Effects of Sho-seiryu-to (Xiao-Qing-Long-Tang) on experimental allergic reactions

Hiroichi NAGAI,*[a] Hiroshi TAKEDA,[a] Takeshi NISHIYORI,[a] Takashi OCHI,[a] Masatsugu SAWADA,[a] Toshinobu KATO,[a] Naoki INAGAKI,[a] Masufumi MORITA[b] and Yasuhiro KOMATSU[b]

[a] *Department of Pharmacology, Gifu Pharmaceutical University*
[b] *R & D Division, Tsumura & Co.*

(*Received November 5, 1999. Accepted January 17, 2000.*)

Abstract

Effects of a traditional Chinese herbal medicine, Sho-seiryu-to (Xiao-Qing-Long-Tang ; TJ-19) on experimental allergic reactions were studied.

1. As type I allergic reaction models, experimental allergic rhinitis in guinea pigs and biphasic cutaneous reaction in mice were carried out. Biphasic increase in nasal airway resistance and skin edema (immediate phase reaction : IPR, and late phase reaction : LPR) were clearly inhibited by TJ-19.

2. To investigate the inhibitory mechanisms of TJ-19 for type I allergic IPR and LPR, some *in vivo* and *in vitro* experiments were carried out. TJ-19 clearly inhibited histamine- and tumor necrosis factor-α (TNF-α)-induced cutaneous reactions in mice. Although TJ-19 did not affect the IgE-dependent histamine release from mouse, rat and human mast cells, it inhibited the generation of granulocyte-macrophage colony stimulating factor from human mast cells. In addition, TJ-19 inhibited the antigen-induced expression of TNF-α mRNA, but not interleukin (IL)-1β mRNA in the skin of sensitized mice. TJ-19 had no effect on the anti-CD3 antibody-induced production of interferon-γ and IL-4 by mouse splenic $CD4^+$ T cells.

3. In order to know the effect of TJ-19 on other types of allergic reactions, effects of TJ-19 on Forssman cutaneous reaction in guinea pigs (Type II), passive Arthus reaction in rats (Type III) and dinitrofluorobenzene-induced contact dermatitis in mice (Type IV) were investigated. TJ-19 showed no effect on these three allergic cutaneous reactions.

These results indicate that TJ-19 selectively inhibits Type I allergic IPR and LPR. IPR is inhibited by an antagonistic action to histamine, and LPR is inhibited by either antagonistic action to cytokines or inhibition of pathological cytokine production.

Key words Sho-seiryu-to, allergic reaction, rhinitis, dermatitis, cytokine, mast cell.

Abbreviations BMMC, bone marrow-derived mast cells ; CHMC, cultured human mast cells; DNFB, dinitrofluorobenzene ; DNP, dinitrophenyl ; GM-CSF, granulocyte-macrophage colony stimulating factor ; IFN, interferon ; IL, interleukin ; IPR, immediate phase reaction ; LPR, late phase reaction; PEMC, peritoneal mast cells ; TNF, tumor necrosis factor.

Introduction

Recently, many Chinese herbal medicines have been applied for the treatment of chronic, allergic and inflammatory diseases. Some are reported to be effective for the treatment of bronchial asthma, allergic rhinitis and atopic dermatitis.[1-3]

In general, glucocorticoid is the most effective drug for the therapy of chronic inflammatory diseases. Glucocorticoids show the inhibitory effect on many points during inflammatory process and

*〒502-8585 岐阜市三田洞東5-6-1
岐阜薬科大学薬理学教室 永井博弌
5-6-1 Mitahorahigashi, Gifu 502-8585, Japan

immune response.[4-12] The main anti-inflammatory mechanism is probably based on the inhibition of gene expression of phlogistic enzymes and cytokines. However, the long term treatment with glucocorticoids 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 anti-inflammatory activities.[1-3,13-17] Whereas they show valuable clinical efficacy, most of their pharmacological mechanisms are still obscure.

Sho-seiryu-to (TJ-19) is a novel Chinese medicine that is often used for the treatment of chronic allergic diseases including allergic rhinitis and bronchial asthma.[18-20] The basic pharmacological properties on TJ-19 are still not clear also. The present study, therefore, was carried out to elucidate the pharmacological actions and basic mechanisms of TJ-19 on allergic reactions.

Materials and Methods

Animals: Male Hartley guinea pigs weighing approximately 300 g, female BALB/c mice weighting about 20 g, and Wistar rats weighing about 180 g were purchased from Japan SLC (Hamamatsu, Japan). They were fed in our laboratory until use. Animal experiments were performed in accordance with the animal care guideline of Gifu Pharmaceutical University.

Drugs: Sho-seiryu-to (TJ-19) provided by Tsumura Co. Ltd. (Tokyo, Japan) is a spray-dried preparation of water extract of 8 crude drugs (Zingiberis Siccatum Rhizoma 3 g, Glycyrrhizae Radix 3 g, Cinnamomi Cortex 3 g, Schisandrae Fructus 3 g, Asiasari Radix 3 g, Paeoniae Radix 3 g, Pinelliae Tuber 6 g, Ephedrae Herba 3 g). Analysis data on major constituents of the TJ-19 preparation by HPLC were shown in Fig. 1.

As reference drugs, disodium cromoglycate, dexamethasone, isoproterenol, prednisolone and terfenadine were used.

