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Anti-allergic action of gomisin A (TJN-101)

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Abstract

The effect of gomisin A (TJN-101) on experimental allergic cutaneous reactions in mice and rats and antigen-induced contraction of guinea pig tracheal muscle was investigated. Homologous passive cutaneous anaphylaxis (PCA) reactions in mice and rats were clearly inhibited by TJN-101. Besides PCA reaction, TJN-101 inhibited reversed cutaneous anaphylaxis in rats, passive Arthus reaction in rats and picrylchloride-induced contact dermatitis in mice. The doses required to inhibit the PCA reactions were slightly lower than that required to inhibit other cutaneous reactions. In addition to cutaneous reactions, TJN-101 inhibited the antigen-induced contraction of guinea pig tracheal muscle. The contractile responses of guinea pig tracheal muscle induced by histamine, leukotriene D_4 and $CaCl_2$ in vitro were also inhibited by TJN-101. Moreover, high K-induced contraction of guinea pig taenia coli was inhibited by TJN-101 in a dose related fashion. Additionally, TJN-101 inhibited the antigen-induced histamine release from sensitized guinea pig lung tissue and compound 48/80-induced histamine release from rat peritoneal mast cells. These results indicate that TJN-101 shows an anti-allergic action in mouse and rat skin and the tracheal muscle of guinea pigs. Anti-allergic mechanism of TJN-101 in guinea pig tracheal muscle seems to be related to the inhibition of histamine release, antagonisms to the chemical mediators and inhibition of calcium movement.

Key words Gomisin A, TJN-101, allergic cutaneous reaction, allergic bronchoconstriction, anti-allergic action.

Abbreviations DNP-As, dinitrophenylated ascaris ; PCA, passive cutaneous anaphylaxis ; OA, ovalbumin ; BPO-BGG, bezylpenicilloyl bovine-γ-globulin ; RCA, reveresed cutaneous anaphylaxis ; PC, picrylchloride ; LT, luekotriene.

Introduction

Gomisin A (TJN-101 ; (+)-(6s,7s, R-biar)-6,7-dimethyl-10,11-methylenedioxy-1,2,3,12-tetramethoxy-5,6,7,8-tetrahydro-6-dibenzo (a, c) cyclooctenol) is isolated from *Schizandra* fruits.[1-3] We previously reported an inhibitory effect of TJN-101 on immunologic (allergic)-induced liver injury in mice.[4] The results in previous study strongly suggest that a cytoprotective action of TJN-101 participates to aid the appearance of an anti-hepatotoxic action.

The present study was, therefore, conducted to investigate the effect of TJN-101 on some allergic reactions in the experimental animals.

Materials and Methods

Animals : Male ddY mice weighing 18 to 20 g, male Wistar rats weighing 120 to 150 g and male Hartley guinea pigs weighing 350 to 400 g were used. Animals were purchased from Japan SLC Inc. (Hamamatsu, Japan). Animals were housed in wire mesh cages in an air-conditioned room at 24℃ and fed the usual laboratory diet and water

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ad libitum as provided.

Drugs : TJN-101 was kindly supplied from Tsumura & Co. (Tokyo, Japan). Prednisolone (Nippon-Merck Banyu, Tokyo, Japan), papaverine (Wako, Osaka, Japan) and diltiazem (Nakarai, Kyoto, Japan) were purchased. Tranilast and ketotifen which are potent mast cell stabilizers were kindly supplied from Kissei Pharmaceutical Co., Ltd. (Matsumoto, Japan) and Sandz Pharmaceutical Co., Ltd. (Basel, Switzerland). All drugs were suspended in 0.2% carboxy methyl cellulose saline. The control group was treated with 0.2% carboxy methyl cellulose saline as the same manner to the group treated with drugs.

