The Combination of Rat Mast Cell and Rabbit Aortic Smooth Muscle Is the Simple Bioassay for the Screening of Anti-allergic Ingreditent from Methanolic Extract of Corydalis Tuber

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We have assessed the release of histamine from mast cells by smooth muscle contraction. 0.3 μg/ml compound 48/80 showed no effect on concentration–response relationship of histamine in rabbit aorta. Compound 48/80 induced release of histamine from rat mast cells. When aorta was stimulated by compound 48/80 in the presence of mast cells, contraction was evoked in concentration-dependent manner. This mast cell-dependent contraction was completely blocked by H_1 receptor antagonist, 1 μM diphenhydramine. When mast cells was treated with compound 48/80 inhibitor benzalkonium chloride, mast cell-dependent contraction was inhibited, although benzalkonium chloride itself showed no effect on concentration–response relationship of histamine in rabbit aorta. At high concentration of 10 μg/ml, benzalkonium chloride itself evoked histamine release from mast cells and indeed inhibitory effect of 10 μg/ml benzalkonium chloride on mast cell-dependent contraction was lower than that of 3 μg/ml. We have applied this bioassay to search anti-allergic ingredient from a total methanolic extract of Corydalis tuber (*Corydalis turtschaninovii* BESSER forma *yanhusuo* Y. H. CHOU *et* C. C. HSU). Successively, we have isolated five fractions. The fractions I—IV are identified to be corybulbine (1), tetrahydropalmatine (2), corydaline (3) and yuanhunine (4), respectively. Main component of fraction V is the mixture of 3 and canadine (5). Fractions II and V significantly inhibited mast cell-dependent contraction in rabbit aorta as well as inhibited histamine release from rat mast cells. Furthermore, fractions I, III and V inhibited histamine-induced contraction in rabbit aorta at non-competitive manner. From these results, combination of rat mast cells and rabbit aorta is good bioassay to search the anti-allergic ingredient, and we have obtained effective fractions from Corydalis tuber using this assay.

Key words Corydalis tuber; rat mast cell; rabbit aorta; anti-allergic ingredient

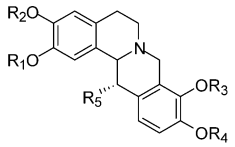
Allergic inflammation is orchestrated by antigen-specific $CD4^+$ T cells, eosinophils and mast cells, and is a characteristic feature of bronchial asthma, rhinitis and atopic dermatitis.[1,2] Allergy is an immunological reaction to a foreign antigen (allergen) that causes tissue inflammation and organ dysfunction. Inflammation is often accompanied by tissue injury and the pathogenesis of many chronic disease states, including those of an autoimmune nature.[3] Regardless of etiology or localization, inflammation involves changes in vascular permeability, with concomitant recruitment of components of the immune system.[4] Edema, redness, pain and heat are the four cardinal symptoms of inflammation. The early-phase mediators of inflammation are histamine and serotonin, and the late-phase mediators are prostaglandins, lymphokines and monokines.

Therefore, to search the anti-allergic compound, inhibitory effect on histamine release from mast cells or inhibitory effect on histamine-induced contraction of smooth muscle is good indice for the screening. In this report, we have performed the combined assay. In this assay we can stimulate mast cells but obtained the response by the contraction of smooth muscle which mimicking the part of inflammation. With the combination we can screen more wide scope not only the effect on component but also on interaction between components with simple bioassay.

Corydalis tuber has been used not only as analgesic but also used for the treatment of inflammatory, hemorheological and allergic diseases in the traditional Chinese system of medicine. Matsuda *et al.* reported that methanolic extract from Corydalis tuber shows anti-allergic effect.[5] Therefore, Corydalis tuber is atractive source to screen anti-allergic ingredient.