Allergic rhinitis in guinea pigs: Experimental allergic rhinitis was caused in guinea pigs according

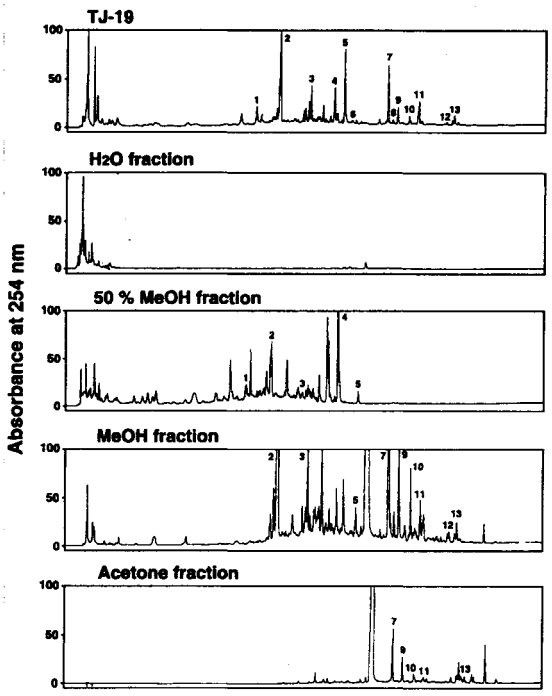

Figure 1 HPLC profiles of TJ-19 and its fractions
TJ-19 was applied on a Diaion HP-20 column and eluted with water, 50 % methanol, methanol and then acetone. Major constituents of TJ-19 ant its fractions were analyzed by HPLC. Pump: Shimadzu LC-10AD, column: TSK-gel ODS-80TM (4.6×150 mm), mobile phase: A [CH_3CN-H_2O-CH_3COOH, 10:90:1], B [CH_3CN], A→B [60 min, linear gradient] →B [15 min], 1 ml/min, monitor: Shimadzu SPD-M10AVP. 1: paeoniflorin, 2: liquiritin, 3: isoliquiritin, 4: cinnamic acid, 5: glycyrrhizin, 6: cinnamaldehyde, 7: schizandrin, 8: methyleugenol, 9: gomisin A, 10: asarinin, 11: saishin amide, 12: glycyrrhetic acid, 13: gomisin N.

to the method of Fujita *et al.*[21] Guinea pigs were systemically sensitized by intraperitoneal injections of 0.5 mg ovalbumin in 0.5 ml saline on days 0 and 2. Sensitized guinea pigs were repeatedly inhaled with either ovalbumin solution (0.1, 0.2, 0.4, 0.5, 1 and 1 %) or saline on days 22, 24, 27, 31, 36 and 38, respectively. On days 41, 44, 46 and 49, the guinea pigs were repeatedly challenged intranasally with 40 μl of 1 % ovalbumin solution. Respiratory resistance, estimated using the modified method described by Fujita *et al.* (1999) was considered to be the nasal resistance.[21] Briefly, after the challenge, the guinea pigs were placed inside a body plethysmograph with their heads outside the chamber. The gap between the animal and the chamber was sealed with silicone rubber. The respiratory airflow from the face mask at the snout

and oscillating pressure in the body chamber at 2 cm H_2O with 30 Hz sine wave pressure were recorded with a differential pressure transducer (Nihon Kohden, Tokyo, Japan) at various time points. These signals were displayed simultaneously on an X-Y oscilloscope and recorded on polygraph. The nasal airway resistance was calculated by following formula: nasal airway resistance pressure/flow (cm H_2O/ml/s). After baseline measurement of nasal airway resistance, the percentage increase in nasal airway resistance was measured 0.5, 2, 3, 4, 5 and 6 hr after antigen instillation. The animals were placed in the chamber several minutes before the measurement to allow them to settle down and become familiarized with the experimental condition.

IgE antibody-mediated biphasic cutaneous reaction in mice: The cutaneous reaction was elicited by a method previously described.[22,23] Briefly, BALB/c mice were passively sensitized by an intravenous injection of anti-dinitrophenyl (DNP) monoclonal IgE antibodies 24 hr before the test. The anti-DNP monoclonal IgE antibodies were prepared in our laboratory by establishing the monoclonal antibody-producing cell line, EC-1. Biphasic cutaneous reaction was elicited by painting 25 μl of 0.15 % dinitrofluorobenzene (DNFB) acetone-olive oil (3 : 1) solution to each side of both ears. The ear thickness was measured by a micrometer, Upright Dial Gauge (Peacock, Ozaki, Tokyo, Japan) at before, and 1 hr (immediate phase) and 24 hr (late phase) after challenge. To investigate the role of cytokines, the expression of interferon (IFN)-γ and interleukin (IL)-1β mRNAs in the skin lesion was measured by reverse transcriptase-polymerase chain reaction (RT-PCR) 4 hr after the antigen challenge.

Histamine- and tumor necrosis factor (TNF)-α-induced ear edema in mice: Female BALB/c mice received 10 μl of histamine at a concentration of 10^{-4} g/ml or TNF-α at a concentration of 10^5 units/ml into both ears. Ear thickness was measured by the same method described above. The ear thickness was measured 10 min after the injection of histamine, and 24 hr after the injection of TNF-α.

IgE-dependent histamine release from mast cells: IgE-dependent histamine release was examined in murine bone marrow-derived mast cells (BMMC), rat peritoneal mast cells (PEMC) and cultured human mast cells (CHMC).

Murine BMMC was prepared according to the previously described method.[24] Briefly, bone marrow cells from femurs of BALB/c mice were cultured in IL-3 containing RPMI 1640 medium. More than 95 % pure mast cells were obtained after 4 weeks of culture. BMMC were passively sensitized with murine anti-DNP monoclonal IgE antibodies by overnight incubation at 37°C. After washing with Tyrode solution, cells (10^5 mast cells) were challenged with DNP-conjugated bovine serum albumin (BSA) by an incubation at 37°C for 30 min. The amount of histamine in the supernatant of reaction mixture was assayed fluorometrically by the method of post-column derivatization as previously reported.[25] The minimal detectable concentration of histamine was 0.2 ng/ml. Histamine release was expressed in percentage of total histamine content determine by cell lysis with perchloric acid.

Sensitized rat PEMC was prepared as reported previously.[26] The rats were passively sensitized by an intraperitoneal injection of 2 ml of 200-fold diluted rat monoclonal IgE preparation. Rat monoclonal IgE antibodies against DNP were prepared from the supernatant of a cell line, REC, established in our laboratory. Two days after the injection of monoclonal antibodies, PEMC was recovered using Tyrode solution containing 5 units/ml of heparin. Mast cells were purified on Percoll gradient and suspended in Tyrode solution at a concentration of 10^5 cells/ml. Histamine release was initiated by adding DNP-BSA 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. The quantitative analysis of histamine was carried out by the same method described above.