Antiserum : Anti-dinitrophenylated ascaris (anti-DNP-As) mouse homocytotropic antiserum was prepared in BALB/c mice by two injections of antigen and alum at an interval of 30 days. The IgE antibody titre of antiserum estimated by 48 hour homologous passive cutaneous anaphylaxis (PCA) in mouse ear was $2 : 10^8$. Rat homocytotropic antibody against ovalbumin (OA) was prepared according to the method of Tada, Okumura and Mota.[5,6] The immunizing procedure is similar to that described above in rat anti-DNP-As antiserum. Anti-benzylpenicilloyl bovine-γ-globulin (BPO-BGG) antiserum was prepared according to the method of Levine *et al*.[7] by immunizing guinea pigs with BPO-BGG containing alum. The IgE antibody titre of antiserum estimated by 7 days homologous PCA was $1 : 2^{10}$. Sensitized guinea pigs were prepared with an i.v. injection of this homocytotropic antibody 48 hours before the experiment. Anti-rat-serum antibody was prepared with 5 injections of 1 ml of rat serum intravenously into rabbits every other day. The antiserum was obtained 7 days after the last injection.

Homologous PCA in mouse ear : Mouse ear PCA was carried out according to the method described previously.[8] Briefly, 10 μl of diluted antiserum (anti-DNP-As mouse serum) was injected into sites in both ears of ether anesthetized mice. After 48 hours PCA was elicited by an intravenous injection of 0.25 mg antigen (DNP-bovine serum albumin, as a hapten specific antigen) dissolved in 0.5% Evens blue saline solution in a volume of 0.25 ml. Thirty minutes after the challenge, mice were sacrificed by cervical dislocation and ears were removed. To measure the amount of extravasated dye, a pair of ears were dissolved with 0.7 ml of 1 N KOH solution in a stoppered tube at 37℃ overnight and 9.3 ml of mixture of 0.6 N H_3PO_4 solution and acetone (5 : 13) was added. After vigorous shaking, precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

Homologous PCA in rat back skin : In homologous PCA in rat using anti-OA antiserum, 1/10, 1/20, 1/30 and 1/40 diluted antiserum (0.1 ml) was injected into the back skin for sensitization. And OA saline solution containing 0.5% Evans blue was used as a challenging antigen. The following procedure was almost the same as described in mouse homologous PCA.

Reversed cutaneous anaphylaxis (RCA) in rats : RCA was carried out according to the method of Unger *et al*.[9] The lyophilized rabbit antirat-serum was dissolved in 0.01% Evans blue saline solution to make 14% (W/v) antiserum solution. This solution was injected intradermally in a 0.1 ml dose. The same dose of physiologic saline containing 0.01% Evans blue was similarly injected. Two hours later, the animals were sacrificed by exaguination, and the skin was removed. The inflamed areas were cut out with a leather punch (12 mm in a diameter) and the skin discs were compared with that of the saline-treated skin.

Passive Arthus reaction in rats : Rabbit anti-OA serum was diluted 50% in physiologic saline. One hundred microliters of the solution were injected into the planter pad of the hind paw of a rat. Immediately afterwards, 25 mg/kg OA was injected intravenously. The volume of the paw was measured with a mercury plethymometer (KN-357, Natsume, Tokyo, Japan) at times of zero and 2 hours. At time zero, the over-range rat paw volume amounted to 1.63 ml.

Picrylchrolide (PC)-induced contact dermatitis in mice : The experiment was carried out according to the method of Asherson and Ptak.[10] DDY mice were sensitized with an application of 0.1 ml of 7% PC in ethanol solution to the skin of the

abdomen which had been shaved one day before. After 6 days, a reaction was caused by the application of 0.015 ml each of 1% PC in olive oil solution to both ear lobes (primary challenge). The thickness of the lobe after 24 hours was measured with a dial thickness gauge (Ozaki, Tokyo, Japan) and the thickness before the challenge was subtracted from this thickness to obtain the swelling rate due to PC-induced contact dermatitis. In the experiment on secondary sensitization, animals were sensitized again 2 days after the measurement of lobe thickness in the same way and a reaction was caused 6 days later (secondary challenge).

