Effect of Byakko-ka-ninjin-to on experimental allergic cutaneous reaction

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Abstract

The effects of traditional Chinese herbal medicine, Byakko-ka-ninjin-to (白虎加人参湯, TJ-34), on allergic cutaneous reactions were investigated in mice. In the first experiment, the effect on IgE-mediated biphasic cutaneous allergic reaction was studied. Mice were passively sensitized by an intravenous injection of monoclonal anti-dinitrophenyl (DNP) IgE antibody. Biphasic cutaneous reaction with peak responses at 1 hour (early phase) and 24 hours (late phase) was elicited by epicutaneous challenge with antigen in passively sensitized mice. TJ-34 clearly inhibited IgE-mediated biphasic immediate and late phase cutaneous reaction. In the second experiment, the effect of TJ-34 on dinitrofluorobenzene (DNFB)-induced contact dermatitis was examined. TJ-34 inhibited DNFB-induced contact dermatitis. In order to investigate the inhibitory mechanism of TJ-34, the effects of TJ-34 on the production and the action of histamine and cytokines were investigated. TJ-34 inhibited histamine- and TNF-α-induced cutaneous responses but did not affect an anaphylactic histamine release and anti-CD3 antibody- or LPS-induced cytokine production except for IFN-γ production. These data indicate that TJ-34 shows an inhibitory action on IgE-mediated biphasic cutaneous allergic reaction by mainly interfering with the cutaneous response caused by histamine and TNF-α, and on contact dermatitis through the inhibition of IFN-γ production.

Key words Allergic late phase reaction, IgE, Dermatitis, Byakko-ka-ninjin-to, Chinese herbal medicine.

Introduction

Much attention has been paid to the application of some Chinese herbal medicines for the treatment of chronic, allergic and inflammatory diseases. Some medicines are reported to be effective for the treatment of the above diseases.[1-3]

In general, glucocorticoid is the most effective drug for the therapy of chronic allergic diseases. Glucocorticoids showed the inhibitory effect on many points during inflammatory process and immune responses.[4-12] However, a long term treatment with glucocorticoid leads to many severe side effects and problems, including skin thinning and decreased resistance to infection.

Instead of glucocorticoids, some Chinese herbal medicines have been used for long term treatment of chronic inflammation because of their immunomodulating and antiinflammatory activities.[1-3,13-17] Whereas they show valuable clinical efficacy, some of their pharmacological mechanisms are still obscure.

Previously we reported on the IgE-mediated biphasic cutaneous dermatitis model and its pharmacological usefulness.[18,19] Briefly, mice were passively sensitized with monoclonal anti-dinitrophenyl (DNP) IgE antibody. Antigen (dinitrofluorobenzene, DNFB) caused biphasic skin reaction and reached peaks at 1 hr and 24 hr. Chemical mediators, especially histamine secreted from mast cells play an

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important role for the onset of early phase reaction (EPR). Contrary to EPR, some kinds of cytokines including TNF-α play a role in late phase reaction (LPR). This LPR is one of the experimental models for sub-acute allergic dermatitis as well as contact dermatitis.

In the present study, we investigated the effect of Chinese herbal medicine, Byakko-ka-ninjin-to (白虎加人参湯, TJ-34) on biphasic skin reaction and DNFB-induced contact dermatitis in mice and its inhibitory mechanism in animal models.

Materials and Methods

Animals : Balb/c mice (female, 9-10 week old) and Wistar rats (female, 10-12 week old), barrier-derived and specific pathogen-free, were purchased from Japan SLC (Hamamatsu, Japan). They were fed in our laboratory until use.

Materials : 2.4-Dinitrofluorobenzene (DNFB) was purchased from Nakalai Tesque (Kyoto, Japan) and dissolved in acetone : olive oil (3 : 1) mixture before use.

Monoclonal IgE preparation : The monoclonal antibody was prepared as described previously. Briefly, the monoclonal antibody-producing cell lines, EC1 for mouse IgE and REC for rat IgE, were cultured in a medium (mixture of equal volumes of RPMI 1640 and DMEM) until a confluent state. The supernatant was harvested, centrifuged at 400×g and stored at -80°C until use. The PCA titer was 1 : 1,024 or more.

