Effect of some Kampo medicines, including Tokaku-joki-to (Tao-He-Cheng-Qi-Tang), on IgE-mediated triphasic skin reaction in passively sensitized mice

Tomohiro YAMADA[a] Eiichi TAHARA[b] Hiroichi NAGAI[c] Katsutoshi TERASAWA[b] Tadato TANI[d] Shinyu NUNOME[e] and Ikuo SAIKI*[a]

[a] *Department of Pathogenic Biochemistry,* [d] *Department of Pharmacognosy, Institute of Natural Medicine;*
[b] *Department of Japanese Oriental (Kampo) Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University*
[c] *Department of Pharmacology, Gifu Pharmaceutical University*
[e] *Kampo and Pharmacognosy Laboratory, Tsumura & Co.*

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Abstract

Previous studies have reported that the mice passively sensitized with anti-DNP IgE antibody exhibited IgE-mediated biphasic cutaneous reaction with an immediate phase response (IPR) at 1 h and a late phase response (LPR) at 24 h after the challenge of DNFB (dinitrofluorobenzene). We recently found that the third phase inflammatory response with intense and persisting infiltration of eosinophils, named very late phase response (vLPR), was induced following IPR and LPR in response to DNFB in passively sensitized mice, and that the peak response of vLPR was on the 8th day after the challenge. The inhibitory effect of Kampo medicines on the triphasic cutaneous inflammatory reaction was divided into several groups in terms of their inhibition rate of ear swelling. Among the formulations, Tokaku-joki-to (Tao-He-Cheng-Qi-Tang) was effective at inhibiting IPR, LPR and vLPR (+/+/+ group) and scratching behavior in IPR. The Inhibitory effect of Tokaku-joki-to on triphasic cutaneous reaction primarily depends on its composed crude drugs, Glycyrrhizae Radix and Cinnamomi Cortex. These findings indicate that Tokaku-joki-to formulation is usefull for the inhibition of cutaneous inflammatory diseases.

Key words vLPR, Kampo formulation, Tokaku-joki-to, Allergic inflammation, Triphasic reaction, Scratching, Glycyrrhizae Radix , Cinnamomi Cortex.

Abbreviations DNP, dinitrophenol; DNFB, dinitrofluorobenzene; IPR, immediate phase response; LPR, late phase response; vLPR, very late phase response; mAb, monoclonal antibody; DTH, delayed type hypersensitivity.

Introduction

A recent increase in the incidence of chronic allergic diseases including atopic dermatitis has been reported.[1,2] To search for new anti-allergic agents, we have reported that spikelets of *Miscanthus sinensis*[3] and some Kampo medicines[4,5] inhibited the IgE-mediated biphasic skin reaction. In this model, passive sensitization with a murine monoclonal IgE antibody specific for dinitrophenyl group (anti-DNP IgE mAb) followed by the challenge of dinitrofluorobenzene (DNFB) to the mouse ear induces a biphasic skin reaction with immediate phase response (IPR) and late phase response (LPR) at 1 and 24 h after the challenge.[6-9] In the process of our study, we recently found a third inflammatory phase response following LPR, temporarily designated "very late phase

*〒930-0194 富山市杉谷2630
富山医科薬科大学和漢薬研究所病態生化学部門 済木育夫
2630 Sugitani, Toyama 930-0194, Japan

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response (vLPR)".[10] The vLPR with intense ear swelling and massive infiltration of eosinophils peaked on day 8 after the challenge, and was mainly mediated by T cells and partially by mast cells.[10]

In the present study, to find effective Kampo formulations for vLPR which is considered to be associated with severe and chronic allergic reactions, we characterized the anti-allergic efficacies of more than 12 Kampo formulations, and also examined the effect of an effective Kampo formulation, Tokaku-joki-to (Tao-He-Cheng-Qi-Tang), and its composing crude drugs on triphasic skin reaction in passively sensitized mice and on scratching behavior which is likely an itch-associated behavior after the challenge.