Experiments on tracheal preparation : After the guinea pigs were stunned and exaguinated, the tracheas were excised, trimmed of excess tissue and cut vertically along the cartilage tissue area. Each open trachea was then cut horizontally into 16 segments. Four segments were tied together to form a chain which was then placed in an organ bath containing Tyrode solution. With an initial resting tension of 0.5 g, isotonic changes in the preparation were recorded (MEC, ME-4013, World Medical Co., Ltd., Nagoya, Japan). In most experiments, the drugs were incubated with tissue for 30 minutes before the addition of antigen, histamine, leukotriene D_4 (LTD_4) and $CaCl_2$. The contractile responses were expressed as a percentage of the contraction induced by 10^{-7} g/ml carbachol. The addition of antigen (10^{-6} g/ml) induced a contraction which was recorded for 15 minutes. For evaluation of antagonistic action against histamine (5×10^{-7} g/ml) and LTD_4 (2×10^{-8} g/ml), contractile levels by agonists in the presence or absence of the drugs were compared. In the case of evaluating the Ca-induced contraction of tracheal muscle, the $CaCl_2$-induced contraction level in high K-depolirized muscle was employed. Briefly, the tracheal muscle was immersed in Ca-free Tyrode solution for 60 minutes, followed by a 6 minute immersion in a high K Tyrode solution omitting Ca ion. Thereafter, a contraction was induced by adding $CaCl_2$ (10^{-2} g/ml) into the medium.

Experiment on taenia coli : Strips of taenia coli were freshly dissected from guinea pigs and strips were immersed in Tyrode solution. The preparations were equilibrated for 60 minutes before replacement of the medium to high K Tyrode solution. When the muscle was treated with the high K-(isotonic 60 mM) solution, it developed a temporal strong contraction where gradually fell within 20 minutes and there remained a steady level. Increasing concentrations of examined agents were cumulatively added to the high K medium. The inhibition percent was calculated to the percentage to the maximum relaxation caused by 10^{-4} M papaverine.

Histamine release from guinea pig lung tissues and rat peritoneal mast cells : The lungs from guinea pigs passively sensitized with anti-BPO-BGG homocytotropic antibody were chopped on a McIlwain tissue chopper and suspended in 10 volumes of Tyrode solution. Histamine release was caused by the addition of antigen into the medium at final concetration of 5×10^{-6} g/ml. The amount of histamine in the incubation medium was assayed by a fluorescence method according to May *et al*.[11] Rat peritoneal mast cells were obtained by the method described previously. Harvested cells were adjusted to 10^5 mast cells/ml. Compound 48/80 (10^{-6} to 10^{-4} g/ml) was added to the cell suspension at 37℃ for 20 minutes. The amount of histamine released from mast cell was measured by the same method as mentioned above.

Statistics : Results were statistically evaluated using the Student's *t*-test.

Results

Effect of TJN-101 on allergic cutaneous reactions

Homologous PCA in mice and rats : The effects of TJN-101 and prednisolone on homologous PCA in mice ear are indicated in Fig. 1. TJN-101 at doses of 50 and 100 mg/kg and prednisolone at a dose of 10 mg/kg inhibited the dye leakage. In order to clarify the time course for inhibition, TJN-101 at a dose of 50 mg/kg was administered intraperitoneally at varying times before challenge. The most potent inhibitory activity was observed at the 1 hour pretreatment. Four hours pretreatment resulted in very little