CHMC was prepared from human umbilical cord blood cells according to the method of Saito *et al.*[27] Briefly, mononuclear cells were separated from heparin-treated umbilical cord blood by Ficoll (Organon Teknika Co., Durham, NC, USA) gradient. Cells were cultured in Media I (IBL, Gunma, Japan) containing 10 % fetal bovine serum (Filtron Pty Ltd., Brooklyn, Australia), 80 ng/ml human recombinant stem cell factor (Kirin Brewery, Maebashi, Japan), 50 ng/ml

human recombinant IL-6 (Kirin), and 300 nmol/l prostaglandin E_2 (GMBH & Co., Frankfurt, Germany). Cells were harvested weekly and resuspended in the fresh media. The purity of mast cell was determined by toluidine blue and by May-Gruenwald and Giemsa staining. The immunoperoxidase staining for tryptase and chymase was performed to confirm the phenotype of cultured mast cells by a previously established method.[25] CHMC were sensitized with IgE-rich atopic patient's serum (Total IgE : 880 units/ml, added at a 1 : 10 dilution with cell suspension) at 37°C overnight. After washing, cells were resuspended at 1×10^5 cells/ml in Tyrode solution containing 1 mmol/l $CaCl_2$, 0.6 mmol/l $MgCl_2$, 0.03 % BSA (Seikagaku Kogyo, Co., Tokyo, Japan), pH 7.4. The cell suspensions were challenged with 4 μg/ml of anti-human IgE (Coopen Biomedical Int., Malvern, PA, USA) for 30 min. The amount of histamine in the supernatant of reaction mixture was assayed by the same method described above.

Granulocyte-macrophage colony stimulating factor (GM-CSF) release from CHMC : CHMC was prepared according to the method described above. CHMC (5×10^5 cells/ml) were sensitized with IgE-rich atopic patient's serum at 37°C overnight and then challenged with 10 μg/ml of anti-human IgE antibody by incubation at 37°C for 6 hr. The amount of GM-CSF in the supernatant was assayed by ELISA system (Amersham, Buckinghamshire, UK).

IFN-γ and IL-4 production by murine CD4$^+$ T cells : $CD4^+$ T cells were prepared by magnetic cell sorting system (MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) from single cell suspension of spleen cells obtained from BALB/c mice. Murine $CD4^+$ T cell suspension (10^6 cells/ml in RPMI containing 10 % fetal calf serum) was incubated in the purified anti-CD3 monoclonal antibody (clone 145-2C11, Pharmingen)-coated 48-well multiplate (Falcon) at 37°C for 48 hr. The amounts of IFN-γ and IL-4 were measured by ELISA systems (Endogen).

Forssman cutaneous reaction in guinea pigs : Forssman cutaneous reaction was carried out according to the method described previously.[28] Guinea pigs were injected 0.1 ml of rabbit anti-sheep red blood cell serum diluted 8-fold with physiological saline intradermally into their shaved backs, followed by an intravenous injection of 1.0 ml 1 % Evans blue. After 1 hr, the animals were sacrificed by exanguination, and the skin was removed. The bluing spot caused by the Forssman cutaneous reaction was evaluated by the amount of extravasated dye.

Passive Arthus reaction in rats : Rabbit anti-ovalbumin serum was diluted with the same volume of physiologic saline. Hundred microliters of the solution were injected into the plantar pad of the hind paw of a rat. Immediately afterwards, 25 mg/kg of ovalbumin was injected intravenously. The volume of the paw was measured with a mercury plethysmometer (KN-357, Natsume, Tokyo, Japan) at times 0 and 4 hr. At time zero, the average rat paw volume amounted 1.84 ml.

DNFB-induced contact dermatitis in mice : Ears of BALB/c mice were painted with DNFB acetone-olive oil (3 : 1) solution or vehicle once each week for 3 weeks. A total of 25 μl of 0.15 % DNFB in vehicle was applied to each side of the ear. Ear thickness was measured using the same method as described above.

Statistics : Data were presented as mean±S.E.M. Differences between two groups were analyzed by Student's or Welch's t-test. Differences among three groups or more were analyzed by Dunnett's test of either parametric or non-parametric type. $P<0.05$ was considered to be significant.

Results

Effect of TJ-19 on experimental rhinitis

Effect of TJ-19 on experimental rhinitis in guinea pigs was examined. TJ-19 at a dose of 300 mg/kg was administered orally 1 hr before every antigen challenge. Figure 2 shows the results on increase in nasal airway resistance after second, third and forth intranasal challenges with antigen. Baseline values of airway resistance were 1.0±0.1 cm H_2O/ml/s. Vehicle-treated control animals exhibited a significant biphasic increase of nasal airway resistance both at 0.5 and 4 hr after the third and forth challenge with antigen. TJ-19 almost completely suppressed the increase in biphasic nasal airway resistance after the second, third and forth challenges.