Materials and Methods

Mice: Specific pathogen-free BALB/c mice (6 weeks old), were purchased from Japan SLC Inc, Hamamatsu, Japan, and maintained in the Laboratory for Animal Experiments, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. This study was conducted in accordance with the standards outlined in the Guidelines for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

Materials: Kampo formulations were kindly donated by Tsumura and Co., (Tokyo, Japan) : Sho-saiko-to (Xiao-Chai-Hu-Tang; TJ-9, lot. no. 24009040), Sho-seiryu-to (Xiao-Qing-Long-Tang; TJ-19, lot. no. 260019030), Shofu-san (Xiao-Feng-San; TJ-22, lot. no. 250022010), Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San; TJ-23, lot. no. 260023020), Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang; TJ-41, lot. no. 930041001PO), Juzen-taiho-to (Shi-Quan-Da-Bu-Tang; TJ-48, lot. no. 260048010), Keigai-rengyo-to (Jing-Jie-Lian-Qiao-Tang; TJ-50, lot. no. 250050010), Unsei-in (Wen-Qing-Yin; TJ-57, lot. no. 260057010), Ji-zuso-ippo (Zhi-Tou-Chuang-Yi-Fang; TJ-59, lot. no. 260057010), Tokaku-joki-to (Tao-He-Cheng-Qi-Tang; TJ-61, lot. no. 250061010), Rokumi-gan (Liu-Wei-Wan; TJ-87, lot. no. 250087010), Inchinko-to (Yin-Chen-Gao-Tang; TJ-135, lot. no. 260135010). The composition of crude drugs in the formulation, which were quality-controlled by Japanese Pharmacopeia XIII, was summarized in Table I. The extracted solution of the formulations was spray-dried by the manufacture's conventional procedures to obtain a dry extract powders.

To prepare the extracts of each crude drug in the Tokaku-joki-to formulation (Table II), Persicae Semen (Japanese name; Tonin, lot no. 180985), Cinnamomi Cortex (Keihi, lot no. 010896), Glycyrrhizae Radix (Kanzo, lot no. 241297) and Rhei Rhizoma (Daio, lot no. 090781) were purchased from Tochimoto Pharmaceuticals, Osaka, Japan. Natrii Sulfus (Bosho, lot no. G90107) was purchased from Tomita Pharmaceuticals, Japan. The extracts of each crude drug were prepared by boiling in water for 50 min and freeze-dried into powder. Each formulation and extract were dissolved in distilled water and administered orally 2 h before and 2-6 days after antigen challenge.

HPLC pattern analysis, so called "fingerprint" method, was performed to assess the homogeneity of the formulation and to prepare batches of constant formulation, as describrd previously.[11] Fig. 1 shows HPLC profile of Tokaku-joki-to by single monitor (220 nm) and contour plot (190-500 nm) using a photodiode array system as a detector.

DNFB was purchased from Nacalai Tesque, Kyoto, Japan, and dissolved in 100 % ethanol. Prednisolone 21-acetate and diphenhydramine were purchased from Sigma Chemical Co., St. Louis, MO. They were suspended in 0.5 % methylcellulose solution, and administered intraperitoneally 2 h before and 4-6 days after the challenge.

Anti-DNP IgE preparation: An anti-DNP mAb-producing cell line (EC1) was cultured in 10 ml of an equal volume mixture of RPMI-1640 and Dulbecco's modified Eagle minimum essential medium with high glucose supplemented with 10 % heat-inactivated fetal bovine serum (GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY) and 2 mM glutamine until reaching a confluent state. The supernatant was harvested, centrifuged at 400×g, and stored at -80°C until use.[12] The IgE antibody titer was estimated to be 1 : 1024 by heterologous passive cutaneous anaphylaxis in rats injected intravenously with DNP-bovine serum albumin as an antigen.[13]