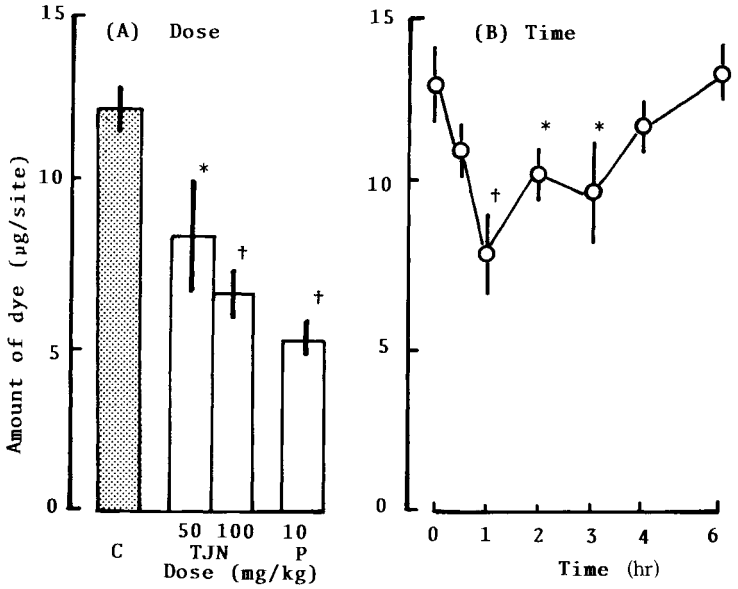

Fig. 1 Effect of TJN-101 on homologous passive cutaneous anaphylaxis in mice.
(A) ; TJN-101 and prednisolone were administered intraperitoneally 1 hour and 6 hour priors to challenge, respectively. (B) ; TJN-101 at a dose of 50 mg/kg was administered intraperitoneally at various time priors to challenge. Each experiment consists of 7 to 14 mice. * ; $p<0.05$, † ; $p<0.01$.

inhibition of PCA. When TJN-101 was administered intraperitoneally 1 hour prior to challenge, dye leakage caused by various concentration of anti-OA antiserum containing homocytotropic IgE antibody in rat was clearly inhibited by TJN-101 at doses between 10 and 100 mg/kg (Fig. 2). Ketotifen used as a reference drug showed the most potent inhibition.

RCA in rats : The effects of TJN-101 and prednisolone on RCA in rats are indicated in Fig. 3-A. TJN-101 at a dose of 100 mg/kg and prednisolone at a dose of 10 mg/kg inhibited the swelling.

Passive Arthus reaction in rats : TJN-101 at a dose of 100 mg/kg and prednisolone at a dose of 10 mg/kg inhibited the reaction as indicated in Fig. 3-B.

PC-induced contact dermatitis in mice : TJN-101 at a dose of 100 mg/kg and prednisolone at a dose of 5 mg/kg clearly inhibited the ear swelling (Fig. 3-C).

Contractile response of guinea pig tracheal muscle and taenia coli : The effect of TJN-101 on

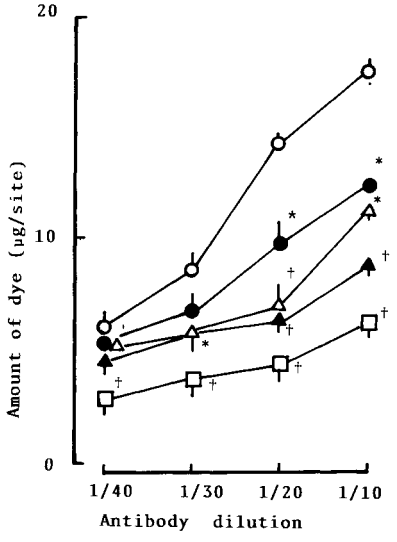

Fig. 2 Effect of TJN-101 and ketotifen on homologous passive cutaneous anaphylaxis in rats.
Each drug was administered intraperitoneally 1 hr prior to challenge. Each point consists of 6 to 8 experiments. ○ : Control, ● : TJN-101 10 mg/kg, △ : TJN-101 30 mg/kg, ▲ : TJN-101 100 mg/kg, □ : Ketotifen 10 mg/kg. * ; $p<0.05$, † ; $p<0.01$.