Effect of TJ-19 on IgE antibody-mediated biphasic cutaneous reaction in mice

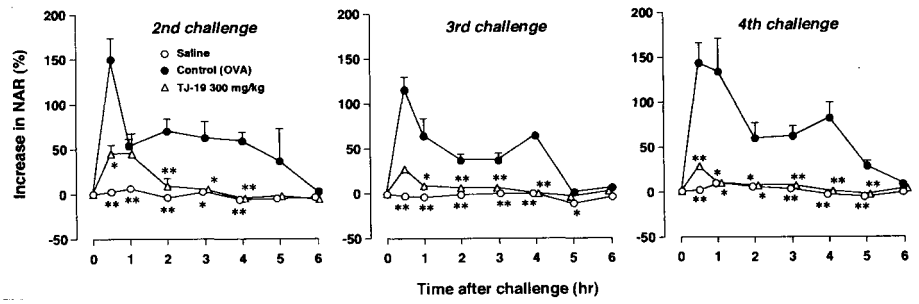

Figure 2 Effect of TJ-19 on experimental rhinitis in guinea pigs

Guinea pigs were systemically sensitized with ovalbumin and then repeatedly exposed to the antigen. Nasal airway resistance (NAR) was estimated after the second, third and forth antigen challenge. Baseline NAR value was 1.0±0.1 cm H_2O/ml/s. TJ-19 at a dose of 300 mg/kg was administered orally 1 hr before every challenge. Each value represents the mean±S.E.M. of 4 or 5 guinea pigs. $*p<0.05$, $**p<0.01$

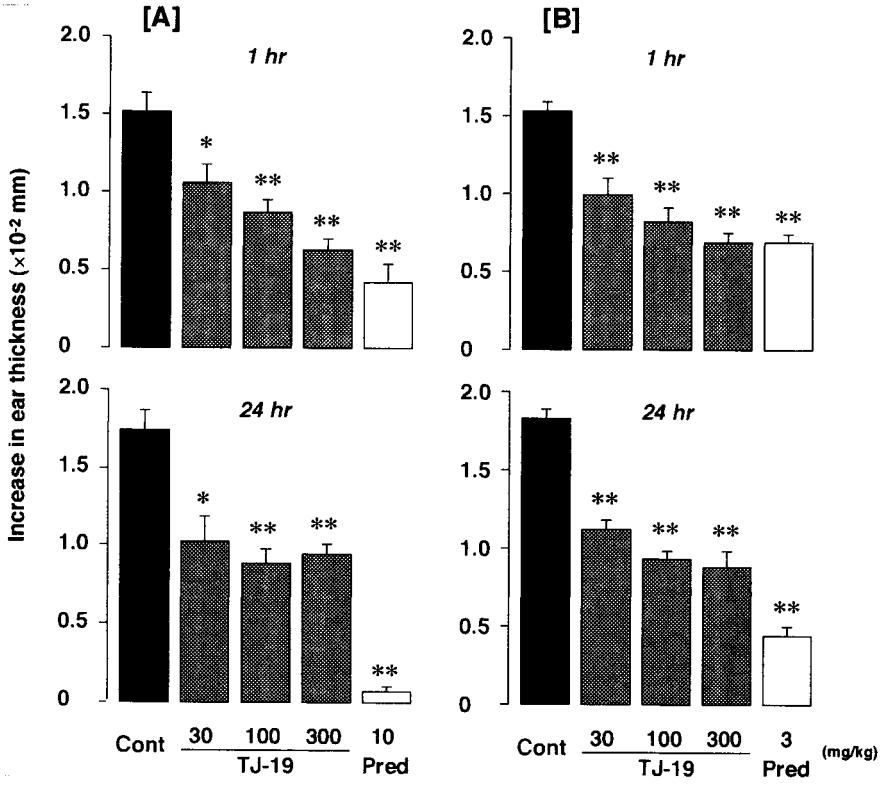

Figure 3 Effects of TJ-19 and prednisolone (Pred) on IgE antibody-mediated biphasic cutaneous reaction in mice

Mice were passively sensitized with anti-DNP monoclonal IgE antibody 24 hr before DNFB challenge. [A] TJ-19 and Pred were administered orally 1 hr and 2 hr, respectively, before challenge. [B] Both drugs were given orally once a day for 6 days before challenge. Each value represents the mean±S.E.M. of 6 mice. $*p<0.05$, $**p<0.01$

Figure 3 shows the effect of TJ-19 on the increase in ear thickness 1 and 24 hr after epicutaneous antigen challenge in sensitized mice. Mice were passively sensitized by an intravenous injection of monoclonal IgE antibody 24 hr before the antigen challenge. TJ-19 was administered 1 hr before [A] or once a day for 6

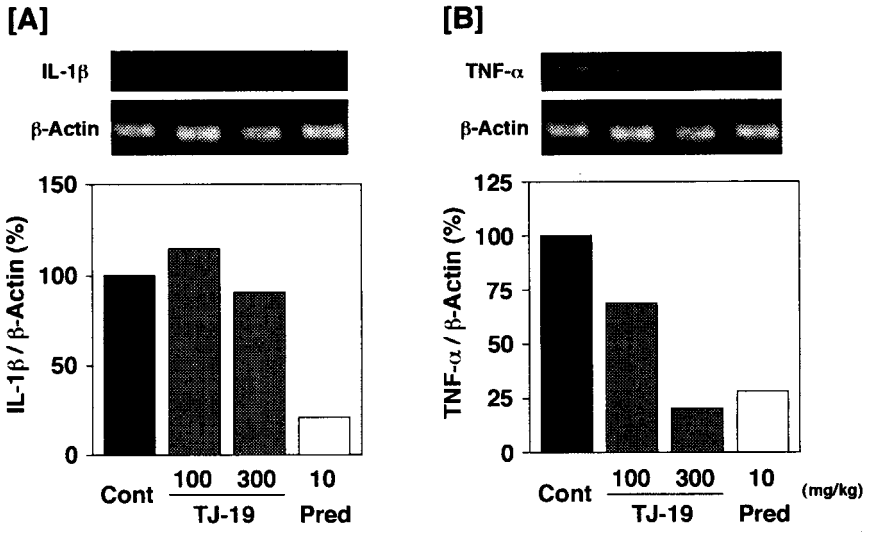

Figure 4 Effects of TJ-19 and prednisolone (Pred) on the expression of IL-1β and TNF-α mRNAs
Mice were passively sensitized with anti-DNP monoclonal IgE antibody 24 hr before DNFB challenge and the skin lesion was excised 4 hr after the challenge, and cytokine mRNA expression was examined by RT-PCR. RT-PCR data were semiquantified by densitometrically scanning photo negatives. TJ-19 and Pred were administered orally 1 hr and 2 hr, respectively, before challenge.