Induction of skin reaction in mouse ears: BALB/c

Table I The composition of crude drugs in the formulations used in this study

Kampo formulation (Chinese name) / Crude drugs (number : ratio of preparing the formulation)
Inchinko-to (Yin-Chen-Gao-Tang) Gardeniae fructus (3.0), Rhei rhizoma (1.0), Artemisiae capillaris spica (4.0)
Unsei-in (Wen-Qing-Yin) Rehmanniae radix (3.0), Paeoniae radix (3.0), Cnidii rhizoma (3.0), Angelicae radix (3.0), Scutellariae radix (1.5), Phellodendri cortex (1.5), Coptitis rhizoma (1.5), Gardeniae fructus (1.5)
Keigai-rengyo-to (Jing-Jie-Lian-Qiao-Tang) Scutellariae radix (1.5), Phellodendri cortex (1.5), Coptitis rhizoma (1.5), Platycodi radix (1.5), Aurantii fructus immaturus (1.5), Schizonepetae spica (1.5), Bupleuri radix (1.5), Gardeniae fructus (1.5), Rehmanniae radix (1.5), Paeoniae radix (1.5), Cnidii rhizoma (1.5), Angelicae radix (1.5), Menthae herba (1.5), Angelicae dahuricae radix (1.5), Ledebouriella radix (1.5), Forsythiae fructus (1.5), Glycyrrhizae radix (1.0)
Juzen-taiho-to (Shi-Quan-Da-Bu-Tang) Astragali radix (3.0), Cinnamomi cortex (3.0), Rehmanniae radix (3.0), Paeoniae radix (3.0), Cnidii rhizoma (3.0), Angelicae radix (3.0), Ginseng radix (3.0), Hoelen (3.0), Glycyrrhizae radix (1.5), Atractylodis lanceae rhizoma (3.0)
Sho-saiko-to (Xiao-Chai-Hu-Tang) Bupleuri radix (7.0), Pinnelliae tuber (5.0), Ginseng radix (3.0), Zizyphi fructus (3.0), Glycyrrhizae radix (2.0), Zingiberis rhizoma (1.0), Scutellariae radix (3.0)
Sho-seiryu-to (Xiao-Qing-Long-Tang) Pinnelliae tuber (6.0), Glycyrrhizae radix (3.0), Cinnamomi cortex (3.0), Schisandrae fructus (3.0), Asiasari radix (3.0), Paeoniae radix (3.0), Ephedrae herba (3.0), Zingibers siccatum rhizoma (3.0)
Shofu-san (Xiao-Feng-San) Gypsum fibrosum (3.0), Rehmanniae radix (3.0), Angelicae radixs (3.0), Atractylodis lanceae rhizoma (2.0), Ledebouriella radix (2.0), Akebiae caulis (2.0), Anemarrhenae radix (1.5), Glycyrrhizae radix (1.0), Sophorae Radix (1.0), Hoelen (1.0), Arctii fructus (2.0), Sesami semen (1.5), Cicadae periostracum (1.0)
Ji-zuso-ippo (Zhi-Tou-Chuang-Yi-Fang) Cnidii rhizoma (3.0), Atractylodis lanceae rhizoma (3.0), Forsythiae fructus (3.0), Ledebouriella radix (2.0), Glycyrrhizae radix (1.0), Hoelen (1.0), Carthtami flos (1.0), Lonicerae caulis et folium (0.5), Rhei rhizoma (2.0)
Tokaku-joki-to (Tao-He-Cheng-Qi-Tang) Persicae semen (5.0), Cinnamomi cortex (4.0), Rhei rhizoma (3.0), Glycyrrhizae radix (1.5), Natrium sulfuricum (0.9)
Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San) Paeoniae radix (4.0), Atractylodis lanceae rhizoma (4.0), Alismatis rhizoma (4.0), Angelicae radix (3.0), Hoelen (4.0), Cnidii rhizoma (3.0)
Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang) Astragali radix (4.0), Atractylodis lanceae rhizoma (4.0), Ginseng radix (3.0), Angelicae radix (3.0), Bupleuri radix (2.0), Zizyphi fructus (2.0), Aurantii nobilis pericarpium (2.0), Glycyrrhizae radix (1.5), Cimicifugae rhizoma (1.0), Zingiberis rhizoma (0.5)
Rokumi-gan (Liu-Wei-Wan) Rehmanniae radix (5.0), Corni fructus (3.0), Dioscoreae radix (3.0), Alismatis rhizoma (3.0), Hoelen (3.0), Moutan radix (3.0)

mice were given an intravenous injection of a 1-ml aliquot of anti-DNP IgE mAb-containing fluid 24 h before DNFB challenge. Skin reaction was elicited by applying 10 μl of 0.1 % DNFB in 100 % ethanol to each side of each ear of sensitized mice. The reaction to DNFB was evaluated by measuring ear thickness

Table II Botanical origin of crude drugs of Tokaku-joki-to (lot no. 250061010)

Crude drugs (Japanese name)	Botanical origin[a] (Family name)	Specimen no.
Persicae Semen (Tonin)	*Prunus perisica* BATSCH[b] or *Prunus persica* BATSCH var. davidiana MAXIMOWICS (Rosaceae)	180985
Cinnamomi Cortex (Keihi)	*Cinnamomum cassia* BLUME[b] or other species of the same genus (Lauraceae)	010896
Rhei Rhizoma (Daio)	*Rheum palmatum* LINNE[b], *Rheum tanguticum* MAXIMOWICS, *Rheum officinale* BAILLON, *Rheum coreanum* NAKAI, or their interspecific hybrids (Polygonaceae)	090781
Glycyrrhizae Radix (Kanzo)	*Glycyrrhiza uralensis* FISCHER[b], *Glycyrrihiza glabra* LINNE, or other species of the same genus (Leguminosae)	241297
Natrii Sulfus (Bosho)	Natural hydrous sodium sulfate $Na_2SO_4 \cdot 10H_2O$ or $Na_2SO_4 \cdot 2H_2O$[b]	G90107

