Inhibitory Effects of Moutan Cortex on Immediate Allergic Reactions

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The anti-allergic effect of an ethanol extract from Moutan Cortex was evaluated in some animal models. The Moutan Cortex extract (30, 100 mg/kg, i.p.) dose-dependently inhibited systemic anaphylactic shock induced by compound 48/80 in mice. It also inhibited dose-dependently the scratching behavior induced by compound 48/80 or histamine at a dose of 100 mg/kg. An increase in the vascular permeability induced by compound 48/80 or histamine was also inhibited by the Moutan Cortex. In addition, *in vitro* studies, the Moutan Cortex inhibited histamine release from rat peritoneal mast cells induced by compound 48/80. To investigate the active component of Moutan Cortex extract, it was suspended in water and extracted with EtOAc to yield EtOAc insoluble (A) and soluble (B) fractions. The effect of extract (B) was more potent than that of extract (A) in inhibiting histamine release. From these findings, it seems likely that the Moutan Cortex extract is effective in antagonizing certain pharmacological effects induced by compound 48/80, which is probably mediated by inhibiting the release of histamine from mast cells and antagonizing the effect on histamine. The main active component of Moutan Cortex is considered to be contained in extract (B). In conclusion, Moutan Cortex may be useful for the relief of symptoms of atopic dermatitis and other allergy-related diseases.

Key words Moutan Cortex; anaphylactic shock; mast cell; compound 48/80; scratching behavior; vascular permeability

Atopic dermatitis, allergic rhinitis, food allergy and asthma are classified as type I allergy. Atopic dermatitis is a common disorder in the population, and recent studies have shown that the disease is increasing, its prevalence increasing by 2- to 3-fold during the past three decades in industrialized countries,[1] and the reported prevalence among children up to the age of 16 ranges between 15% and 30% in Europe.[2] Atopic dermatitis is a condition manifesting eczema, serous papules, scaling and crust and in severe cases, erosion of the affected skin. Itching, a sensation causing the urge to scratch, is the most significant outcome of atopic dermatitis. This unpleasant sensation stimulates scratching of the lesioned skin, thus worsening the lesions.[3] In the treatment of atopic dermatitis, anti-histamine and anti-allergy drugs are generally used, but they do not allay a severe itching sensation in atopic dermatitis.[4,5] Corticosteroid is also used as external medicine to relieve cutaneous pruritics, but it has many adverse effects including skin atrophy, rosacea, acne, purpura and so on[6]; therefore, it is worth developing new treatment drugs besides the generally used medicines.

Traditional Chinese medicines have a long history dating back several thousands of years. There are many herb medicines which have been applied to cutaneous disease and some traditional Chinese herbal medicines are prescribed for dermatitis.[7] Among these, Moutan Cortex, the root bark of *Paeonia suffruticosa* ANDREWS, is a widely used drug for cutaneous disease in traditional Chinese medicine.[8,9] In association with this, Moutan Cortex is effective in cardiovascular and female genital disease.[10,11] In addition, Moutan Cortex has been described to show analgesic, sedative, anti-inflammatory and antimicrobial properties[12]; however, we have as yet very little information as to whether Moutan Cortex is effective in experimental allergy models.

In the present study, therefore, we demonstrated the inhibitory effects of ethanol extract from Moutan Cortex on experimental immediate allergic reactions both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Animals Female ICR mice (6—10 weeks old) and male Wistar rats (7 weeks old) were obtained from Japan SLC, Inc., Shizuoka, Japan. The animals were housed in an air-conditioned room maintained at 24±2 °C with a relative humidity of 55±15%. They were given standard laboratory rodent food chow (Oriental Yeast, Tokyo) and water *ad libitum*. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Chemicals and Reagents The following reagents were used in this study and their sources are shown in parentheses: histamine dihydrochloride (Sigma, St. Louis, MO, U.S.A.), compound 48/80 (Sigma) and Evans blue (Wako, Tokyo, Japan). Compound 48/80 and histamine were dissolved in physiological saline and administered intradermally. The drugs used were azelastine hydrochloride (Sigma), ketotifen fumarate (Sigma) and disodium cromoglycate (DSCG) (Sigma). The test drugs were dissolved in physiological saline and administered intraperitoneally.