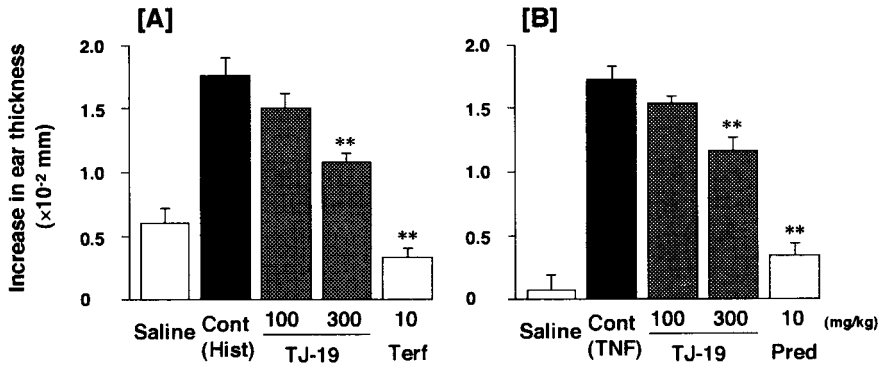

Figure 5 Effects of TJ-19 and reference drugs on cutaneous reactions caused by histamine [A] and TNF-α [B] in mice
The ear thickness was measured 10 min after the injection of histamine, and 24 hr after the injection of TNF-α. TJ-19 and terfenadine (Terf) were administered orally 1 hr before the cutaneous reaction. Prednisolone (Pred) was given orally 2 hr before the reaction. Each value represents the mean±S.E.M. of 6 mice. $^{**}p<0.01$

days [B] before antigen challenge. TJ-19 inhibited the biphasic increases in ear thickness in a dose-related fashion. Prednisolone also inhibited the biphasic cutaneous reaction significantly. Since our previous study showed that the expression of cytokine mRNAs corresponds well to the magnitude of late phase reaction, the effect of TJ-19 on the expression of IL-1β and TNF-α mRNAs in the skin lesion at 4 hr after antigen challenge was investigated. TJ-19 clearly inhibited the increase in TNF-α mRNA, but not IL-1β-mRNA (Fig. 4). In contrast, prednisolone inhibited the expression of both cytokine mRNAs.

These results indicate the inhibitory action of TJ-19 on Type I allergic mechanism-induced immediate

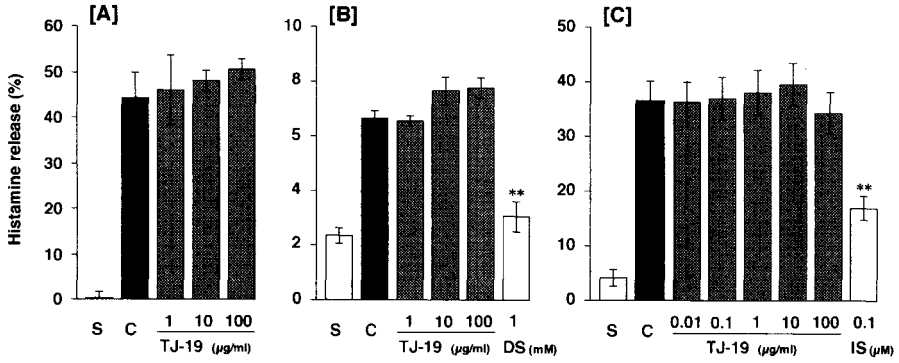

Figure 6 Effects of TJ-19 and reference drugs on IgE-dependent histamine release from sensitized mouse, rat and human mast cells

Mast cells of three origines were sensitized with IgE antibodies and stimulated with antigen or anti-IgE. TJ-19 and isoproterenol (IS) were added to the cell suspension 10 min before, and disodium cromoglycate (DS) was added immediately before the stimulation. Each value represents the mean±S.E.M. [A] murine BMMC, n=3, [B] rat PEMC, n=3, [C] CHMC, n=6, S : spontaneous, C : control, $^{**}p<0.01$

and late phase reactions. In order to investigate the mechanism of inhibition, following experiments were further carried out.

Effect of TJ-19 on histamine- and TNF-α-induced cutaneous reactions in mice

Figure 5 shows the effect of TJ-19 on the increase of ear thickness caused by histamine [A] or TNF-α [B]in mice. TJ-19 inhibited both histamine- and TNF-α-induced ear edemas. Terfenadine and prednisolone, as reference drugs, also inhibited histamine- and TNF-α-induced cutaneous reactions, respectively.

Effect of TJ-19 on IgE-dependent histamine release from mouse, rat and human mast cells

Figure 6 shows the effect of TJ-19 on IgE-dependent histamine release from sensitized murine BMMC, rat PEMC and CHMC. TJ-19 had no effect on the IgE-dependent histamine release from each mast cell type. In contrast, disodium cromoglycate and isoproterenol apparently inhibited histamine release from rat PEMC and CHMC, respectively.

Effect of TJ-19 on the release of cytokines from CHMC and murine T cells

Figure 7 shows the effect of TJ-19 on the generation of GM-CSF from CHMC. TJ-19 at a concentration range of 0.01-100 μg/ml inhibited the release of GM-CSF. Isoproterenol at a concentration of 0.1 μM completely suppressed the generation of GM-CSF. Figure 8 shows the effect of TJ-19 on the release of IL-4 and IFN-γ from mouse $CD4^+$ T cells caused by anti-

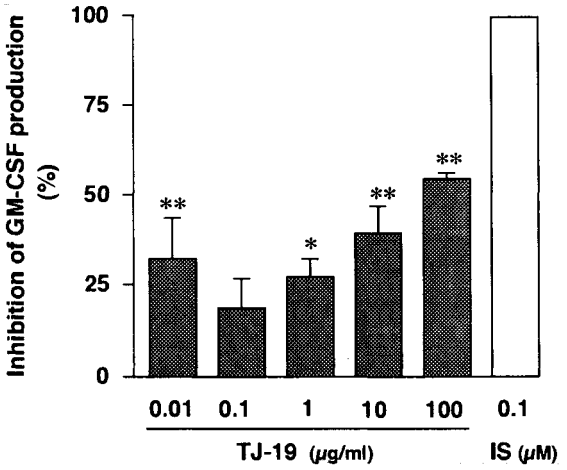

Figure 7 Effects of TJ-19 and isoproterenol (IS) on the generation of GM-CSF from human mast cells