[a]The botanical origins are based on Japanese Pharmacopeia XIII.
[b]These crude drugs were used in the Tokaku-joki-to.

using a dial thickness gauge (G-1A type, Peacock, Ozaki MFG., Co., LTD., Osaka, Japan) immediately before and at appropriate times after the challenge. The results were expressed as average ear swelling (increase in ear thickness, μm) $\pm$S.D. of 3 mice.

Scratching behavioral experiments: For scratching behavioral examination,[14,15] 6 passively sensitized mice/group were put into an acrylic cage (13×9×30 cm) for about 2 h for acclimation before the experiment. Immediately after the DNFB challenge, they were put back into the same cage and, for the observation of scratching, behaviors were recorded using an 8-mm video camera (CCD-TRV60, Sony, Tokyo, Japan) under unmanned conditions. Scratching of the challenged site with the hind paws was counted. Each mouse was used for only one experiment. The mice generally scratched several times for about 1 sec, and a series of this behavior was counted as one incident of scratching.

Statistical analysis: Statistical significance of difference between the groups was determined by using Mann-Whitney's U-test for the ear swelling experiments and two-way repeated-measures ANOVA for the behavioral experiments.

Results and Discussion

We recently found that passive sensitization with anti-DNP IgE antibody followed by the challenge of DNFB to mouse ear can induce the triphasic cutaneous reactions (ear swelling) of IPR, LPR and vLPR peaking at 1 h, 24 h and 8 days after antigen challenge, respectively (Fig. 2).[10] IPR was absent in mast cell-deficient mice but LPR was sufficiently observed, and vLPR was partly attenuated.[10] LPR is a T cell-independent response, while vLPR is almost completely absent in T cell-deficient nude mice.[10] Thus, a third phase response (vLPR) with massive eosinophil infiltration actually represents an important inflammatory reaction mediated by T cells and partially mast cells.

We have previously reported the effect of more than 20 Kampo formulations on IgE-mediated biphasic (IPR and LPR) cutaneous reaction.[4,5] In the