MATERIAL AND METHODS

Preparation of Rat Peritoneal Mast Cells The rats were sacrificed and peritoneal cells were harvested according to the procedure described by Ohuchi *et al.*[6] Briefly, 10 ml of ice-cold PBS (137 mM NaCl, 2.68 mM KCl, 0.91 $CaCl_2$, 8.1 mM Na_2PO_4, 1.47 mM KH_2PO_4, 0.91 mM $MgCl_2$, 5.6 mM glucose and 20.0 mM HEPES) was injected i.p. into male Wistar rats (200—250 g). 90—120 s later, PBS was collected and peritoneal was washed by 5 ml and subsequently 10 ml of PBS. PBS for washing were also collected and combined with first PBS, and centrifuged (200×***g***, 5 min at 4 °C). Pellet was washed twice and finally resuspended into 10 ml PBS. Mast cells represented approximately more than 10% of the cellular population (predominately lymphocytes) isolated from the rat peritoneal lavage.



	R_1	R_2	R_3	R_4	R_5
1	CH_3	H	CH_3	CH_3	CH_3
2	CH_3	CH_3	CH_3	CH_3	H
3	CH_3	CH_3	CH_3	CH_3	CH_3
4	CH_3	CH_3	CH_3	H	CH_3
5	-OCH_2O-		CH_3	CH_3	H

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Measurement of Histamine-Release 1.8 ml of mast cell suspensions are preincubated for 5 min at 37 °C before applying 0.1 ml of test solution. After incubating 10 min, mast cells were stimulated by 0.1 ml of compound 48/80 for 10 min. Histamine-release were terminated by cooling cells in ice. Cells and solution are separated by centrifuge (100× ***g***, 10 min at 4 °C), equal volume of 0.8 N $HClO_4$ and twice volume of 0.4 N $HClO_4$ was added to supernatant and pellet, respectively. To 1 ml of sample, a mixture of 125 μl of NaOH (5 N), 0.4 g of NaCl and 2.5 ml of *n*-butanol was added. Then, the samples were centrifuged at 200×***g*** for 1 min at room temperature. The upper organic phase was transferred to tubes containing 2 ml of NaOH (0.1 N) saturated with NaCl, and the sample was centrifuged to remove contaminated materials from the organic phase; the procedure was then repeated. Next, the upper organic phase was transferred to tubes containing 2 ml HCl (0.1 N) and 7.6 ml *n*-heptane. To the 1 ml of the lower aqueous phase, 0.1 ml of NaOH (10 N) was added. The histamine-*o*-phthalaldehyde (OPT) reaction[7] was carried out by incubation with 0.1 ml of OPT (10 mg/ml methanol) for 4 min at room temperature, and terminated by addition of 0.6 ml HCl (3 N). The fluorescence of the conjugate was assessed at 450 nm emission excitated at 360 nm.

Tissue Preparation The thoracic aorta was prepared from male Japanese White rabbits (3—3.5 kg). The aorta was cut into helical strips, approximately 4 mm wide and 20 mm long. The endothelium was removed by gently rubbing the endothelial surface with cotton pellets. The strips were mounted and suspended in a 1 ml organ bath containing the modified Krebs–Ringer-bicarbonate solution contained (mM), NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 25.2, KH_2PO_4 1.2 and glucose 5.8.

Force Measurement Muscle tension was recorded isometrically with a force-displacement transducer connected to a polygraph (Nihon Koden, Tokyo, Japan). A passive tension of 1 g was initially applied and tissues were allowed to equilibrate for 60 min. After equilibration, the strips were precontracted with high K^+ solution made by replacing 60 mM NaCl in the modified Krebs–Ringer-bicarbonate solution by equimolar KCl. After the response reached a steady level the experiment was started. Contractile response to histamine were normalized with that of high K^+. The data were fitted to a logistic equation:

$$R = E_{\max}\frac{A^n}{A^n + K^n}$$

where R is a response, A is a concentration of histamine, $E_{\max}$ is a maximum response, K is a observed EC_{50} (the concentration which cause repines of half maximum) of histamine and n is a Hill's coefficient.

Materials The Corydalis tuber used in this study originated from *Corydalis turtschaninovii* Besser forma *yanhusuo* Y. H. Chou *et* C. C. Hsu in China. A commercial Corydalis tuber was obtained from Tochimoto (Osaka, Japan). Compound 48/80, benzalkonium chloride, histamine and diphenhydramine were purchased from Sigma-Aldrich (Tokyo, Japan).