Reagent : Recombinant murine TNF-α (rm TNF-α) was purchased from Genzyme Corporation (Boston, USA).

TJ-34 was kindly donated from Tsumura Co. LTD. TJ-34 was administered orally 1 hr prior to antigen challenge. TJ-34 was a lyophyrized material of the water extract from the mixture containing Gypsum Fibrosum (15), Aenmarrhenae Rhizoma (5), Oryzae Fructus (8), Glycyrrhizal Radix (2) and Ginseng Radix (13). The number in parentheses is the ratio of each crude drug.

Prednisolone acetate (Shionogi, Osaka, Japan) was purchased. Prednisolone was injected intraperitoneally 2 hr before each of the skin tests. Recombinant murine TNF-α and histamine were injected into both ears in a volume of 10 μl. Amlexanox (Solfa) was a gift from Takeda Pharmaceutical Co. Ltd. (Osaka, Japan).

IgE-mediated cutaneous reaction : The cutaneous reaction was elicited by a method previously described.[18,19] Briefly, Balb/c mice were passively sensitized by intravenous injection of anti-DNP monoclonal IgE antibody 24 hr before the test. Cutaneous reaction was elicited by painting 25 μl of 0.15 % DNFB acetone-olive oil solution to each side of both ears.

The ear thickness was measured by micrometer, Upright Dial Gauge (Peacock) at before and appropriate time after challenge.

DNFB-induced contact dermatitis : Mice ears were painted with DNFB or vehicle once each week for 5 weeks. A total of 25 μl of 0.15 % DNFB in vehicle [acetone : olive oil (3 : 1)] was applied to each side of both ears. Ear thickness was measured using the same method as described above.

Histamine- and TNF-α-induced mouse ear edema: Female Balb/c mice received 10 μl of histamine at a concentration of 10^{-4}g/ml or TNF-α at a concentration of 10^5U/ml into both ears. Ear thickness was measured by the same method described above.

Histamine release from peritoneal mast cells : *In vitro* histamine release from peritoneal mast cells was examined as reported previously.[20] Briefly, the rats were passively sensitized by an intraperitoneal injection of 2 ml of 200-fold diluted rat monoclonal IgE preparation. Two days later, peritoneal exudate cells were recovered using Tyrode solution containing 5 U/ml of heparin. Cells were washed twice with Tyrode solution, and suspended in Tyrode solution at a concentration of 10^5 mast cells/ml. Histamine release was initiated by adding DNP-conjugated bovine serum albumin at a final concentration of 1 μg/ml. After incubation with antigen at 37°C for 15 min, the reaction was terminated and the supernatant was separated by centrifugation at 350×g for 10 min. To assess the amount of total histamine, cell-associated histamine was extracted in the presence of 1.2 % $HClO_4$. The amount of histamine released in the supernatant was measured fluorometrically. The mast cell histamine content was 30-40 μg/10^6 cells, and the spontaneous release of histamine was 3 % of total histamine

or lower. Peritoneal mast cells released 10~20 % of their total histamine upon challenge with 1 μg/ml DNP-conjugated bovine serum albumin. Duplicate experiments with the same or a similar design were repeated at least twice, and a representative experiment is shown.

The production of TNF-α from J774.1 cell in vitro : Murine derived macrophage-like cell, J774.1, was cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, 50 μM mercaptoethanol, 50 U / ml penicillin, and 50 μM streptomycin (FCS - RPMI). The cells were adjusted to a concentration of 2×10^5 cells/ml using FCS-RPMI and plated into 24-well plates (Corning Coster Co., MA., U.S.A.) at 1 ml/well. After incubation at 37°C for 30 min in 5 % CO_2, lipopolysaccharide (LPS ; Difco Laboratories, MI, U. S.A.) was added to a final concentration of 1 μg/ml. After incubation for 24 hr, the supernatant was harvested. The supernatant was centrifuged to remove cell debris and then TNF-α was assayed by ELISA (Endogen, Inc., Woburn, MA, U.S.A.).