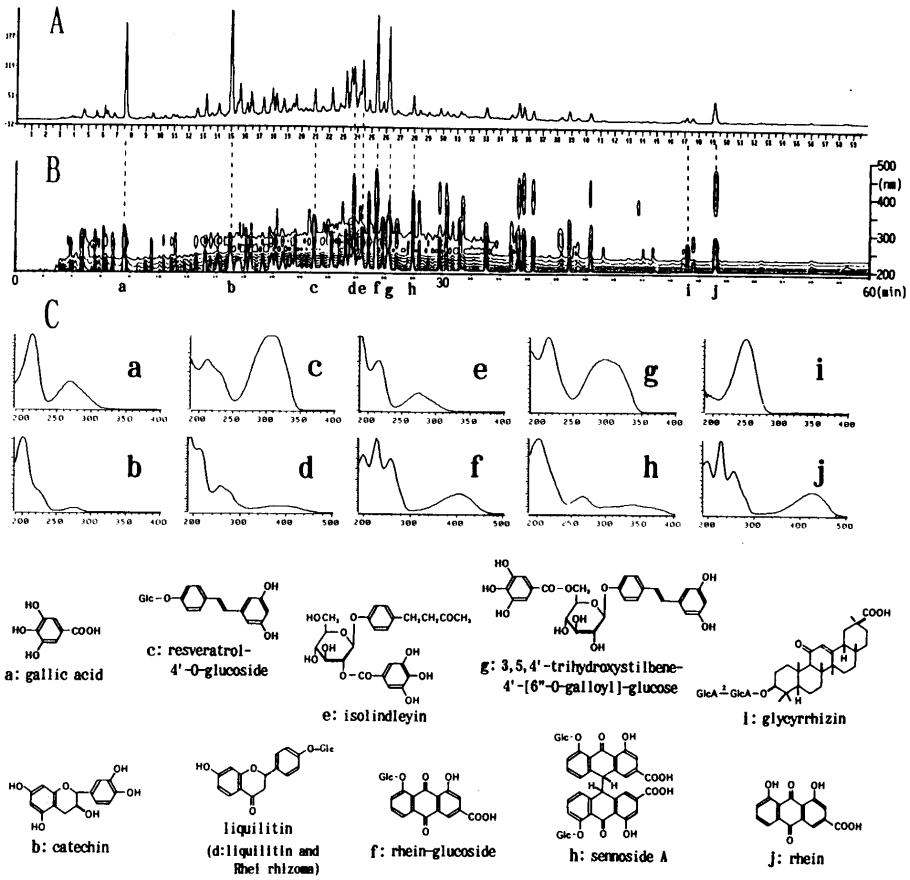

Fig. 1 HPLC profile of Tokaku-joki-to and UV spectra. A dosage of Tokaku-joki-to was extracted with H_2O-EtOH (9 : 1, 200 ml), filtered and analyzed by HPLC (HP-1090, Series II, Hewlett-Packard) under the following conditions: column, TSK gel ODS-80Ts (4.6×250 mm) ; mobile phase, 10 mM phosphoric acid : CH_3CN (linear gradient, 95 : 5→35 : 65, linear gradient, for 1 h); flow rate, 0.8 ml/min ; oven temperature, 40°C ; injection volume, 5 μl.
A : HPLC pattern analyzed by absorbance at 220 nm,
B : Contour plot of HPLC pattern by UV absorbance (190-500 nm),
C : UV spectra of main peaks, origins of peak [a : Rhei rhizoma (gallic acid), b : Rhei rhizoma (catechin), c : Rhei rhizoma (resveratrol-4'-O-glucoside), d : Glycyrrhizae radix (liquilitin) and Rhei rhizoma, e : Rhei rhizoma (isolindleyin), f : Rhei rhizoma (rhein-glucoside), g : Rhei rhizoma (3,5,4'-trihydroxystilbene-4'-[6"-O-galloyl]-glucose), h : Rhei rhizoma (sennoside A), i : Glycyrrhizae radix (glycyrrhizin), j : Rhei rhizoma (rhein)

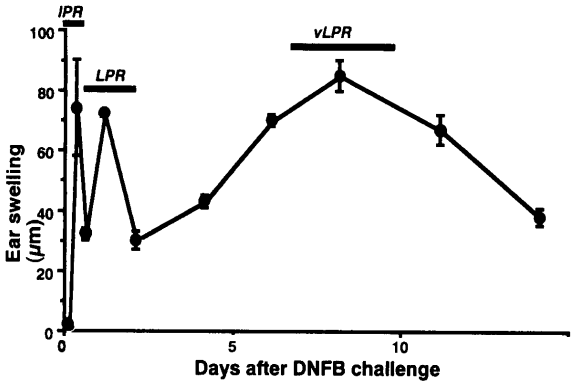

Fig. 2 Time course study of DNFB-specific skin reaction in mice. BALB/c mice were passively sensitized with 1.0 ml anti-DNP IgE mAb preparation, 24 h before antigen challenge. Skin reaction was elicited by applying 0.1 % DNFB in 100 % ethanol to the ear skin of passively sensitized mice. Each value represents mean ear swelling (μm) ±S.D. of 3 mice.

present study, we used 12 Kampo formulations to examine the efficacy on a third phase response vLPR following IPR and LPR (Fig. 3). These formulations were determined by "**HEAT**" and "**COLD**" symptoms divided by pathologic condition and nature of disease, as diagnosed by the system of Kampo medicine. For instance, 4 formulations in upper panels of Fig. 3 (Tokaku-joki-to, Shofu-san, Ji-zuso-ippo and Inchin-ko-to) were for clearing away heat, while lower 4 formulations were for warming and benefiting vital energy. The inhibitory effects of the Kampo formulations on the triphasic cutaneous reaction were divided into several groups according to the efficacies for IPR/LPR/vLPR (Fig. 3 and 4). The group consisting