Isolation and Identification Dried plant material (3.0 kg) was extracted with MeOH (3 l×3) at the room temperature to yield a methanolic extract (39 g) and the extract was suspended in H_2O and then partitioned with EtOAc. The EtOAc-soluble fraction (12 g) of the methanol extract was subjected to silica gel column chromatography (C_6H_{14}–EtOAc, 7 : 3→ 0 : 1) to give subfractions 1—8. These subfractions were tested by the mast cell based bioassay and found the subfractions 1, 2, 4 and 5 to have positive activities. These active subfractions were continually separated by a series of bioassay-directed chromatographic separations, employing preparative TLC (Silica gel) and reversed-phase semipreparative HPLC on YMC-AM 324 column (ODS, 30×1 cm i.d. stainless column, MeOH–H_2O, 1 ml/min) to obtain the corybulmine (**1**, 21.2 mg), tetrahydropalmatine (**2**, 300.0 mg), corydaline (**3**, 23.0 mg) and yuanhunine (**4**, 22.1 mg) and a mixture (40.2 mg). Portions (10.0 mg) of the mixture was further separated by a semipreparative HPLC (YMC-AM 324, ODS, 30×1 cm i.d. stainless column, 45% MeOH in H_2O, 1 ml/min) to afford corydaline **3** (4.6 mg) and canadine (**5**, 3.8 mg), respectively.

Corybulmine (**1**): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.93 (3H, d, J=6.9 Hz, H-15), 2.54 (1H, td, J=10.3, 2.7 Hz, H-5), 2.56 (1H, dd, J=10.3, 3.4 Hz, H-6), 3.02 (1H, m, H-5), 3.13 (1H, ddd, J=10.3, 3.4, 2.7 Hz, H-6), 3.18 (1H, qd, J=6.8, 3.4 Hz, H-13), 3.47 (1H, d, J=15.8 Hz, H-8), 3.66 (1H, br s, H-14), 3.84 (6H, s, OCH_3-9 and OCH_3-10), 3.86 (3H, s, OCH_3-2), 4.12 (1H, d, J=15.8 Hz, H-8), 6.64 (1H, s, H-4), 6.65 (1H, s, H-1), 6.80 (1H, d, J=8.3 Hz, H-11), 6.88 (1H, d, J=8.3 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 18.4 (q, C-15), 29.2 (t, C-5), 38.5 (d, C-13), 51.4 (t, C-6), 54.5 (t, C-8), 55.9 (q, OCH_3-10), 56.2 (q, OCH_3-9), 60.2 (q, OCH_3-2), 63.2 (d, C-14), 108.0 (d, C-1), 111.0 (d, C-11), 114.1 (d, C-4), 124.0 (d, C-12), 128.1 (s, C-4a), 128.6 (s, C-14a), 129.3 (s, C-8a), 135.0 (s, C-12a), 143.7 (s, C-2), 144.9 (s, C-3), 145.5 (s, C-9), 150.1 (s, C-10). EI-MS *m*/*z*: 355 (64, M^+), 340 (17), 324 (9), 191 (1), 178 (100), 163 (23).

Tetrahydropalmatine (**2**): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.59 (1H, td, J=11.0, 2.8 Hz, H-6), 2.63 (1H, d, J=17.8 Hz, H-5), 2.80 (1H, dd, J=15.8, 11.7 Hz, H-13), 3.11 (1H, m, H-5), 3.17 (1H, m, H-6), 3.24 (1H, dd, J=15.8, 3.4 Hz, H-13), 3.51 (2H, d, J=15.1 Hz, H-8 and H-14), 3.81 (3H, s, OCH_3-9), 3.82 (3H, s, OCH_3-2), 3.83 (3H, s, OCH_3-10), 3.85 (3H, s, OCH_3-3), 4.21 (1H, d, J=15.1 Hz, H-8), 6.59 (1H, s, H-4), 6.71 (1H, s, H-1), 6.75 (1H, d, J=8.2 Hz, H-11), 6.84 (1H, d, J=8.2 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 28.9 (t, C-5), 36.1 (d, C-13), 51.3 (t, C-6), 53.8 (t, C-8), 55.66 (q, OCH_3-2), 55.68 (q, OCH_3-10), 55.9 (q, OCH_3-3), 59.1 (q, C-14), 60.0 (q, OCH_3-9), 108.5 (d, C-1), 110.8 (d, C-11), 111.2 (d, C-4), 123.7 (d, C-12), 126.6 (s, C-4a), 127.6 (s, C-8a), 128.5 (s, C-12a), 129.6 (s, C-14a), 144.9 (s, C-9), 147.26 (s, C-2), 147.32 (s, C-3), 150.1 (s, C-10). EI-MS *m*/*z*: 355 (100, M^+), 340 (11), 324 (20), 192 (9), 190 (23), 164 (64), 149 (43).