Preparing the Extract from Moutan Cortex Dried root bark of Moutan Cortex (lot number: 010405) was purchased from Tochimoto Tenkaido Company (a herbal drug company), Osaka, Japan. This powdered Moutan Cortex Bark was the product which standardized by the Japanese Pharmacopoeia fifteenth edition. A voucher specimen was deposited at Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Okayama, Japan. Five hundred grams of Moutan Cortex were ground and refluxed with 600 ml of ethanol for 2 h. The solution was separated by filtration through filter paper, and the extract process was repeated twice. The combined solutions were concentrated under reduced pressure at 40 °C, and the residue was lyophilized to give a dry extract (yield 9.02%). The dried extract was suspended in 500 ml of water and extracted with 500 ml of EtOAc to yield EtOAc insoluble (A) (yield 5.01%)

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and soluble (B) fractions (yield 3.96%). The dried Moutan Cortex extract was freshly suspended in physiological saline and administered intraperitoneally.

Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice The experiment was carried out according to the method described by Shin *et al.*[13] Briefly, each mouse was given an intraperitoneal injection of compound 48/80 (8 mg/kg) to evoke a systemic anaphylactic reaction. Moutan Cortex extract or DSCG (positive control drug) was administered intraperitoneally 1 h or 30 min before the injection of compound 48/80, respectively. Mortality was monitored for 1 h after the induction of anaphylactic shock.

Histamine Release Induced by Compound 48/80 from Isolated Rat Peritoneal Mast Cells The peritoneal mast cells of male Wistar strain rats were harvested and purified by Percoll density centrifugation.[14] The collected mast cells (2.5×10^4 cells/tube) were then incubated with physiological buffered solution (PBS; in mM: NaCl 154, KCl 2.7, $CaCl_2$ 0.9, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) 5; glucose 5.6; pH 7.4) for 10 min at 37 °C. The test drugs dissolved in PBS were added (0.1 ml) 10 min and DSCG were added (0.1 ml) 30 s before compound 48/80 (final concentration: 0.5 μg/ml), respectively. The reaction was stopped 10 min later by cooling the tubes in ice water, and then centrifuging them for 15 min at 200×***g***. The histamine contents were measured by a fluorometric assay.[15]

Scratching Behavior Induced by Compound 48/80 or Histamine in Mice Scratching behavior was observed using the same method as Takubo *et al.*[16] Moutan Cortex extract was suspended in physiological saline and administrated intraperitoneally 1 h before the start of behavioral observation. Compound 48/80 (10 μg/0.02 ml) or histamine (100 nmol/0.02 ml) was injected intradermally into the rostral part of the back of the mice. Immediately after injection, the mice were placed in the observation chamber and their behavior was observed for 1 h. In the present study, scratching behavior was automatically detected and; objectively evaluated with a new apparatus, MicroAct (Neuroscience, Tokyo, Japan). A small magnet (diameter 1 mm, length 3 mm) was implanted subcutaneously into both hind paws of a mouse under ether anesthesia at least 12 h before the measurement of scratching behavior. Mice were placed in an observation chamber (11 cm in diameter, 18 cm high), which was surrounded by a round coil. The electric current induced in the coil by the movement of magnets attached to the hind paws was amplified and recorded.[17]

Vascular Permeability of the Skin in Mice The increase in vascular permeability was assessed as described by Inagaki *et al.*[18] After the intradermal injection of 0.5 μg/0.02 ml compound 48/80 or 10 nmol/0.02 ml histamine into the rostral part of the back, 1.25 mg of Evans blue was intravenously injected into each animal. The mice were sacrificed 30 min after elicitation of the cutaneous reactions, and the reaction site was excised. The skin samples were incubated with 0.7 ml of 1 N KOH for 24 h, and then 9.3 ml of a mixture of 0.2 M phosphoric acid solution and acetone (5 : 13, v/v) was added. After vigorous shaking, the precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

Statistical Analysis The data are presented as the means±S.E.M. Statistical significance was tested by one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability value less than 0.05 was considered significant.