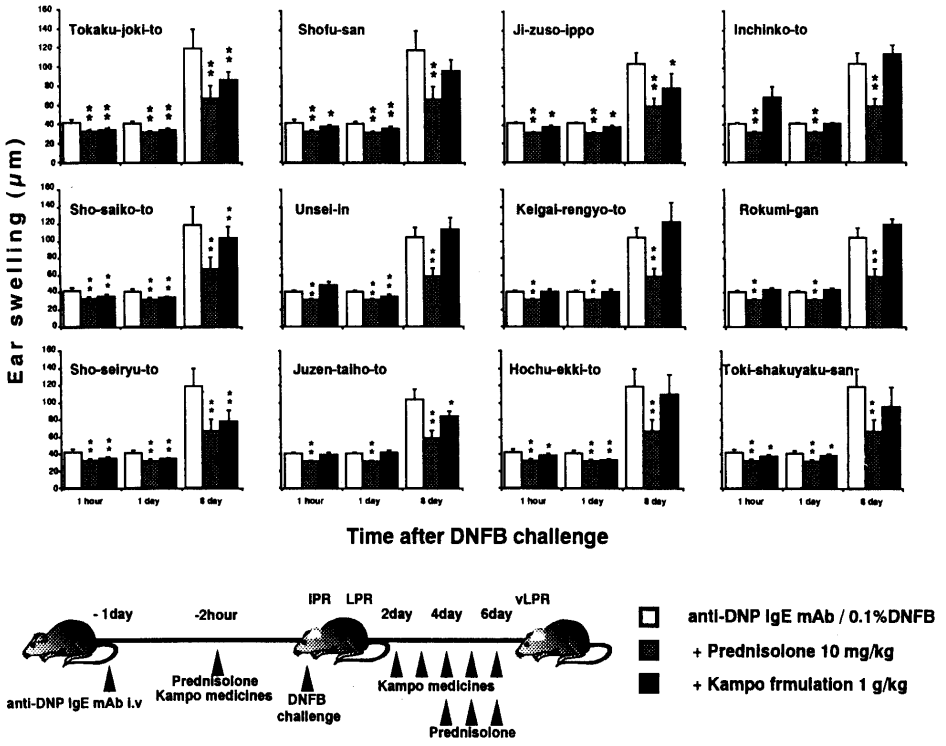

Fig. 3 Effect of Kampo formulations on triphasic skin reaction in passively sensitized mice. Mice received intravenous injection of 1.0 ml of anti-DNP IgE mAb preparation 24 h before skin testing with 0.1 % DNFB in 100 % ethanol. Each Kampo formulation (1 g/kg) was given orally 2 h before and 2 to 6 days after DNFB challenge. Prednisolone (10 mg/kg) was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S.D. of 3 mice. *, $p<0.05$, **, $p<0.005$ by Mann-Whitney's U-test.

of Tokaku-joki-to, Ji-zuso-ippo, Sho-sei-ryu-to and Sho-saiko-to significantly inhibited IPR, LPR and vLPR (*i.e.* +/+/+ group of IPR/LPR/vLPR), similar to the effect of prednisolone as a positive control. Shofu-san, Hochu-ekki-to and Toki-shakuyaku-san were included in +/+/- group of IPR/LPR/vLPR. Oral administration of Inchinko-to, Keigai-rengyo-to and Rokumi-gan did not show any inhibition of the triphasic cutaneous reaction (-/-/- group). Thus, in this study, there was no specific relationship between the efficacy on triphasic cutaneous reaction and the formulations determined according to Kampo diagnosis ("**HEAT**" and "**COLD**" symptoms). The effect of other Kampo formulations, in addition to the formulations tested in this study, was also summarized in Fig. 4.

On the other hand, we have recently reported the differential effect of several synthetic anti-allergic drugs with different mechanisms of action on the triphasic cutaneous reaction,[16] and the pattern of the effectiveness of these drugs was additionally listed in Fig. 4. Since a platelet activating factor (PAF) receptor antagonist (Y-24180) and a leukotriene B4 (LTB4) receptor antagonist (ONO-4057) were effective at inhibiting the triphasic cutaneous reaction,[16] Kampo formulations in +/+/+ and/or -/+/+ groups may show such anti-PAF and LTB4 activities. Interestingly, +/-/- group, which inhibited IPR only, included H1 receptor antagonists (terfenadine and diphenhydramine) and a membrane stabilizer (amlexanox), whereas any tested Kampo formulations were not comprised in this group (+/-/-).

Sho-seiryu-to is the representative medicine for improving the stasis of body fluids which is referred to as "**WATER IMBALANCE**", a concept that includes overall water metabolism and various functions such as the defense system, and mainly used for respiratory diseases including bronchial asthma or rhinitis.[17,18] Figs. 3 and 4 suggest that Sho-seiryu-to (+/+/+ group) may also be effective for patients with atopic dermatitis. Toki-shakuyaku-san (+/+/- group) and Tokaku-joki-to (+/+/+ group) have been used to