The production of cytokines in vivo : The effect of TJ-34 on the production of cytokine *in vivo* was examined by two different methods. The productions of TNF-α, IL-6 and IL-1 β was examined by P. acnes and LPS system. In brief, P. acnes at a dose of 0.3 mg (in 0.2 ml saline) was injected intravenously into male C57BL/6 mice. Seven days later, LPS at a dose of 5 mg/kg was injected intravenously into the mice. Blood samples were obtained 2 hr after the LPS injection. Each level of TNF-α, IL-6 and IL-1 β was measured by ELISA (Endogen). To measure the production of IFN-γ and IL-4, anti-CD3 monoclonal antibody (1 mg/head) was injected into C57BL/6 mice. Blood samples were obtained 90 min after the injection of antibody. The amount of IFN-γ and IL-4 was measured by ELISA.

Statistics : Results were expressed as mean±S.E.M. Either Student's or Aspin-Welch's t-test was employed for evaluation of data.

Results

IgE-mediated biphasic cutaneous reaction

Balb/c mice were passively sensitized with anti-DNP IgE antibody, and challenged with DNFB epicutaneously. As shown in Fig. 1, TJ-34 inhibited the EPR and LPR clearly at a dose of 1,000 mg/kg. Prednisolone also inhibited both reactions at a dose of 10 mg/kg.

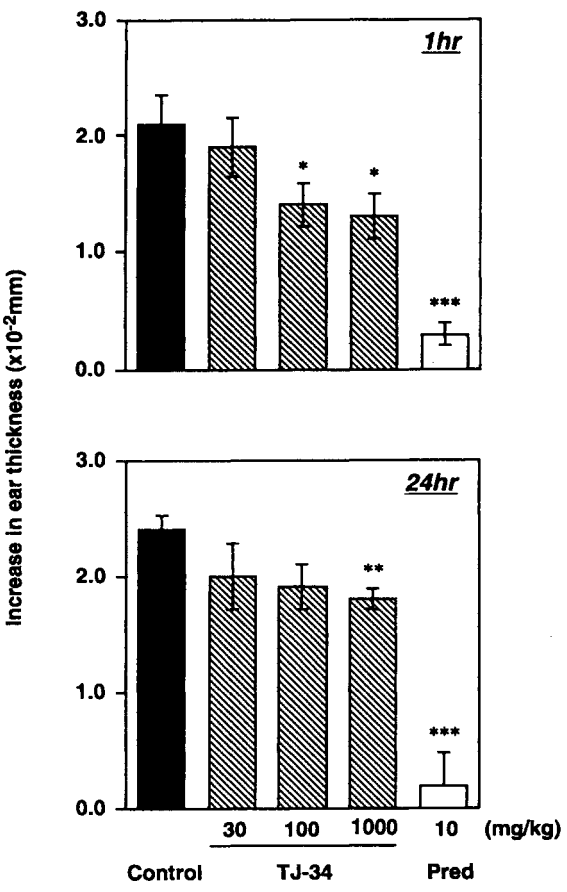

Fig. 1 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on IgE-mediated cutaneous reaction in Balb/c mice. TJ-34 was given orally 25 and 1 hr before the antigen challenge. Prednisolone was given intraperitoneally 2 hr before the antigen challenge. Each value represents the mean ±S.E.M. of 6 animals. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

DNFB-induced contact dermatitis

Balb/c mice were repeatedly stimulated by painting with DNFB on their ears, and the effects of TJ-34 and prednisolone on the dermatitis were examined. As shown in Fig. 2, TJ-34 apparently inhibited the dermatitis and the inhibition was significant at 24 hr after fifth antigen stimulation. Prednisolone also inhibited the reaction. The inhibition of 3 mg/kg of prednisolone was more potent than that of 300 mg/kg of TJ-34.