CHMC was sensitized with IgE antibodies and stimulated with anti-IgE for 6 hr in the presence of TJ-19 or IS. Each value represents the mean ± S.E.M. of 5 experiments. $^{*}p<0.05$, $^{**}p<0.01$

CD3 monoclonal antibodies. TJ-19 showed a tendency to inhibit the production of IL-4 and IFN-γ by $CD4^+$ T cells. Glucocorticoids inhibited the production of both cytokines.

Effect of TJ-19 on the allergic cutaneous response caused by Type II, III and IV mechanisms

Figure 9 shows the effect of TJ-19 on Forssman cutaneous reaction in guinea pigs (Type II), Arthus reaction in rats (Type III) and contact dermatitis in mice (Type IV). Although TJ-19 had no effect on

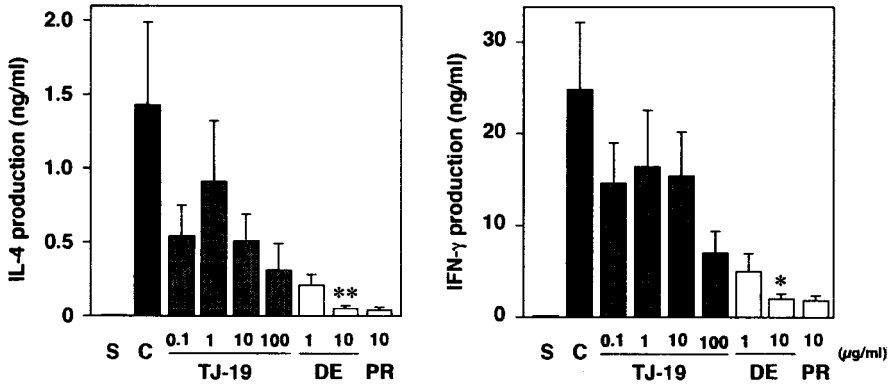

Figure 8 Effects of TJ-19 and glucocorticoids on the generation of IL-4 and IFN-γ from CD4$^+$ T cells caused by anti-CD3 antibodies

CD4$^+$ T cells were purified from mouse spleen cell suspensions, and stimulated with anti-CD3 antibodies for 48 hr in the presence of TJ-19, dexamethasone (DE) or prednisolone (PR). Each value represents the mean±S.E.M. of 4 experiments. $*p<0.05$, $**p<0.01$

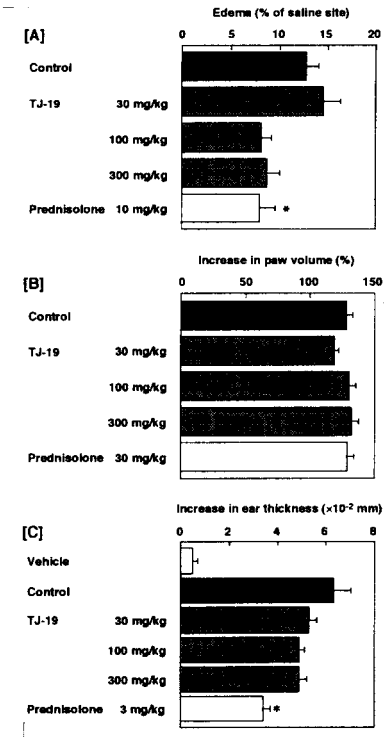

Figure 9 Effects of TJ-19 and prednisolone on Forssman cutaneous reaction in guinea pigs [A], Arthus reaction in rats [B] and contact dermatitis in mice [C]

Forssman cutaneous reaction was caused in guinea pigs using rabbit anti-sheep red blood cell serum. Passive Arthus reaction was induced by ovalbumin in rats treated with rabbit anti-ovalbumin serum. Contact dermatitis was evoked in mice by repeated paintings of DNFB. TJ-19 and prednisolone were administered orally 1 hr before challenge in the experiments of Forssman cutaneous reaction and Arthus reaction. In the case of contact dermatitis, drugs were administered orally once a day throughout. Each value represents the mean±S.E.M. of 5 or 6 animals. $*p<0.05$

each cutaneous response, prednisolone inhibited Forssman cutaneous reaction and contact dermatitis apparently.

Discussion

The present study indicates the following four evidences on the anti-allergic action of TJ-19. 1) TJ-19 selectively inhibited Type I allergic reaction rather than other allergic reactions mediated by Type II, III and IV mechanisms. 2) TJ-19 clearly inhibited both IPR and LPR mediated by Type I mechanism. 3) TJ-19 inhibited the IPR of Type I allergic reaction by an antagonistic action to histamine but not inhibition of histamine release from mast cells. 4) TJ-19 inhibited the LPR of Type I allergic reaction mainly by both antagonistic actions to cytokines and inhibition of pathological cytokine production.

TJ-19 is a novel Chinese medicine which is often used for the treatment of allergic diseases, including rhinitis and bronchial asthma.[18-20] The clinical efficacy of TJ-19 is widely accepted, but little information about the basic pharmacological profile of TJ-19 was obtained. In the present study, therefore, the basic pharmacological actions of TJ-19, especially the effects on experimental allergic reactions were investigated. TJ-19 showed a selective inhibition of Type I allergic reaction in the nose and skin. It failed to affect the Type II, III and IV skin reactions.

