OPEM

Oriental Pharmacy and Experimental Medicine 2002 **2(1)**, 58-63

Effect of *Rubus coreanus* Miq. fruit extract on compound 48/80-induced anaphylactic reactions

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SUMMARY

The effect of the aqueous extract of *Rubus coreanus* Miq. (Rosaceae) fruits (AERC) on anaphylactic reaction was investigated. AERC completely inhibited compound 48/80-induced systemic anaphylactic shock at dose of 0.1 to 1.0 g/kg. When AERC was pretreated at concentrations ranging from 0.01 to 1.0 g/kg, the plasma histamine levels induced by compound 48/80 were reduced in a dose-dependent manner. AERC also inhibited the histamine release from rat peritoneal mast cells (RPMC) by compound 48/80. The level of cAMP in RPMC, when AERC (1.0 mg/ml) was added, transiently and significantly increased about 6-fold compared with that of basal cells. These results indicate that AERC may be beneficial in the treatment of non-specific anaphylactic reactions.

Key words: *Rubus coreanus*; Anaphylaxis; Compound 48/80; Histamine, cAMP

INTRODUCTION

The dried unripe fruits of *Rubus coreanus* Miq. (Rosaceae), well known as "Bok-bun-ja" in Korea, has been used for centuries as traditional medicine. This crude drug is used for the management of impotence, spermatorrhea, enuresis, asthma, allergic diseases and it also has been used as a stomachic and tonic in Korea; (Lee, 1966; But *et al.*, 1997). The condensation product of *N*-methyl-*p*-methoxyphenyl ethylamine with formaldehyde produces a potent histamine-liberating agent called compound 48/80 (Paton, 1951). This compound is employed as a classic mast cell secretagogue that releases histamine (Read and Lenney, 1972). Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells (Allansmith *et al.*, 1989). Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mast cell-mediated non-specific anaphylactic reaction (Shin *et al.*, 2001). In the present study, we examined the effect of AERC on compound 48/80-induced anaphylactic reaction *in vivo* and histamine release from RPMC *in vitro*. To find the reason for the mechanism of the action, we also investigated the effect of AERC on compound 48/80-induced intracellular cAMP level in RPMC.

MATERIALS AND METHODS

Reagents

Compound 48/80, o-phthaldialdehyde (OPA) and metrizamide were purchased from Sigma Chemical Co. (St. Louis, MO), cAMP was purchased from Amersham Pharmacia Biotech (UK).

Animals

The original stocks of male SD rats (200-250 g) and ICR mice (20-25 g) were purchased from Dae Han Experimental Animal Center (Taejeon, South Korea), and the animals were maintained at the College of Pharmacy, Woosuk University. The animals were housed 5-10 per cage in a laminar air flow room

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maintained under a temperature of 22±2°C and relative humidity of 55±5% throughout the study.

Preparation of AERC

Rubus coreanus Miq. fruits were obtained from the Oriental drug store, Bohwa Dang of Chonju, South Korea, and identified by T.K. Kim, College of Pharmacy, Woosuk University. A voucher specimen (number WSP-99-37) was deposited at the Herbarium at the College of Pharmacy, Woosuk University. The *Rubus coreanus* Miq. was extracted with distilled water at 70°C for 5 hr (two times). The extract was filtered, lyophilized, and kept at -4°C. The yield of dried extract from starting crude materials was about 8.8%. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic anaphylactic reaction

Compound 48/80-induced systemic anaphylactic reaction was examined as previously described (Shin *et al.*, 1999). Mice were given an intraperitoneal injection of 0.008 g/kg of the mast cell degranulator compound 48/80. AERC was dissolved in saline and administered by intraperitoneal injection 0.005 to 1.0 g/kg at 1 hr before the injection of compound 48/80 (n=10/group). In the time dependent experimental model, AERC was injected intraperitoneally at 5 min and 10 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 hr after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mice.