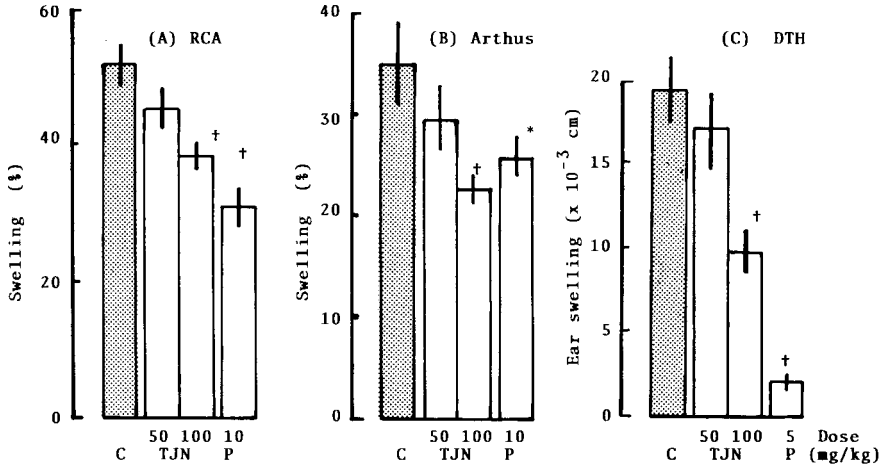

Fig. 3 Effect of TJN-101 and prednisolone on (A) reversed cutaneous anaphylaxis (RCA) in rats, (B) Arthus foot pad reaction in rats and (C) delayed type hypersensitivity (DTH) in mice.
Each experiment consists of 7 to 12 animals. (A) and (B) ; Each drug was administered intraperitoneally 1 hour prior to challenge. (C) ; Each drug was administered intraperitoneally immediately before and 16 hours after challenge. * ; $p<0.05$, † ; $p<0.01$.

antigen-induced contraction of guinea pig trachea is indicated in Fig. 4. Pretreatment of tracheal muscle with TJN-101 (5×10^{-5} and 10^{-4} g/ml), significantly but not completely, inhibited the amplitude of the contraction caused by antigen. On the non-sensitized guinea pig trachea, histamine (5×10^{-7} g/ml) and LTD_4 (2×10^{-8} g/ml) elicited the contraction with registered amplitude of approximately 80 to 90% of the maximum contraction caused by 10^{-7} g/ml carbachol. TJN-101 inhibited the histamine- and LTD_4-induced contraction at concentrations of 10^{-5} and 10^{-4} g/ml (Figs. 5-A and B). Moreover, TJN-101 inhibited Ca-induced contraction in K-depolirized tracheal smooth muscle (Fig. 5-C). In the guinea pig taenia coli, high K Tyrode solution produced a sustained contraction. When TJN-101 (5×10^{-6} and 10^{-4} g/ml) was applied to the muscle, K-induced contraction was inhibited in a concentration-dependent manner (Fig. 6). Papaverine showed more potent inhibition and diltiazem showed the most potent activity among three agents.

Histamine release : Fig. 7-A shows the effect of TJN-101 on antigen-induced histamine release

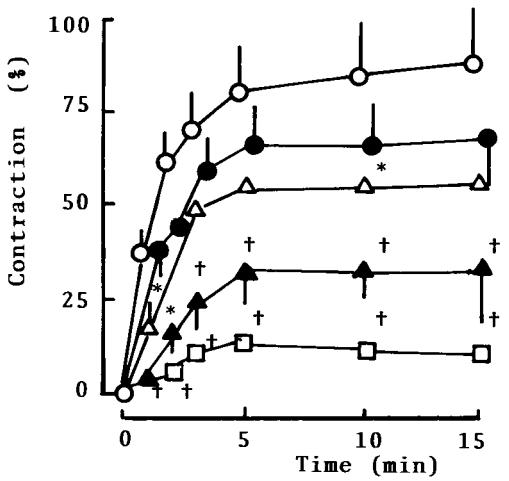

Fig. 4 Effect of TJN-101 on antigen-induced contraction of guinea pig tracheal muscle (Shultz-Dale reaction).
Each point consists of 6 to 8 experiments. ○ : Control, ● : 10^{-5} g/ml, △ : 2×10^{-5} g/ml, ▲ : 5×10^{-5} g/ml, □ : 10^{-4} g/ml. * ; $p<0.05$, † ; $p<0.01$.

from lung tissue of passively sensitized guinea pigs when treated with the drug at concentrations between 10^{-6} and 10^{-4} g/ml 10 minutes prior to