RESULTS

Effect of Moutan Cortex Extracts on Systemic Anaphylactic Shock Induced by Compound 48/80 As shown in Table 1, an intraperitoneal injection of compound 48/80 (8 mg/kg) resulted in a fatal shock in all mice, and Moutan Cortex extract pretreatment (30, 100 mg/kg) dose-dependently reduced the mortality rate. The positive drug DSCG also inhibited the compound 48/80-induced systemic anaphylactic reaction at a dose of 100 mg/kg.

Effect of Moutan Cortex Extract on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 As show in Fig. 1, compound 48/80 (0.5 μg/ml) elicited 59.9±2.6% of histamine release from peritoneal mast cells of rats. Moutan Cortex extract pretreatment clearly inhibited compound 48/80-induced histamine release at concentrations of 10, 30 and 100 μg/ml.

Effect of Moutan Cortex Extract on Scratching Behavior Induced by Compound 48/80 or Histamine Compound 48/80 was used at a dose of 10 μg/site and histamine was used at a dose of 100 nmol/site according to our previous observation.[14] As shown in Fig. 2A, Moutan Cortex extract caused a dose-related inhibition of scratching behavior induced by compound 48/80 (10 μg/site) and a significant effect was observed at a dose of 100 mg/kg. Azelastine, used as positive control at a dose of 10 mg/kg, also caused an inhi-

Table 1. Effect of Moutan Cortex Extract on Compound 48/80-Induced Systemic Anaphylaxis

	Dose (mg/kg, i.p.)	Mortality (%)		
		10	20	60 min
Control		20	70	100
Extract	10	0	30	100
	30	0	20	50
	100	0	0	20
DSCG	100	0	0	50

Moutan Cortex extract or DSCG was administered intraperitoneally 1 h or 30 min before compound 48/80 (8 mg/kg), respectively. Mortality within 1 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice ($n=10$).

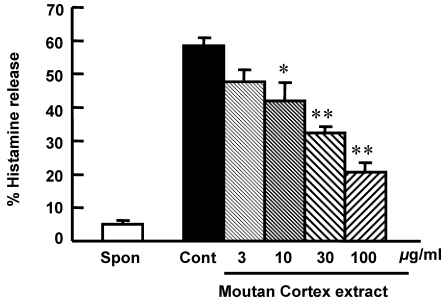

Fig. 1. Effect of Moutan Cortex Extract on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

Each column and vertical bar shows the means±S.E.M. ($n=8$). Compound 48/80 (0.5 μg/ml) elicited 59.9±2.6% of histamine release. *,** Significantly different from the control group at $p<0.05$ and $p<0.01$, respectively. Spon: Spontaneous histamine release. Cont: Control.

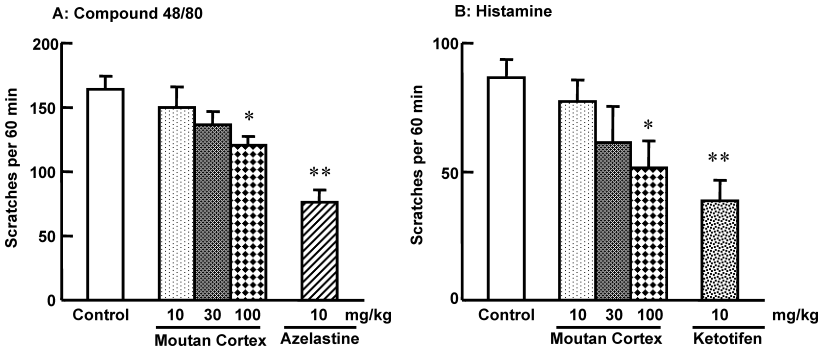

Fig. 2. Effect of Moutan Cortex Extract on the Scratching Behavior Induced by Compound 48/80 (A) or Histamine (B) in ICR Mice