IPR / LPR / vLPR	Kampo medicines		Anti-allergic agents
+ + +	Tokaku-joki-to Ji-zuso-ippo Sho-seiryu-to Sho-saiko-to	Byakko-ka-ninjin-to	Predonisolone (steroid) Y-24180 (PAF receptor antagonist) Cyclosporin A, FK-506
- + +		Shimotsu-to Ogi-kenchu-to	ONO-4057 (LTB4 receptor antagonist)
- - +	Juzen-taiho-to		
+ + -	Shofu-san Hochu-ekki-to Toki-shakuyaku-san		Azelastine (Membrane stabilizer) ONO-1078 (LTC4, D4, E4 receptor antagonist)
+ - -			Diphenhydramine, Terfenadine, (H1 receptor antagonist) Amlexanox (Membrane stabilizer)
- + -	Unsei-in		
- - -	Inchin-ko-to Keigai-rengyo-to Rokumi-gan	Oren-gedoku-to Sho-kenchu-to Yoku-kan-san	

Fig. 4 Summary of the effect of Kampo formulations and anti-allergic agents on triphasic cutaneous reaction. The effect was divided into several groups according to the efficacy for IPR/LPR/vLPR (for example, +/+/+ or -/-/-).

improve "**OKETSU**", to a state of insufficient blood-circulation and blood stasis resulting in chronic autoimmune and allergic inflammatory, and thrombopoietic diseases as diagnosed by the system of Kampo medicine.[17] Terasawa *et al.*[19] have reported four cases of atopic dermatitis successfully treated with Tokaku-joki-to. Therefore, it may be of particular interest to investigate the possible effects of other "**OKETSU**"-improving medicines on IgE-mediated triphasic cutaneous reaction. Shimotsu-to (-/+/+ group in Fig. 4) is a key formulation which has been used in some Kampo medicines such as Unsei-in and Juzen-taiho-to and consists of four crude drugs. Among the four constituents, Cnidii Rhizoma (Senkyu, in Japanese) and Angelicae Radix (Toki) have been used to improve "**OKETSU**", and are considered to stimulate the circulation of "**BLOOD**" (refers to blood, hormones, autonomic nervous system and other regulatory functions of the body's internal environment) and "**KI**" (a concept that encompasses mental nervous activity, especially the appetite for food and actual process of digesting and absorbing nutrients) in Kampo medicine. Our recent study indicated that Cnidii Rhizoma (Senkyu) inhibited ear swelling in LPR and vLPR, wheras Angelicae Radix did inhibit scratching behavior which is considered to be associated with pruritis in IPR. Shimotsu-to containing

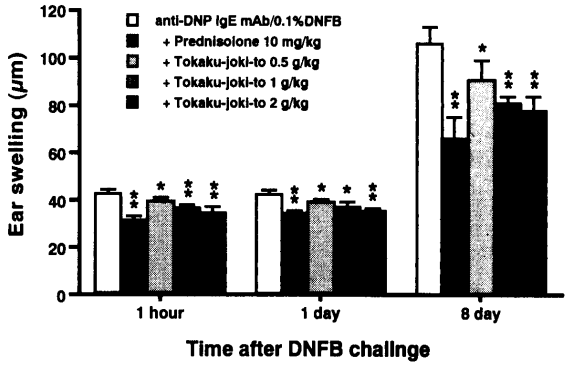

Fig. 5 Effect of Tokaku-joki-to on triphasic skin reaction in passively sensitized mice. Tokaku-joki-to was given orally 2 h before and 2 to 6 days after DNFB challenge in the sensitized mice. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S.D. of 3 mice.
*, $p<0.05$, **, $p<0.005$ by Mann-Whitney's U-test.

both crude drugs evidently showed both inhibitory properties.[20]

As shown in Fig. 5, oral administration of Tokaku-joki-to resulted in a significant inhibition of triphasic cutaneous reaction in a dose-dependent manner. Among the five crude drugs in the Tokaku-joki-to formulation, the extracts of Glycyrrhizae Radix (Kanzo) and Cinnamomi Cortex (Keihi) significantly inhibited IPR, LPR and vLPR (Fig. 6). Persicae Sememn (tonin) extract significantly inhibited vLPR,