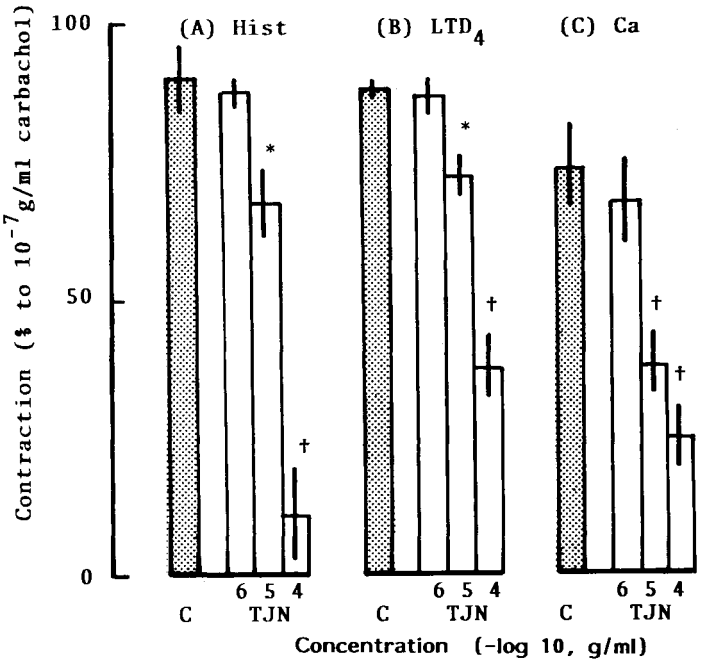

Fig. 5 Effect of TJN-101 on histamine-, LTD_4- and $CaCl_2$-induced contraction of guinea pig tracheal muscle.
Each column consists of 6 to 8 experiments. * ; $p<0.05$, † ; $p<0.01$.

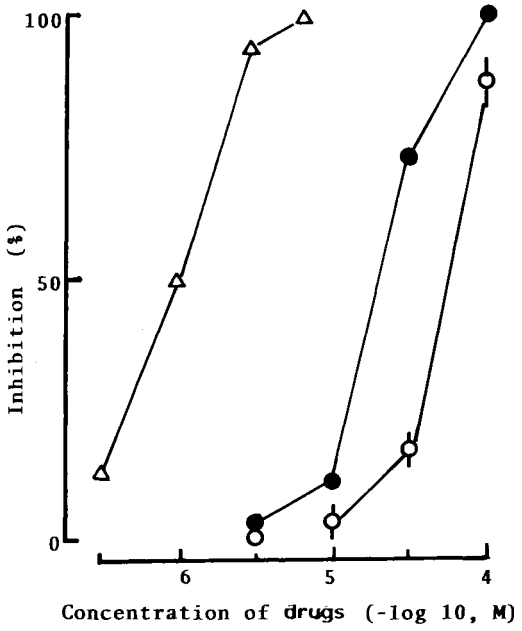

Fig. 6 Effect of TJN-101 (○), papaverine (●) and diltiazem (△) on high K-induced contraction of guinea pig taenia coli.
Each experiment consists of 4 to 6.

challenge. TJN-101 at concentrations between 10^{-6} and 10^{-4} g/ml clearly inhibited the release of histamine. Fig. 7-B shows the effect of TJN-101 (5×10^{-5} g/ml) on compound 48/80-induced histamine release from rat peritoneal mast cells. Compound 48/80 caused the release of histamine in a concentration-related fashion, and the release of histamine due to compound 48/80 at the concentrations between 5×10^{-6} and 5×10^{-5} g/ml was inhibited by TJN-101.

Discussion

The inhibitory action of TJN-101 on allergic cutaneous reactions in mice and rats are exemplified by the results of this report. In addition, TJN-101 inhibits the contraction induced by antigen, histamine and LTD_4 in guinea pig tracheal muscle and the release of histamine from guinea pig lung tissue and rat peritoneal mast cells. Moreover, TJN-101 shows clear calcium antagonistic action in high K depolirized guinea