Moutan Cortex extract was administered intraperitoneally 1 h before compound 48/80 (10 μg/site) or histamine (100 nmol) injection. Azelastine or ketotifen was administered intraperitoneally 30 min before the injection, respectively. Each column and vertical bar shows the means±S.E.M. (n=10). *,** Significantly different from the control group at $p<0.05$ and 0.01, respectively.

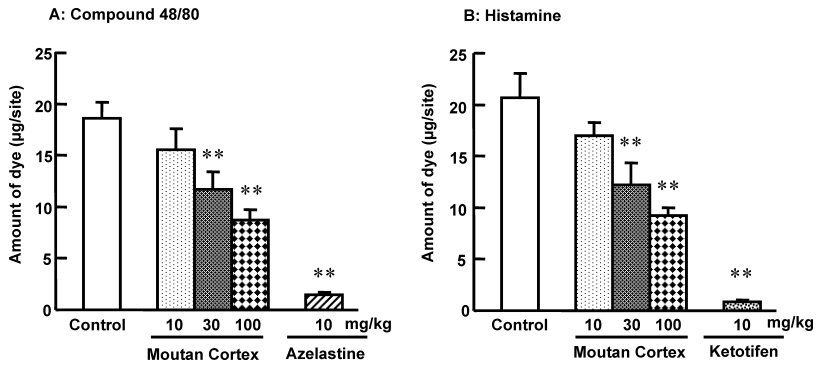

Fig. 3. Effect of Moutan Cortex Extract on the Vascular Permeability Induced by Compound 48/80 (A) or Histamine (B) in ICR Mice

Moutan Cortex extract was administered intraperitoneally 1 h before compound 48/80 (0.5 μg/site) or histamine (10 nmol/site) injection. Azelastine or ketotifen was administered intraperitoneally 30 min before the injection, respectively. Each column and vertical bar shows the means±S.E.M. (n=8). ** Significantly different from the control group at $p<0.01$.

bition of this response. At the same dose, the extract also showed a significant inhibition of scratching behavior induced by histamine (100 nmol/site) (Fig. 2B). Ketotifen, used as positive control, significantly inhibited the histamine-induced scratching behavior at a dose of 10 mg/kg.

Effect of Moutan Cortex Extract on Vascular Permeability Induced by Compound 48/80 or Histamine Vascular permeability reaction was elicited by an intradermal injection of 0.5 μg/site compound 48/80 or 10 nmol/site histamine, respectively. As shown in Fig. 3A, Moutan Cortex extract caused a dose-related inhibition of vascular permeability induced by compound 48/80, and a significant effect was observed at doses of 30 and 100 mg/kg. Azelastine, at a dose of 10 mg/kg, also significantly inhibited an increase in vascular permeability induced by compound 48/80. The extract also reduced histamine-induced vascular permeability at doses of 30 and 100 mg/kg (Fig. 3B). Ketotifen, at a dose of 10 mg/kg, also caused an inhibition of the vascular permeability induced by histamine.

Effect of Moutan Cortex Extract (A) and Extract (B) on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 As shown in Fig. 4, compound 48/80 elicited 52.5±2.4% of histamine release from peritoneal mast cells of rats. Moutan Cortex extract (A) inhibited compound 48/80-induced histamine release at concentrations of 30 and 100 μg/ml. Extract (B) also inhibited histamine release at concentrations of 10, 30 and 100 μg/ml.