Preparation of plasma and determination of histamine

The blood was centrifuged at 400×g for 10 min. The plasma was withdrawn and the histamine content was measured by the OPA spectrofluorometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301, Japan).

Preparation of rat peritoneal mast cells (RPMC)

RPMC were isolated as previously described (Kanemoto *et al.*, 1993). In brief, rats were anesthetized by ether, and injected with 20 ml of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin), into the peritoneal cavity, and the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150×g for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1.0 ml Tyrode buffer B were layered on 2.0 ml of 22.5 w/v% metrizamide (density, 1.120 g/ml) and centrifuged at room temperature for 15 min at 400×g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1.0 ml tyrode buffer A (10 mM/l HEPES, 130 mM/l NaCl, 5 mM/l KCl, 1.4 mM/l $CaCl_2$, 1 mM/l $MgCl_2$, 5.6 mM/l glucose) containing calcium. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

Inhibition of histamine release

Purified RPMC were resuspended in Tyrode buffer A for the treatment of compound 48/80. RPMC (2×10^5 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80. The cells were preincubated with AERC for 10 min at 37°C and washed with Tyrode buffer A, and then incubated (10 min) with compound 48/80. The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

Measurement of cAMP level

The cAMP level was measured according to the method of Peachell *et al.* (1988). In brief, purified mast cells were resuspended in prewarmed (37°C)

Tyrode buffer A. Typically, an aliquot of cells (2×10^5 cells) were added to an equivalent volume (50 μl) of prewarmed buffer containing the drug in an eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by the addition of ice-cold acidified ethanol (0.9 ml of 86% ethanol/1 M HCl, 99:1) with brief vigorous vortexing and then snap frozen in liquid nitrogen. The sample was later thawed and vortexed, then the debris was sedimented in a centrifuge (400×g at 4, for 5 min), and an aliquot (0.9 ml) of the supernatant was removed and evaporated to dryness under reduced pressure. The dried sample was reconstituted in assay buffer (150-200 μl) and stored frozen. The cAMP level was determined by enzyme immunoassay (EIA), using a commercial kit (Amersham Pharmacia Biotech).

Statistical analysis

The results obtained were expressed as mean± SEM. The Student's *t*-test was used to make a statistical comparison between the groups. Results with $P<0.05$ were considered statistically significant.

RESULTS

Effect of AERC on compound 48/80-induced systemic anaphylactic reaction

Initial experiments examined the effects of AERC on compound 48/80-induced systemic anaphylactic reaction in mice. As shown in Table 1, when AERC was pretreated at concentration ranging from 0.01 to 1.0 g/kg for 1 hr, the mortality with compound 48/80 was reduced. Especially, AERC inhibited compound 48/80-induced anaphylactic reaction 100% with dose of 0.1 to 1.0 g/kg. Treatment with AERC (1.0 g/kg) detected no physiological differences by outward appearance.

Effect of AERC on plasma histamine release

To confirm that the effect of AERC on systemic anaphylactic reaction was due to an inhibited plasma histamine release, we analyzed the histamine content after injection of mice with compound 48/80. AERC was given from 0.01 to 1.0 g/kg 1 hr before compound 48/80 injection. The plasma from the mice was uniformly gathered with a heart puncher 15 min after compound 48/80 injection in all groups. The correlative results with those of the mortality test were shown when their plasma histamine contents were measured (Table 2). The inhibition rate by treatment with AERC was significant at doses of 0.1 to 1.0 g/kg.

Time-dependent effect of AERC on compound 48/80-induced systemic anaphylactic reaction

When AERC (1.0 g/kg) was administered 5 or 10 min after compound 48/80 injection, mortality was increased in a time-dependent manner (Table 3). We also analyzed the plasma histamine content. The correlative results with those of the mortality test were shown when their plasma histamine contents were measured (Table 4).