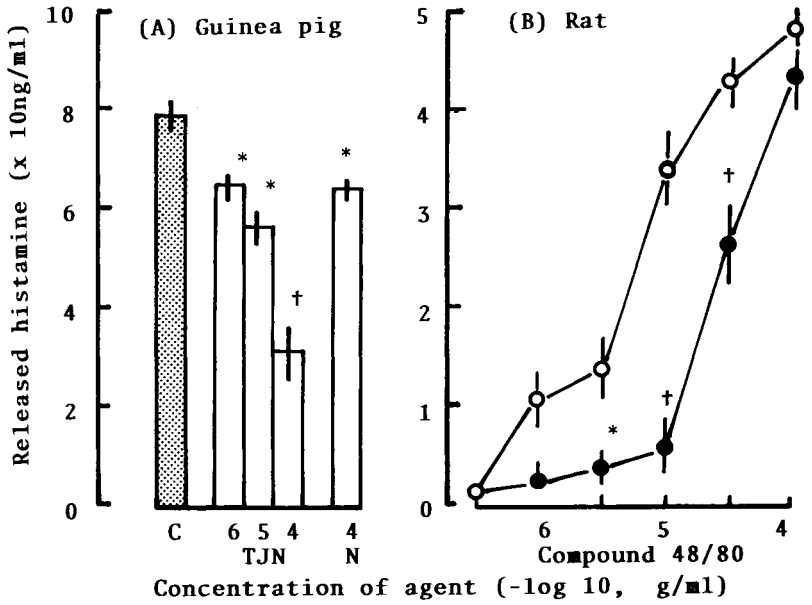

Fig. 7 Effect of TJN-101 and tranilast (N-5') on (A) antigen-induced histamine release from sensitized guinea pig lung tissue and (B) compound 48/80-induced histamine release from rat peritoneal mast cells.
(A) ; C : Control TJN : TJN-101, N : N-5' (tranilast), (B) ; ○ : Control ● : TJN-101 5×10^{-5} g/ml. * ; $p<0.05$, † ; $p<0.01$.

pig tracheal muscle and taenia coli.

While these results clarify an anti-allergic action of TJN-101, the anti-allergic mechanism of the agent is still obscure. Regarding the anti-allergic mechanism of TJN-101, the results from the experiments using guinea pig tracheal muscle and lung tissue offer a little explanation for this. The anti-allergic action seems to be mainly due to the antagonistic action to the mediators and the inhibition of antigen-induced histamine release. In addition to above actions, TJN-101 shows a calcium antagonistic action in the smooth muscles. As well known, calcium ion plays an important role for the onset of allergic reaction, especially the allergic contraction of smooth muscle and the release of chemical mediators.[12] Calcium antagonistic action, therefore, may participate for TJN-101-induced inhibition of smooth muscle contraction and mediator release. So far disodium cromoglycate, tranilast and oxatomide are reported to inhibit the anaphylactic mediator release due to the inhibition of calcium movement.[13-15] TJN-101 indicates similar properties to them. The calcium antagonistic property, however, is not so high that more detailed experiments using ^{45}Ca are necessary for clarifying the mechanism.

In addition to calcium antagonistic action, TJN-101 indicated the cell membrane stabilizing activity in mast cells and hepatocytes.[4] This pharmacological property may participate the anti-allergic action of TJN-101. Since little evidence has been reported the direct relationship between membrane stabilizing activity and anti-allergic actions, detailed experiments are necessary to clarify the relationship.

In conclusion, TJN-101 inhibits Type I, II, III and IV allergic cutaneous reactions and antigen-induced contraction of guinea pig tracheal muscle. This inhibitory action is due to the inhibition of the release of anaphylactic chemical mediators and antagonistic action to the anaphy-

lactic mediators and calcium ion.