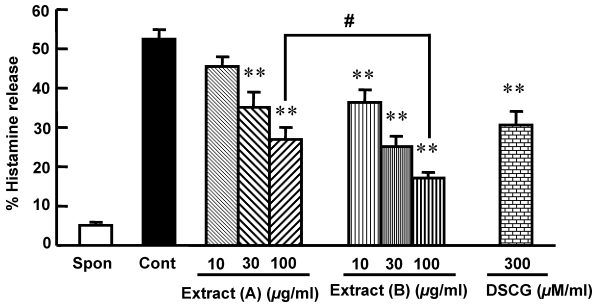

Fig. 4. Effects of Moutan Cortex Extract (A) and Extract (B) on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

Each column and vertical bar shows the means±S.E.M. (n=8). Compound 48/80 (0.5 μg/ml) elicited 52.5±2.4% of histamine release. ** Significantly different from the control group at $p<0.01$. # Significantly different between the extract (A) and extract (B). Spon: Spontaneous histamine release. Cont: Control.

The effect of extract (B) was more potent than that of extract (A), and a significant difference was observed at a dose of 100 μg/ml. DSCG used as a positive control at a dose of 300 μmol/ml also caused an inhibition of histamine release.

DISCUSSION

In the present study, we first demonstrated that Moutan Cortex extract significantly inhibited the systemic anaphylaxis reaction induced by compound 48/80 at doses of 30 and

100 mg/kg (Table 1). It is well known that anaphylaxis reaction induced by compound 48/80 is attributable to histamine released from mast cells.[19] From these findings, it seems likely that Moutan Cortex extract inhibited histamine release from mast cells; therefore, we next studied the effect of Moutan Cortex extract on histamine release using rat peritoneal mast cells. As a result, Moutan Cortex extract dose-dependently inhibited histamine release from rat peritoneal mast cells induced by compound 48/80 (Fig. 1). These results clearly indicate that Moutan Cortex extract inhibited mast cell-mediated immediate-type allergic reactions.

Compound 48/80 is well known to cause skin responses such as scratching behavior or increased vascular permeability by released histamine from mast cells[20]; therefore, it is reasonable to presume that an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis. As shown, Moutan Cortex extract also significantly inhibited the scratching behavior induced by compound 48/80 (Fig. 2A). Among the chemical mediators released from mast cells, histamine is considered to be one of the most important, and it can cause all the pathological features of atopic dermatitis. In this study, we also demonstrated that Moutan Cortex extract inhibited the scratching behavior induced by histamine (100 nmol/site) at a dose of 100 mg/kg (Fig. 2B), suggesting that Moutant Cortex extract directly antagonizing the effect of histamine.

Increased permeability of the microvasculature to macromolecules is one of the earliest events of acute inflammation. Mast cell mediators such as histamine increase vascular permeability in various species.[21] One of the consequences of increased vascular permeability is edema, which is the accumulation of plasma proteins, water and electrolytes in the interstitium. In this study, the effect of Moutan Cortex extract on the vascular permeability induced by compound 48/80 or histamine was investigated, revealing that Moutan Cortex extract inhibited vascular permeability induced by compound 48/80 or histamine at doses of 30 and 100 mg/kg (Figs. 3A, B).

In order to gain more insight into the definite chemical identity of active components in Moutan Cortex, we performed polarity-based fractionalization. As shown in Fig. 4, both Moutan Cortex extracts (A) and extract (B) inhibited histamine release from rat peritoneal mast cells induced by compound 48/80. The inhibitory effect of extract (B) was more potent than extract (A), and a significant difference was observed at a dose of 100 μg/ml, suggesting that the active component of Moutan Cortex is mainly included in extract (B). The chemical components of Moutan Cortex are known to be paeonol, paeonoside, paeonolide, paeoniflorin and oxypaeoniflorin.[22] Among those compounds, paeonol is the major phenolic component of Moutan Cortex. It has been reported that paeonol can inhibit histamine and TNF-α release from rat mast cells and RBL-2H3 cells, respectively.[23] Therefore, it may be that paeonol is one of the active components of Moutan Cortex which contribute to anti-allergic activity.