Corydaline (**3**): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.92 (3H, d, J=6.9 Hz, H-15), 2.55 (1H, m, H-5), 2.57 (1H, m, H-6), 3.06 (1H, m, H-5), 3.15 (1H, ddd, J=10.3, 3.4, 2.7 Hz, H-6), 3.21 (1H, qd, J=6.8, 3.4 Hz, H-13), 3.50 (1H, d, J=15.8 Hz, H-8), 3.67 (1H, br s, H-14), 3.82 (6H, s, OCH_3-9 and OCH_3-10), 3.83 (3H, s, OCH_3-2), 3.84 (3H, s, OCH_3-3), 4.20 (1H, d, J=15.8 Hz, H-8), 6.59 (1H, s, H-4), 6.67 (1H, s, H-1), 6.80 (1H, d, J=8.3 Hz, H-11), 6.88 (1H, d, J=8.3 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 18.9 (q, C-15), 30.0 (t, C-5), 38.9 (d, C-13), 52.1 (t, C-6), 55.1 (t, C-8), 56.5 (q, OCH_3-10), 56.6 (q, OCH_3-9), 56.8 (q, OCH_3-3),

60.8 (q, OCH_3-2), 63.7 (d, C-14), 109.4 (d, C-1), 111.6 (d, C-11), 111.9 (d, C-4), 124.7 (d, C-12), 129.1 (s, C-4a), 129.2 (s, C-14a, C-8a), 135.6 (s, C-12a), 145.6 (s, C-9), 147.9 (s, C-2), 148.3 (s, C-3), 150.7 (s, C-10). EI-MS *m/z*: 369 (67, M^+), 354 (23), 338 (12), 192 (8), 190 (7), 178 (100), 163 (25).

Yuanhunine (**4**): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.89 (3H, d, J=6.8 Hz, H-15), 2.56 (1H, m, H-5), 2.58 (1H, m, H-6), 3.06 (1H, m, H-5), 3.14 (1H, m, H-6), 3.18 (1H, qd, J=6.8, 3.4 Hz, H-13), 3.50 (1H, d, J=15.8 Hz, H-8), 3.67 (1H, br s, H-14), 3.80 (3H, s, OCH_3), 3.85 (6H, s, 2×OCH_3), 4.14 (1H, d, J=15.8 Hz, H-8), 6.58 (1H, s, H-4), 6.65 (1H, s, H-1), 6.81 (1H, d, J=8.3 Hz, H-11), 6.83 (1H, d, J=8.3 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 18.4 (q, C-15), 29.4 (t, C-5), 38.4 (d, C-13), 51.5 (t, C-6), 54.5 (t, C-8), 55.9 (q, OCH_3-2), 56.2 (q, OCH_3-3), 60.5 (q, OCH_3-9), 63.3 (d, C-14), 108.8 (d, C-1), 111.2 (d, C-11), 114.3 (d, C-4), 125.1 (d, C-12), 127.8 (s, C-4a), 128.3 (s, C-8a), 128.5 (s, C-14a), 134.3 (s, C-12a), 143.0 (s, C-9), 146.6 (s, C-10), 147.3 (s, C-2), 147.8 (s, C-3). EI-MS *m/z*: 355 (71, M^+), 354 (26), 340 (18), 324 (11), 192 (100), 190 (11), 164 (31), 149 (26).