和文抄録

生薬五味子のリグナン成分 gomisin A のマウスおよびラットにおける皮膚でのアレルギー反応および感作モルモット気管筋の抗原による収縮反応に及ぼす影響を検討した。TJN-101 はマウスおよびラットの同種皮膚アナフィラキシー反応，ラットの逆アナフィラキシー反応，ラットの受身アルチュス反応およびマウスの picrylchloride による遅延型皮膚反応を明らかに抑制した。さらに TJN-101 は抗原による感作モルモット気管筋の収縮も抑制した。そこで気管筋における抑制作用機序について検討した。TJN-101 はモルモット気管筋のヒスタミンおよび LTD_4 による収縮および感作モルモット肺切片からの抗原によるヒスタミン遊離を抑制した。これらの作用に加えて TJN-101 はモルモット気管筋および盲腸紐においてCa 拮抗作用およびラット腹腔マスト細胞からの compound 48/80 によるヒスタミン遊離抑制を示した。これらのことより気管筋における TJN-101 の抗アレルギー作用はアナフィラキシー性ケミカル mediator の遊離抑制作用と拮抗作用および Ca 拮抗作用によるものとおもわれる。

Reference

1) Taniguchi, H. and Ikeya, Y. : The constituents of Schizandra chinensis Baill I. The structure of gomisin A, B and C. *Chem. Pharm. Bull.* **23**, 3296-3298, 1975.
2) Taniguchi, H. and Ikeya, Y. : The constituents of *Schizandra chinensis* BAILL. The structure of two new lignans, gomisin F and G and the absolute structures of gomisin A, B and C. *Chem. Pharm. Bull.* **25**, 364-366, 1977.
3) Nakajima, K., Taniguchi, H., Ikeya, Y., Endo, T. and Yoshioka, I. : The constituents of *Schizandra chinensis* BAILL. XII Quantitative analysis of lignans in the fruits of *Schizandra chinensis* BAILL. by high performance liquid chromatography. *Yakugaku Zasshi* **103**, 743-749, 1983.
4) Nagai, H., Yakuo, I., Aoki, M., Teshima, K., Ono, Y., Sengoku, T., Shimazawa, T., Aburada, M. and Koda, A. : The effect of Gomisin A on immunologic liver injury in mice. *Planta Medica* **55**, 13-17, 1989.
5) Tada, T. and Okumura, K. : Regulation of homocytotropic antibody formation in rats. I. Feedback regulation of passively administered antibody. *J. Immunol.* **106** 1002-1011, 1971.
6) Mota, I. : The mechanism of anaphylaxis. I. Production and biological properties of mast cell sensitizing antibody. *Immunology* **7**, 681-699, 1964.
7) Levine, B.B. and Ovary, Z. : Studies on the mechanism of the formation of the penicillin antigen. III. The N-(D-a-benzylpenicilloyl) group as an antigen determinant responsible for hypersensitivity to penicillin G. *J. Exp. Med.* **114**, 875-903, 1962.
8) Nagai, H., Yakuo, I., Nakatomi, I., Inagaki, N. and Koda, A. : The role of thromboxane A_2 in allergic cutaneous reactions and the effect of (E)-3p-(1H-imidazol-1Ylmethyl) phenyl-2-propenoic acid hydrochloride (OKY-046), a TxA_2 synthetase inhibitor. *Prostaglandins Leukotrienes and Essential Fatty Acid* **35**, 125-130, 1989.
9) Unger, G., Korbin, S. and Sezesny, B.R. Measurement of inflammation and evaluation of anti-inflammatory agents. *Arch. int. Pharmacodyn.* **123**, 71-82, 1959.
10) Asherson, G.L. and Ptak, W. : Contact and delayed hypersensitivity in mouse. I. Active sensitization and passive transfer. *Immunology* **15**, 405-416, 1968.
11) May, C.D., Lyman, M., Alberto, R. and Cheng, J. : Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J. Allergy* **46**, 12-20, 1970.
12) Middleton, E. Jr. : Antiasthmatic drug thrapy and calcium ion. *J. Pharm. Sci.* **69**, 243-251, 1980.
13) Middleton, E. Jr. : Airway smooth muscle, asthma and calcium ions. *J. Allergy Clin. Immunol.* **73**, 643-650, 1984.
14) Nagai, H., Kelly, K. and Sehon, A.H. : The inhibition of histamine release by anti-allergic drugs. *Int. Archs. Allergy Appl. Immunol.* **56**, 307-315, 1978.
15) Koch, H. : Oxatomide ; anti-allergic/anti spasmodic. *Pharmacy international* **81**, 50-88, 1981.