Histamine-induced ear edema

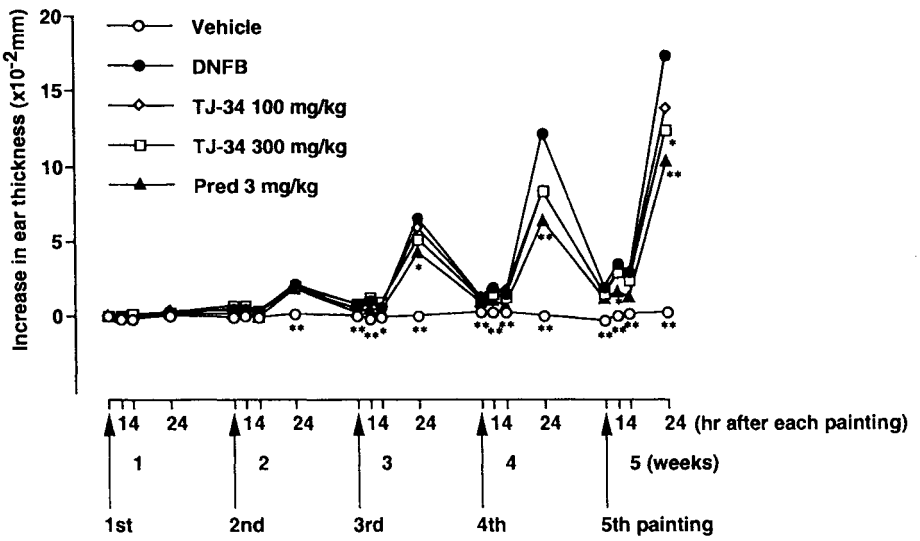

Fig. 2 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on ear swelling caused by repeated painting of DNFB in mice. TJ-34 and Pred were given orally. Each point indicates the mean of 6 or 7 mice. $*p<0.05$, $**p<0.01$

In order to search the inhibitory mechanisms of EPR, histamine induced cutaneous reaction was examined. As shown in Fig. 3, increased ear thickness was observed after the injection of histamine into the skin with a peak at 10 min. TJ-34 suppressed the histamine induced edema at a dose of 1,000 mg/kg.

TNF-α-induced ear edema

In order to confirm the role of TNF-α in the cutaneous reaction, rmTNF-α-induced ear edema was examined. Increased ear thickness was observed after an injection of rmTNF-α at a dose of 1,000 U/ear into the skin with a peak at 24 hr. As shown in Fig. 4, TJ-34 inhibited an increase in TNF-α-induced ear

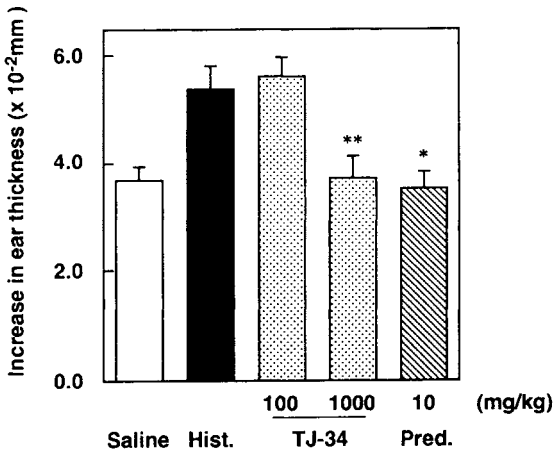

Fig. 3 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on histamine-induced cutaneous reaction in mice ear. TJ-34 was given orally 1 hr before histamine injection. Prednisolone was administered intraperitoneally 2 hr before histamine injection. Each value represents the mean ±S.E.M. of 4-6 animals. $*p<0.05$, $**p<0.01$

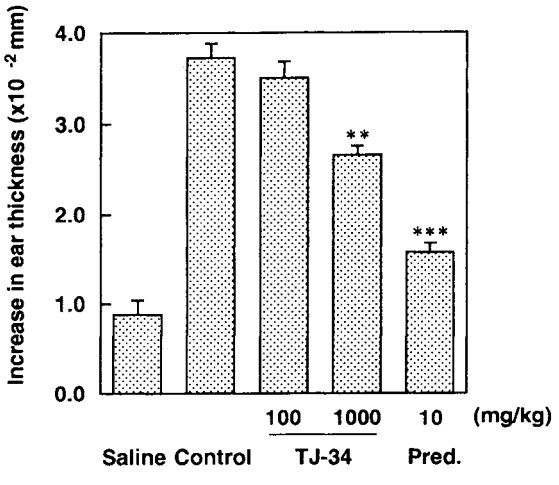

Fig. 4 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on TNF-α-induced cutaneous reaction in mice ear. TJ-34 was given orally 1 hr before TNF-α injection. Prednisolone was administered intraperitoneally 2 hr before TNF-α injection. Each value represents the mean±S.E.M. of 3-6 animals. $**p<0.01$, $***p<0.001$