Canadine (**5**): Yellow powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.60 (1H, m, H-5), 2.64 (1H, J=16.4 Hz, H-6), 2.80 (1H, dd, J=15.8, 11.0 Hz, H-13), 3.10 (1H, m, H-5), 3.16 (1H, m, H-13), 3.20 (1H, dd, J=15.8, 4.1 Hz, H-6), 3.52 (2H, d, J=15.1 Hz, H-8, H-14), 3.83 (6H, s, 2×OCH_3), 4.22 (1H, d, J=15.1 Hz, H-8), 5.89 (2H, s, O–CH_2–O), 6.57 (1H, s, H-4), 6.71 (1H, s, H-1), 6.77 (1H, d, J=8.3 Hz, H-11), 6.84 (1H, d, J=8.3 Hz, H-12). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 29.4 (t, C-5), 36.3 (t, C-13), 51.3 (t, C-6), 53.8 (t, C-8), 55.8 (q, OCH_3-10), 59.5 (d, C-14), 59.9 (q, OCH_3-9), 105.4 (d, C-1), 108.3 (d, C-4), 110.9 (d, C-11), 123.8 (d, C-12), 127.6 (s, C-12a), 127.7 (s, C-4a), 128.5 (s, C-8a), 130.6 (s, C-14a), 145.0 (s, C-9), 145.8 (s, C-3), 146.1 (s, C-2), 150.2 (s, C-10). EI-MS *m/z*: 339 (100, M^+), 338 (56), 308 (17), 256 (15), 213 (5), 174 (17), 164 (74), 149 (49).

Statistics Results of the experiments are expressed as means±S.E.M. Significance was tested with Student's *t*-test, or Dunnett's multiple comparison test when comparisons involved more than two groups. p values smaller than 0.05 were considered significant.

RESULTS

Mast Cell-Depend Contraction One milliliter of mast cells suspended in PBS were introduced to organ bath where aorta was suspended. Compound 48/80 evoked contraction in concentration dependent manner and 1 μM diphenhydramine, H_1 receptor antagonist, completely inhibited this contration (Fig. 1).

In the presence of mast cells, benzalkonium chloride (BAC), a typical inhibitor of histamine-release,[8] inhibited the contraction evoked by 0.3 μg/ml compound 48/80 in rabbit aorta, in concentration-dependent manner (Fig. 2A). Since Read and Kiefer reported that higher concentration of BAC itself releases histamine,[8] we measured the effect of BAC on histamine-release from mast cell. As a result, only at highest concentration of BAC (10 μg/ml) showed fascilitating effect (Fig. 2B).

As shown in Fig. 3, we have assessed the effect of compound 48/80 and BAC with the concentrations which used in this bioassay, on concentration–response relationship of histamine in rabbit aorta. Neither 0.3 μg/ml compound 48/80, 3 μg/ml nor 10 μg/ml BAC had affected on concentration–contraction relationship of histamine.

Fractionation The methanolic extract from Corydalis tuber was suspended in H_2O, and partitioned with EtOAc. We assessed each fraction on mast cell-dependent contraction of rabbit aorta (Table 1).

Each fraction was subfractioned by TLC pattern and finally isolated five fractions using HPLC (Chart 1).

Pharmacological Profile 50 μg/ml of five fractions has applied to study the effect on mast cell-dependent contraction in rabbit aorta (Fig. 4A). Fractions II and V showed significant inhibition. Fractions II and V also inhibited compound 48/80-induced histamine-release from mast cells (Fig. 4B).

To evaluate direct effect on smooth muscle, five fractions has applied to concentration–contraction relationship of histamine in rabbit aorta. Fractions I, III and V significantly inhibited the histamine-induced contraction without affecting

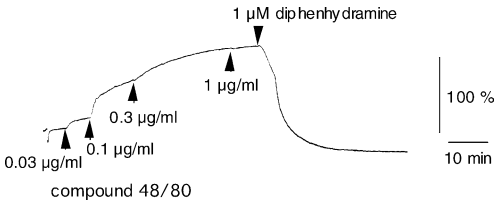

Fig. 1. Representative Trace of Compound 48/80-Induced Contraction of Rabbit Aorta in the Presence of Rat Mast Cells

Contraction was normalized with high K^+-induced contraction.

