both EPR and LPR in the cutaneous reaction. Previously, we reported that the EPR was caused mainly by mast cell-derived chemical mediators such as histamine and serotonin.[19] Therefore, we examined the effect of Sho-fu-san on the histamine-induced cutaneous reaction in mouse ears. When histamine was injected, it produced an edema which peaked at 10 min. This edema terminated within 60 min after injection. Sho-fu-san inhibited histamine-induced edema. The inhibitory pattern and potency of Sho-fu-san on the histamine-induced reaction is similar to its effect on EPR. These results suggests that the inhibition of IgE-mediated EPR by Sho-fu-san is due to an antagonism to histamine. In addition, we investigated the effect of Sho-fu-san on antigen-induced histamine release from sensitized peritoneal mast cells of rats. Sho-fu-san did not affect antigen-induced histamine release from rat peritoneal mast cells (data not shown). This suggests that Sho-fu-san mainly inhibited the histamine-induced reaction in EPR.

Our previous experiments had indicated that cytokines, especially pro-inflammatory cytokines such as IL-1 β, TNF-α and IL-6, play an important role in LPR. In order to investigate the inhibitory mechanisms of Sho-fu-san on IgE antibody-mediated LPR,

we examined the effect of Sho-fu-san on cytokine mRNA expression and cytokine activity. In the first experiment, we examined the expression of IL-1 β and TNF-α mRNA in a skin region IgE-mediated reaction. Since our preliminary experiments indicated the expression of IL-1 β and TNF-α mRNA 4 hr after DNFB challenge, we tested the effect of Sho-fu-san on the expression of mRNA. Sho-fu-san did not influence the expression of IL-1 β and TNF-α mRNA.

We then tested the effect of Sho-fu-san on the edema induced by TNF-α. Sho-fu-san suppressed TNF-α-induced edema. Prednisolone also suppressed the edema. These results suggest that Sho-fu-san inhibits IgE-mediated LPR by inhibiting the action of TNF-α.

In the present study, we examined the effects of only a single administration of Chinese herbal medicines because we were conducting a larger main study at the time. To obtain more detailed results, the Chinese herbal medicines should be administered for several days. therefore, we are presently examining the effects of sequential administration of these medicines on IgE-mediated biphasic reaction.

In conclusion, the present results demonstrate the effectiveness of Sho-fu-san on IgE-mediated biphasic cutaneous reaction, especially LPR. The inhibitory mechanism of Sho-fu-san is related to the inhibition of histamine-induced reaction which is responsible for EPR or TNF-α-induced edema in LPR.

和文抄録

臨床でアレルギー性皮膚炎の治療に応用されている3種の漢方方剤のIgE依存性二相性皮膚反応に及ぼす影響を検討した。BALB/cマウスに抗DNPモノクローナルIgE抗体を注射して受動的に感作し，24時間後に耳殻に抗原を塗布すると，抗原塗布1時間後をピークとする即時相と24時間後をピークとする遅発相の二相性の皮膚反応が観察された。消風散はIgE依存性二相性皮膚反応を明らかに抑制したが，十味敗毒湯および黄連解毒湯は影響を及ぼさなかった。消風散の皮膚反応抑制作用機序を解明するため，histamineおよびTNF-αによる皮膚反応に及ぼす影響を検討した。消風散はhistamineおよびTNF-αによる皮膚反応を抑制したが，反応局所におけるIL-1 βおよびTNF-α mRNAの発現には影響を及ぼさなかった。したがって，消風散はhistamineおよびTNF-αの作用を抑制することによりIgE依存性二相性皮膚反応を抑制するものと考えられる。

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