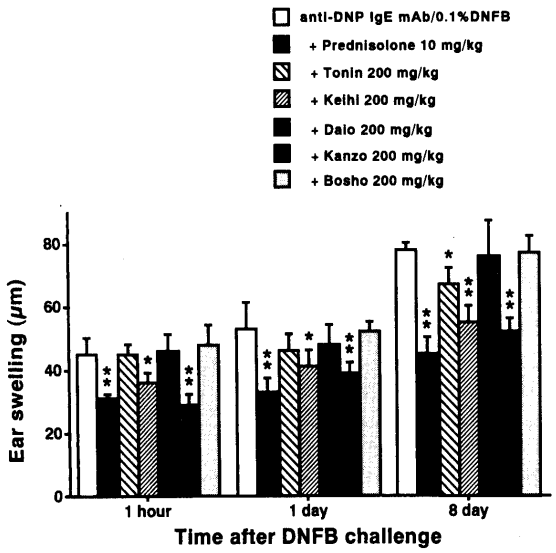

Fig. 6 Effect of Tokaku-joki-to constituents on triphasic skin reaction in passively sensitized mice. Each crude drug in Tokaku-joki-to was given orally 2 h before and 2 to 6 days after DNFB challenge in the sensitized mice. Prednisolone was given intraperitoneally 2 h before and 4 to 6 days after the challenge. Each value represents mean±S.D. of 3 mice. *, $p<0.05$, **, $p<0.005$ by Mann-Whitney's U-test.

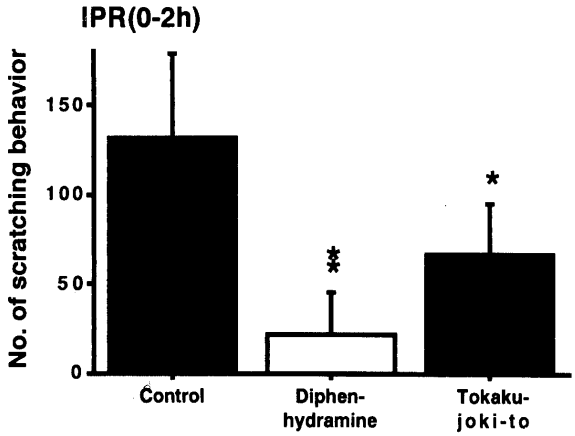

Fig. 7 Effects of Tokaku-joki-to and diphenhydramine on scratching behavior after DNFB challenge in passively sensitized mice. Diphenhydramine (30 mg/kg) was given intraperitoneally 30 min before DNFB challenge. Tokaku-joki-to (2 g/kg) was given orally 2 h before the challenge. Number of scratching incidents was measured at IPR after the challenge. Each value represents mean±S.D. of 6 mice. *, $p<0.05$ by two-way repeated measures ANOVA.

but did not affect IPR and LPR. Natrii Sulfus (Bosho) and Rubarb Rhizome (Daio) extract did not show any effect on triphasic skin reaction. Prednisolone was apparently effective at inhibiting the triphasic skin reaction. Since pruritus produces scratching, worsening the condition of atopic patients,[21,22] we also investigated the effect of Tokaku-joki-to on scratching behavior elicited by DNFB challenge in passively sensitized mice (Fig. 7). Tokaku-joki-to and diphenhydramine (an H1 receptor antagonist) significantly inhibited scratching behavior in IPR.

In conclusion, we found the differential pattern of the efficacy of Kampo medicines on triphasic cutaneous inflammatory reaction. Among the formulations, Tokaku-joki-to was effective at inhibiting IPR, LPR and vLPR (ear swelling) and scratching behavior. The detailed mechanism of the inhibitory effect of Tokaku-joki-to and its constituents (Glycyrrhizae Radix and Cinnamomi Cortex) is now under investigation.

和文抄録

抗DNP IgE抗体で受動感作したマウスの耳介にDNFB（ジニトロフルオロベンゼン）を塗布することにより，1時間および24時間目をピークとする即時相反応（IPR）および遅発相反応（LPR）からなるIgE介在二相性皮膚反応を示すことがすでに知られている。我々は最近，この受動感作マウスにおいてDNFBによる反応惹起後にIPR，LPRに続く，三相目の強い炎症性反応を見出し，超遅発相反応（vLPR）と名付けた。これは抗原塗布から8日目をピークとする，著明かつ持続的な好酸球の浸潤を伴う腫脹反応である。種々の漢方方剤を用いて，この三相性皮膚反応に対する抑制効果を検討した結果，各相の耳介腫脹の抑制率に基づき，いくつかのグループに分類された。検討した方剤中，桃核承気湯はIPR，LPR，vLPRの三相反応に対して抑制を示し（+/+/+群），さらにIPRで観察される耳介の掻き行動（痒みの指標と考えられる）を抑制した。桃核承気湯の三相性皮膚反応に対する抑制効果の発現は，主として構成生薬である甘草および桂皮に基づくことが示唆された。これらの知見から，漢方方剤：桃核承気湯が炎症性皮膚疾患に有効であることが示された。

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