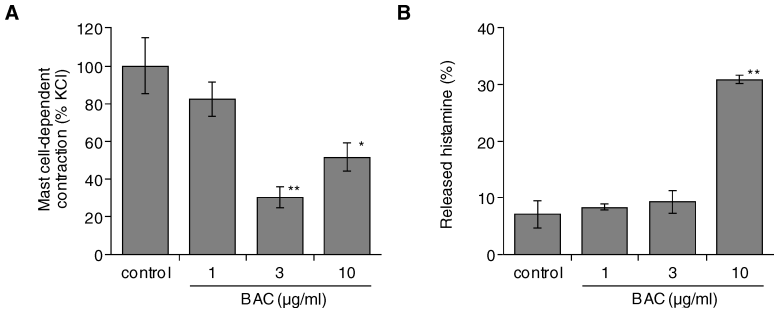

Fig. 2. Effect of Benzalkonium Chloride on Rat Mast Cell

(A) Mast cells with rabbit aorta were incubated with benzalkonium chloride or DMSO for 5 min. Subsequently 0.3 μg/ml compound 48/80 was applied. (B) Mast cells were incubated with benzalkonium chloride 10 min prior to applying 0.3 μg/ml compound 48/80. The amounts of histamine accumulated in buffer for 10 min and remained in mast cell were determined. $*p<0.05$, $**p<0.01$.

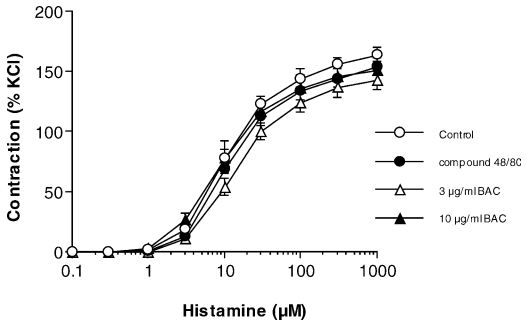

Fig. 3. Effects of Compound 48/80 and Benzalkonium Chloride on Concentration–Response Relationship of Histamine in Rabbit Aorta

Aorta was incubated with 0.3 μg/ml compound 48/80, 3 μg/ml or 10 μg/ml benzalkonium chloride for 15 min. Subsequently, histamine was applied in cumulative manner.

Table 1. Effect of Fractions of Methanol Extract of Corydalis Tuber on the Mast Cell-Dependent Contractile Response of the Rabbit Aorta

Fraction	Contraction (% KCl)	Fraction	Contraction (% KCl)
Control	73.7	Control	50
F-1	21.3	F-6	21.4
F-2	6.1	F-7	15.4
F-3	0	F-8	44
F-4	11.1		
F-5	54.3		

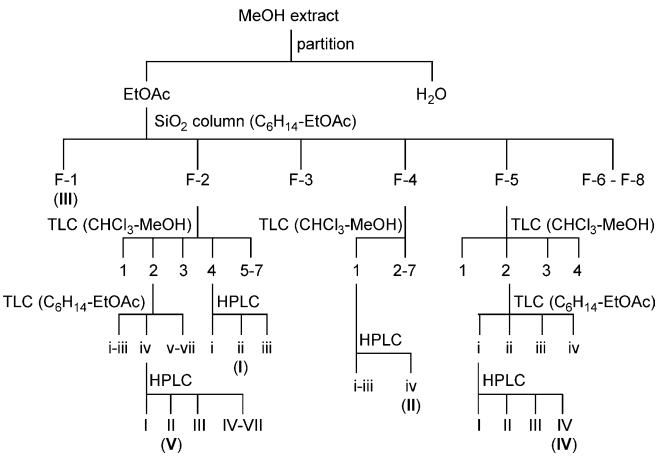

Chart 1

EC_{50} value of histamine (Table 2). Fractions II and IV did not significantly but tended to inhibit maximum contraction of histamine (Table 2).