In addition, TJ-19 exhibited the inhibition of

immediate and late phase allergic reaction in an allergic rhinitis model in guinea pigs and IgE-mediated biphasic cutaneous reactions in mice. Previously, our studies demonstrated the main role of mast cell-derived mediators (histamine or serotonin) in the onset of IPR in Type I allergic reaction.[22,23] In contrast, we have also demonstrated the main role of cytokines in the onset of LPR.[23,24]

In the present study, we examined the effect of TJ-19 on the release of histamine using mast cells of three different origins. The main reason why we have examined the effect in three different mast cells is that some anti-allergic drugs indicate the different effects on different mast cells.[29] TJ-19 showed no effect of histamine release from all three mast cells. There are, however, some reports to indicate the inhibition of histamine release from mast cells by TJ-19.[30,31] These data were obtained from the experiments by using chemical histamine liberators, such as calcium ionophore and compound 48/80. TJ-19 may suppress the pathway of histamine release activated by chemical liberators, but not by IgE-dependent stimuli. Although TJ-19 had no effect on the histamine release in the present study, it showed a clear antagonistic action to histamine in the mouse ear. This means that the mechanism for suppression of IPR in Type I allergic reaction is mainly based on the antagonistic action to histamine.

Cytokine is believed to be one of potent inflammatory mediators in some situations. In the LPR of Type I allergic reaction, many investigations indicate that cytokines are main mediators to develop the reaction. In the present study, TJ-19 clearly inhibited the antigen-induced in vivo expression of TNF-α mRNA in the mice ear, but not IL-1β mRNA. From our previous study using anti-IL-1β monoclonal antibody, IL-1β was not an important cytokine to cause a late phase reaction. Our previous preliminary experiment indicates the importance of IFN-γ, TNF-α and GM-CSF for the onset and development of LPR. TJ-19 clearly indicates an antagonistic action to TNF-α, and the inhibition of TNF-γ mRNA expression and GM-CSF production. Although the GM-CSF production from CHMC was inhibited by TJ-19 at concentration between 0.01-100 μg/ml, a clear concentration-dependency was not observed at the lower concentration range. Our information about the role of cytokines in the LPR is mainly obtained from the experiments of cutaneous reaction. Further experiments to investigate the role of cytokines in the onset of airway late phase allergic reaction will be necessary.

In conclusion, the present data indicate the efficacy of TJ-19 on Type I biphasic allergic reactions in experimental animals. The inhibitory mechanism for the immediate phase of Type I allergic reaction by TJ-19 is mainly due to the antagonistic action to histamine in the tissue. The late phase of Type I allergic reaction is inhibited by mainly through antagonistic actions to cytokines and inhibition of cytokine production from many cell types.

和文抄録

小青竜湯（TJ-19）のアレルギー反応に及ぼす影響を検討した。TJ-19はモルモットのアレルギー性鼻炎の即時相および遅発相をともに抑制した。また，マウスIgE依存性皮膚反応においても即時相および遅発相を抑制した。TJ-19はhistamineおよびtumor necrosis factor (TNF)-αによるマウス皮膚反応を抑制した。また，マウス，ラットおよびヒト肥満細胞からのhistamine遊離には影響を及ぼさなかったが，ヒト肥満細胞からのgranulocyte-macrophage colony stimulating factor産生を抑制した。さらに，TJ-19は抗原刺激によるマウスのinterleukin (IL)-1β mRNAの発現には影響を及ぼさなかったが，TNF-α mRNAの発現を抑制した。TJ-19はマウス脾臓のCD4$^+$ T細胞の抗CD3抗体刺激によるinterferon-γおよびIL-4産生には影響を及ぼさなかった。TJ-19はモルモットForssman反応（II型），ラットArthus反応（III型）およびマウス接触過敏反応（IV型）には影響を及ぼさなかった。したがって，TJ-19はI型アレルギー反応を選択的に抑制するものと考えられ，I型アレルギー反応即時相の抑制には主としてhistamineに対する拮抗作用が，また，遅発相の抑制にはサイトカインの産生抑制あるいは作用発現の抑制が関与すると推定される。

References

1) Higaki, S., Konishi, K., Morohashi, M. and Terasawa, K.: Touka ni okeru Wakan-yaku-gairai no joukyou. *J. Med. Pharm. Soc. Wakan-Yaku* **2**, 652-653, 1985.

2) Mikawa, H. and Ito, S.: Effect of Saiko-seikan-to on atopic dermatitis in children. *Kampo Immuno-aller.* **6**, 80-86, 1992.