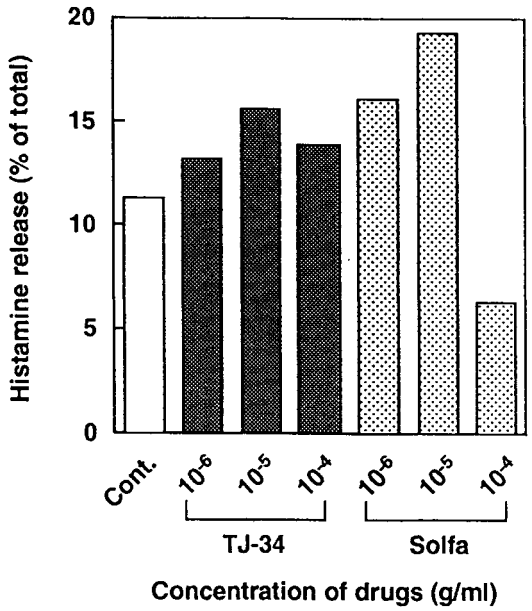

Fig. 5 Effects of Byakko-ka-ninjin-to (TJ-34) and amlexanox (Solfa) on antigen-induced histamine release from rat peritoneal mast cells. Peritoneal mast cells of rats were sensitized *in vivo* by an injection of rat anti-DNP monoclonal IgE antibody. Two days later, mast cells were recovered. Each value represents the mean of duplicate determinations.

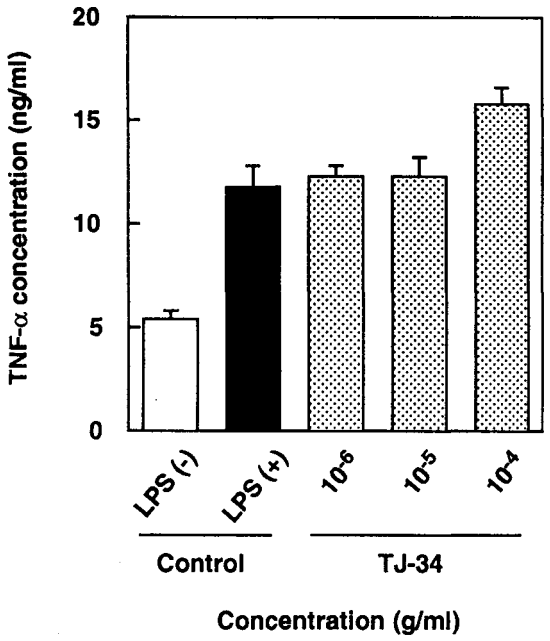

Fig. 6 Effects of Byakko-ka-ninjin-to (TJ-34) on TNF-α production in J744.1 cells. Each column and vertical bar represents the mean±S.E.M. of 8 experiments, respectively. Cells were incubated with TJ-34 for 18 hr at 37°C in 5 % CO_2. TNF-α was measured by ELISA.

thickness. Prednisolone clearly suppressed the TNF-α-induced edema at a dose of 10 mg/kg.

Histamine release and TNF-α production in vitro

Anaphylactic histamine release from rat peritoneal mast cells was clearly inhibited by 10^{-4}g/ml solfa, used as a reference drug. TJ-34 did not affect an antigen-induced release of histamine from rat peritoneal mast cells (Fig. 5). TNF-α production by J774.1 cells was neither affected by TJ-34 at doses between 10^{-6} to 10^{-4} g/ml (Fig. 6).