In conclusion, we demonstrated that the extract from Moutan Cortex exhibited profound anti-allergic potential in mast cell-dependent test models. This work provides experimental evidence for the folk medicine use of Moutan Cortex in the treatment of allergic disease.

REFERENCES

1) Mohrenschlager M., Darsow U., Schnopp C., Ring J., *J. Eur. Acad. Dermatol. Venereol.*, **20**, 503—511 (2006).
2) Schultz Larsen F., Diepgen T., Svensson A., *J. Am. Acad. Dermatol.* **34**, 760—764 (1996).
3) Wahlgren C. F., *Allergy*, **47**, 65—75 (1992).
4) Wahlgren C. F., *Acta Derm. Venereol. Suppl.*, **165**, 1—53 (1991).
5) Berth-Jones J., Graham-Brown R. A., *Br. J. Dermatol.*, **121**, 635—637 (1989).
6) Hengge U. R., Ruzicka T., Schwartz R. A., Cork M. J., *J. Am. Acad. Dermatol.*, **54**, 1—15 (2006).
7) Sheehan M. P., Atherton D. J., *Br. J. Dertamol.*, **130**, 488—493 (1994).
8) Hong S. H., Yi J. M., Kim H., Choi H. Y., Kim Y. K., Chae H. J., Kim H. R., Kim C. H., Kim H. M., *J. Ethnopharmacol.*, **98**, 361—365 (2005).
9) Harper J. I., Yang S. L., Evans A. T., Evans F. J., Phillipson J. D., *Lancet*, **335**, 795 (1990).
10) Hirai A., Terano T., Hamazaki T., Sajiki J., Saito H., Tahara K., Tamura Y., Kumagai A., *Thromb. Res.*, **31**, 29—40 (1983).
11) Sakamoto S., Yoshino H., Shirahata Y., Shimodairo K., Okamoto R., *Am. J. Chin. Med.*, **20**, 313—317 (1992).
12) Harada M., Yamashita A., Aburada M., *Yakugaku Zasshi*, **92**, 750—756 (1972).
13) Shin T. Y., Park J. H., Kim H. M., *J. Ethnopharmacol.*, **66**, 319—325 (1999).
14) Jiang S., Tsumuro T., Takubo M., Fujii Y., Kamei C., *Biol. Pharm. Bull.*, **28**, 2197—2200 (2005).
15) Inoue T., Sugimoto Y., Masuda H., Kamei C., *Biol. Pharm. Bull.*, **25**, 256—259 (2002).
16) Takubo M., Ueda Y., Yatsuzuka R., Jiang S., Fujii Y., Kamei C., *J. Pharmacol. Sci.*, **100**, 285—288 (2006).
17) Inagaki N., Igeta K., Shiraishi N., Kim J. F., Nagao M., Nakamura N., Nagai H., *Skin. Pharmacol. Appl. Skin. Physiol.*, **16**, 165—175 (2003).
18) Inagaki N., Nagao M., Igeta K., Kawasaki H., Kim J. F., Nagai H., *Skin. Pharmacol. Appl. Skin. Physiol.*, **14**, 87—96 (2001).
19) Amir S., English A. M., *Eur. J. Pharmacol.*, **203**, 125—127 (1991).
20) Kuraishi Y., Nagasawa T., Hayashi K., Satoh M., *Eur. J. Pharmacol.*, **275**, 229—233 (1995).
21) Ramos B. F., Zhang Y., Angkachatchai V., Jakschik B. A., *J. Pharmacol. Exp. Ther.*, **262**, 559—565 (1992).
22) Chen G., Zhang L., Yang P., *Anal. Sci.*, **21**, 1161—1165 (2005).
23) Kim S. H., Kim S. A., Park M. K., Kim S. H., Park Y. D., Na H. J., Kim H. M., Shin M. K., Ahn K. S., *Int. Immunopharmacol.*, **4**, 279—287 (2004).