3) Tukamoto, Y., Nakajima, S. and Kunii, Y.: Therapy on atopic dermatitis by Sho-fu-san, Ouren-gedoku-to and Shousaiko-to. *J. Med. Pharm. Soc. Wakan-Yaku* **4**, 242-243, 1987.
4) Beutler, B., Krochin, N., Milsark, I. W., Luedke, C. and Cerami, A.: Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* **232**, 997-980, 1986.
5) Tessier, P., Audetti, M., Cattaruzzi, P. and McColl, S. R.: Up-regulation by tumor necrosis factor alpha of intercellular adhesion molecule 1 expression and function in synovial fibroblasts and its inhibition by glucocorticoids. *Arthritis Rheum.* **36**, 1528-1539, 1993.
6) Schleimer, R. P.: An overview of glucocorticoid anti-inflammatory actions. *Eur. J. Clin. Pharmacol.* **45**, S3-7, 1993.
7) Barnes, P. J. and Adcock, I.: Anti-inflammatory actions of steroids: molecular mechanisms. *Trends. Pharmacol. Sci.* **14**, 436-431, 1993.
8) Guyre, P. M., Girard, M. T., Morganelli, P. M. and Manganiello, P. D.: Glucocorticoid effects on the production and actions of immune cytokines. *J. Steroid Biochem.* **30**, 89-93, 1988.
9) Snijdewint, F. G., Kapsenberg, M. L., Wauben-Penris, P. J. and Bos, J. D.: Corticosteroids class-dependently inhibit in vitro Th1- and Th2-type cytokine production. *Immunopharmacology* **29**, 93-101, 1995.
10) Abe, S., Yamamoto, T., Iihara, S., Yamazaki, M. and Mizuno, D.: A possible role of glucocorticoids: an intrinsic inhibitor of the cytotoxic activity of tumor necrosis factor. *Jpn. J. Cancer. Res.* **79**, 305-308, 1988.
11) Waage, A. and Bakke, O.: Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes. *Immunology* **63**, 299-302, 1988.
12) Marone, G., Stellato, C., Renda, A. and Genovese, A.: Anti-inflammatory effects of glucocorticoids and cyclosporin A on human basophils. *Eur. J. Clin. Pharmacol.* **45**, S17-20, 1993.
13) Iwama, H., Amagaya, S. and Ogihara, Y.: Effect of Kampo on the immune response. *Jpn. J. Inflammation.* **4**, 566-568, 1984.
14) Takenaka, T., Okitu-Negishi, S., Hashira, S., Abe, T. and Yoshino, K.: The effect of Sairei-to on the cytokine production from peritoneal macrophage of mice. *Jpn. J. Inflammation* **14**, 371-377, 1994.
15) Takeuchi, Y., Nishimura, Y., Yoshikawa, T., Kuriyama, J. and Kimura, Y.: A comparison between Chinese blended medicine "Shou-seiryu-to", tranilast ketotifen on the anti-allergic action in guinea pigs. *Jpn. J. Allergol.* **34**, 391-393, 1993.
16) Nishiyori, T., Nakatomi, I., Matsuura, N., Nagai, H. and Koda, A.: Effect of Chinese blended medicine, Saiboku-to, on type IV allergic reaction. *Jpn. J. Allergol.* **32**, 317-323, 1983.
17) Homma, M., Oka, K., Niitsuma, T. and Itoh, H.: A novel 11 β-hydroxysteroid dehydrogenase inhibitor contained in Saiboku-to, a herbal remedy for steroid-dependent bronchial asthma. *J. Pharm.Pharmacol.* **46**, 305-309, 1994.
18) Iikura, Y., Saito, H., Umesato, Y., Shichijo, K., Ebisawa, M. and Nagakura, T.: Bronchial asthma and traditional Japanese herbal medicine: In vitro and in vivo studies. *J. Med. Phram. Soc, Wakan-Yaku* **6**, 220-223, 1989.
19) Terasawa, K., Itoh, T., Tosa, H., Shiroishi, H., and Imadaya, A.: Therapentic effect of Sino-Japanese (kampoh) medicine on bronchial asthma. *J. Med. Phram. Soc, Wakan-Yaku* **4**, 65-72, 1987.
20) Umesato, Y.: Bronchial asthma and traditional Japanese herbal medicine. *Jpn. J. Allergol.* **33**, 1047-1052, 1984.
21) Fujita, M., Yonetomi, Y., Shimouchi, K., Takeda, H., Aze, Y., Kawabata, K. and Ohno, H.: Involvement of cysteinyl leukotrienes in biphasic increase of nasal airway resistance of antigen-induced rhinitis in guinea pigs. *Eur. J. Pharmacol.* **369**, 349-356, 1999.
22) Nagai, H., Sakurai, T., Inagaki, N. and Mori, H.: An immunopharmacological study of the biphasic allergic skin reaction in mice. *Biol. Pharm. Bull.* **18**, 239-245, 1995.
23) Sakurai, T., Inagaki, N. and Nagai, H.: The effect of anti-tumor necrosis factor (TNF)-α monoclonal antibody on allergic cutaneous late phase reaction in mice. *Life Sci.* **54**, PL291-295, 1994.
24) Kawakami, Y., Hartman, S.E., Kinoshita, E., Suzuki, H., Kitaura, J., Yao, L., Inagaki, N., Franco, A., Hata, D., Yamamoto, M., Fukamachi, H., Nagai, H. and Kawakami, T.: Terreic acid, a quinone expoxide inhibitor of Bruton's tyrosine kinase. *Proc. Natl. Acad. Sci. USA* **96**, 2227-2232, 1999.
25) Shichijo, M., Inagaki, N., Nakai, N., Kimata, M., Nakahata, K., Serizawa, I., Iikura, Y., Saito, H. and Nagai, H.: The effect of anti-asthma drugs on mediator release from cultured human mast cells. *Clin. Exp. Allergy* **28**, 1228-1236, 1998.
26) Inagaki, N., Kawasaki, H., Ueno, M., Nagai, H. and Koda, A.: Potentiation of antigen-induced histamine release from rat peritoneal mast cells through a direct interaction between mast cells and non-mast cells. *Life Sci.* **54**, 1403-1409, 1994.
27) Saito, H., Ebisawa, M., Sakaguchi, N., Onda, Y., Iikura, Y., Yanagida, M., Uzumaki, H. and Nakahata, T.: Characterization of cord-blood-derived human mast cells cultured in the presence of steel factor and interleukin-6. *Int. Arch. Allergy Immunol.* **107**, 63-65, 1995.
28) Nagai, H., Nishiyori, T., Ochi, T., Imai, Y., Tanaka, H. and Inagaki, N.: The effect of methanolic extract from Corydalis Tuber on cytokine production and allergic reactions in experimental animals. *J. Trad. Med.* **16**, 51-57, 1999.
29) Brogdem, R.N., Speight, T.M. and Avery, G.S.: Sodium cromoglycate: a review of its mode of action, pharmacology therapeutic efficacy and use. *Drugs* **7**, 164-282, 1974.
30) Nyunt, A.K., Takeuchi, Y., Yokomuro, K. and Miyanaga, Y.: Comparative studies on the anti-allergic effect of Kampo medicines used for the therapy of respiratory diseases. *Jpn. J. Allergol.* **44**, 503-512, 1995.
31) Yamahara, J., Yamada, T., Kimura, H., Sawada, T. and Fujimura, H.: Biological active principles of crude drugs. II anti-allergic principles in "Shouseiryu-to" anti-inflamatory properties of paeoniflorin and its derivatives. *J. Pharmacobiodyn.* **5**, 921-929, 1982.