Production of cytokines in vivo

The injection of bacterial LPS produced a signifi-

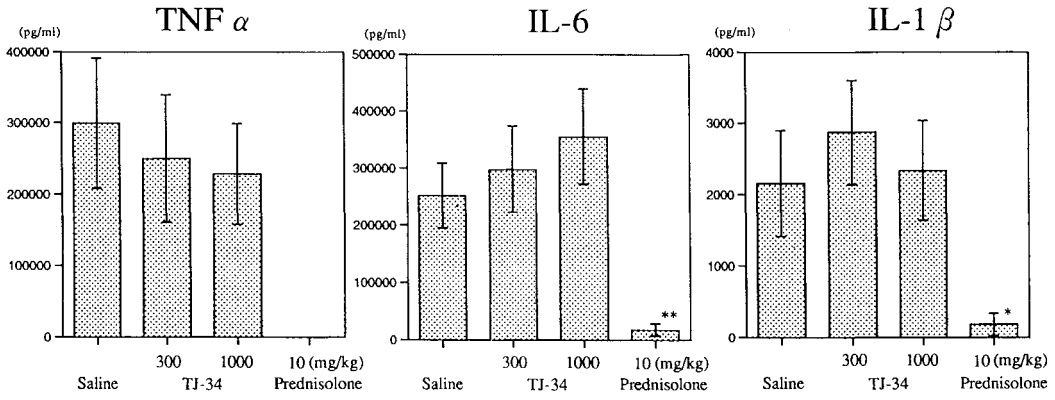

Fig. 7 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on the production of TNF-α, IL-6 and IL-1 β caused by lipopolysaccharide (LPS) in the mice pretreated with P. acnes. TJ-34 was administered orally 1 hr before LPS injection. Pred was administered intraperitoneally 2 hr before LPS injection. Each experiment consisted of 4 to 6 animals. $*p<0.05$, $**p<0.01$

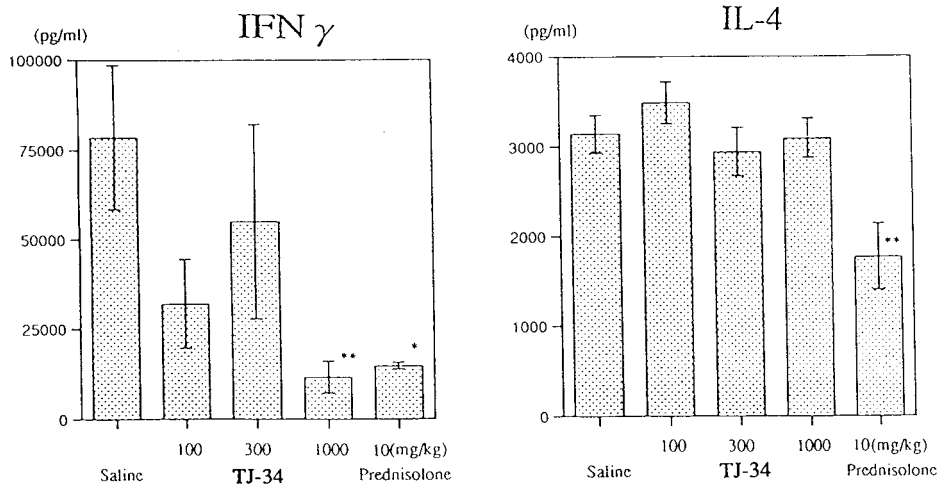

Fig. 8 Effects of Byakko-ka-ninjin-to (TJ-34) and prednisolone (Pred) on anti-CD3 antibody-induced IFN-γ and IL-4 production in mice. TJ-34 was administered orally 1 hr before anti-CD3 antibody injection. Pred was administered intraperitoneally 2 hr before antibody injection. Each experiment consisted of 4 to 6 animals. $^{*}p<0.05$, $^{**}p<0.01$

cant amount of TNF-α, IL-6 and IL-1 β in the serum. TJ-34 at doses of 300 and 1,000 mg/kg did not affect the production of three cytokines (Fig. 7). TJ-34 at a dose of 1,000 mg/kg, however, inhibited the production of IFN-γ but not IL-4 caused by anti-CD3 antibody (Fig. 8). Prednisolone at a dose of 10 mg/kg clearly inhibited the production of all kinds of cytokines examined.

Discussion

Recently, many Chinese herbal medicines have been used for the treatment of patients with chronic inflammatory diseases.[1-3] Some reports have indicated many Chinese plants and Chinese herbal medicines have anti-allergic and antiinflammatory effects.[13,17,22,23] Among many prescriptions, the pharmacological action of Saiboku-to (柴朴湯) was most investigated. Saiboku-to mainly acts on the IgE antibody-[24] and effector T cell-mediated allergic reactions. In addition, Saiboku-to inhibits an antigen-induced accumulation of inflammatory cells in airways and airway hypersensitiveness.[25] In addition to asthma, the effects of some Chinese medicines on allergic skin diseases,[1] for example atopic dermatitis,[2,3] psoriasis and chronic nettle rash have been investigated.