DISCUSSION

Shore *et al.* developed the method to quantify histamine in high-sensitivity.[7] This method is well established and oftenly used. The method is composed from 2 steps; 1) purification of amine, and 2) measurement of fluoresence. Therefore, we should be careful for contamination of ingredient of test sample interfering with fluorescence of histamine-OPT condensated product when using this method. Our bioassay is the alternative one, where histamine can be assayed as the contraction of aorta.

It is generally known that compound 48/80 induces histamine release from mast cells without the participation of an immunological mechanism as caused by an antigen–antibody reaction.[9,10] In our bioassay system, stimulant of mast cell is allowed to be contacted with smooth muscle. From our result, rabbit aorta does not respond to compound 48/80. Therefore, it is safely to use compound 48/80 to stimulate mast cells in this assay.

According to this bioassay, we have successively obtained five fractions from Corydalis tuber. 50 μg/ml of fractions II and V inhibited histamine release and fractions I, III and V inhibited histamine-induced smooth muscle contraction. The main content of fractions I—V were corydaline analogues. Matsuda et al. demonstrated that dehydrocorydaline isolated from Corydalis tuber[11] inhibit some allergic models. Our results suggesting that other corydaline analogues may account for anti-allergic effect of methanolic extract of Corydalis tuber.[5] Since fraction IV showing scarce effect comparing

Table 2. Effect of Fractions I—V on Pharmacaological Parameters of Concentration–Response Relationship of Histamine in Rabbit Aorta

	E_{max} (%KCl)	EC_{50} (μM)
Control	152.8±4.2	10.5±2.0
I	108.3±5.3**	75.4±40.9
II	127.2±5.0	19.2±4.8
III	100.6±12.6**	20.9±1.3
IV	125.7±5.7	12.2±2.0
V	100.5±13.4**	12.8±4.4

Rabbit aorta was incubated with 50 μg/ml of fractions I—V for 15 min. Subsequently, histamine (0.1—1000 μM) was applied in cumulative manner. **: $p<0.01$.

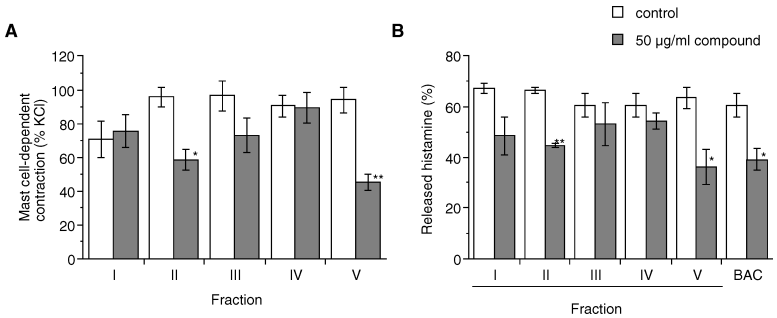

Fig. 4. Effect of Corydalis Tuber Fractions I—V on Rat Mast Cell

(A) Mast cells with rabbit aorta were incubated with 50 μg/ml of fractions for 5 min. Subsequently 0.3 μg/ml compound 48/80 was applied. (B) Mast cells were incubated with 50 μg/ml of fractions I—V or 3 μg/ml benzalkonium chloride 10 min prior to applying 0.3 μg/ml compound 48/80. The amounts of histamine accumulated in buffer for 10 min and remained in mast cell were determined. * $p<0.05$, ** $p<0.01$.

with fraction V, canadine (**5**) may account for inhibitory effect of fraction V on histamine release. Furthermore, corybulmine (**1**), tetrahydropalmatine (**2**) and corydaline (**3**) are also the candidate for developing anti-allergic drug. Further studies are required to evaluate the action and the mechanisms of the effects of these compounds on histamine release and anti-histaminergic effect.

The bioassay represented in this manuscript is sensitive to detect both inhibitory effects on activation of mast cell as well as inhibitory effect on histamine-induced smooth muscle contraction. Therefore, one must keep in mind that this is not the simple alternative method for the detection of histamine released from mast cells. This method is complemental to colorimetric method and also it rather will be *in vitro* model of passive cutaneous anaphylaxis.

From these results, the combination of mast cells and aortic smooth muscle is shown to be good bioassay to search ingredients which affect on histamine-release and/or smooth muscle contraction.

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