Many well known Chinese herbal medicines have been used for the treatment of allergic skin diseases.[1-3] TJ-34 is often used for the therapy of atopic dermatitis. However the basic pharmacological study on TJ-34 is not yet clear.

In the present study, we examined the effect of TJ-34 on IgE-mediated biphasic cutaneous reaction and DNFB-induced contact dermatitis. TJ-34 exhibited the inhibition of EPR and LPR in IgE-mediated biphasic cutaneous reaction and contact dermatitis induced by DNFB. Previously, we reported that the EPR is caused mainly by mast cell derived chemical mediators such as histamine and serotonin.[19] Therefore, we have examined the effect of TJ-34 on histamine-induced cutaneous reaction in mice ear and histamine release from rat peritoneal mast cells. When histamine was injected, it produced an edema with a peak at 10 min. This edema disappears 60 min after the injection. TJ-34 inhibited histamine-induced edema. But TJ-34 did not affect an antigen-induced histamine release from mast cells. These results suggest that the inhibition of IgE-mediated EPR by TJ-34 is due to an antagonistic action to histamine. In addition, we have also indicated cytokines especially pro-inflammatory cytokines such as TNF-α, IL-1 β and IL-6 play an important role in LPR. In order to investigate the inhibitory mechanisms of TJ-34 on IgE antibody-mediated LPR, we have examined the effect

of TJ-34 on cytokine activity and cytokine production. In the first experiment, we tested the effect of TJ-34 on the edema induced by TNF-α. TJ-34 suppressed TNF-α-induced edema. Prednisolone also suppressed the reaction. TJ-34, however, did not affect the production of TNF-α *in vitro* and *in vivo*. These results suggest that TJ-34 inhibits the IgE-mediated LPR by inhibiting the action of cytokines, especially TNF-α. Moreover, TJ-34 clearly inhibited DNFB-induced contact dermatitis. Our recent studies indicate that this contact dermatitis is induced by mainly T helper 1 (Th1) cells. As well known, Th1 cells are characterized by the production of IFN-γ or IL-2. IFN-γ causes an activation of macrophages resulting in the contact dermatitis. From this background, the inhibitory action of TJ-34 on contact dermatitis may be closely related to the inhibition of IFN-γ production.

In the present study, we employed different kinds of experimental systems. But in each experiment, reference drugs showed clear pharmacological action. Therefore, we think that the experimental system employed in the present study is appropriate for evaluating the pharmacological action of TJ-34.

In conclusion the present study demonstrates the effectiveness of TJ-34 on IgE-mediated biphasic cutaneous reaction and contact dermatitis in mice. The inhibitory mechanism of TJ-34 is related to the inhibition of histamine- or TNF-α-induced edema and IFN-γ production.

和文抄録

アレルギー性皮膚反応に対する漢方薬，白虎加人参湯（白湯）の効果をマウスで検討した。まずはじめに，IgE依存性二相性皮膚反応の検討を行った。マウスはモノクローナル抗DNP IgE抗体の静脈内注射によって受動的に感作した。マウスに経皮的に抗原を投与することで，1時間と24時間にピークを示す二相性皮膚反応が発症した。白湯はこのIgE依存性二相性皮膚反応を明らかに抑制した。次に，ジニトロフルオロベンゼン(DNFB)が誘発する接触皮膚炎の効果を検討した。白湯は接触皮膚炎も抑制した。これらの白湯の抑制機構を検討するため，ヒスタミン，サイトカインの産生と作用に対する白湯の効力を検討した。白湯はヒスタミンとTNF-αによる皮膚反応を抑制した。さらにIFN-γ産生を抑制したが，アレルギー性ヒスタミン遊離と抗CD3抗体あるいはLPS誘発のサイトカイン産生に作用しなかった。これらのデータは，白湯がヒスタミンとTNF-αによる皮膚反応を阻害することでIgE依存性二相性皮膚反応を抑制し，IFN-γの産生を抑制して接触皮膚炎を抑制することを示している。

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