Effect of AERC on histamine release from RPMC

The inhibitory effects of AERC on compound 48/80-induced histamine release from RPMC are shown in Table 5. AERC dose-dependently inhibited

Table 1. Effect of AERC on compound 48/80-induced systemic anaphylactic reaction

AERC treatment (g/kg, body weight)	Compound 48/80 (0.008 g/kg)	Mortality (%)
None(saline)	+	100
0.01	+	90±10
0.05	+	10±10
0.1	+	0
0.5	+	0
1.0	+	0
1.0	-	0

Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl saline or AERC. AERC was given at various doses 1 hr before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 hr following compound 48/80 injection was represented as number of dead mice×100/total number of experimental mice. Each datum represents the mean±SEM of three independent experiments.

Table 2. Effect of AERC on compound 48/80-induced plasma histamine release.

AERC treatment (g/kg, body weight)	Compound 48/80(0.008 g/kg)	Histamine release(μg/ml)
None(saline)	+	0.20±0.01
0.01	+	0.19±0.11
0.05	+	0.17±0.03
0.1	+	0.15±0.07*
0.5	+	0.08±0.03*
1.0	+	0.07±0.02*

Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl saline or AERC. AERC was given with various doses 1 hr before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of mice. Each datum represents the mean±SEM of three independent experiments. **P*<0.05: significantly different from the saline value.

Table 3. Time-dependent effect of AERC on compound 48/80-induced systemic anaphylactic reaction.

AERC treatment (g/kg, body weight)	Compound 48/80 (0.008 g/kg)	Mortality (%)	
		5 min after	10 min after
None(saline)	+	100	100
1.0	+	20±10	40±20
1.0	-	0	0

Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl saline or AERC. AERC was given at 5 min or 10 min after the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 hr following compound 48/80 injection was represented as number of dead mice×100/total number of experimental mice. Each datum represents the mean±SEM of three independent experiments.

Table 4. Time-dependent effect of AERC on compound 48/80-induced plasma histamine release.

AERC treatment (g/kg, body weight)	Compound 48/80 (0.008 g/kg)	Histamine release (μg/ml)	
		5 min after	10 min after
None(saline)	+	0.24±0.05	0.27±0.03
1.0	+	0.08±0.03*	0.15±0.02*

Groups of mice (n=10/group) were intraperitoneally pretreated with 200 μl saline or AERC. AERC was given at 5 min or 10 min after the compound 48/80 injection. The compound 48/80 solution was intrateritoneally given to the groups of mice. Each datum represents the mean±SEM of three independent experiments. **P*<0.05: significantly different from the saline value.

the histamine release from RPMC at concentrations of 0.001-1.0 mg/ml. AERC significantly inhibited compound 48/80-induced histamine release at concentrations of 0.1-1.0 mg/ml.

Effect of AERC on cAMP level of RPMC

Finally, we examined the cAMP content to clarify the mechanism by which AERC inhibits histamine release from RPMC. Data in Table 6. show the effect of AERC on the cAMP level of RPMC. The intracellular cAMP content of the mast cells, when incubated with AERC (1.0 mg/ml) increased about 6-fold in comparison with that of basal cells. The increase in cAMP content by AERC was little affected by the addition of compound 48/80.

DISCUSSION

The present study showed that AERC pretreatment profoundly affected compound 48/80-induced systemic anaphylactic reaction. AERC inhibited the compound 48/80-induced histamine release from RPMC. The results demonstrate that AERC inhibits

Table 5. Effect of AERC on compound 48/80-induced histamine release from RPMC

AERC treatment (mg/ml)	Compound 48/80(5 μg/ml)	Histamine release(μg/ml)
None(saline)	+	0.210.01
0.001	+	0.180.02
0.01	+	0.160.03
0.1	+	0.100.09*
1.0	+	0.060.01*

RPMC (2×10^5 cells/ml) were preincubated with AERC at 37°C for 10 min prior to incubation with compound 48/80 for 10 min. Each datum represents the mean±SEM of three independent experiments. *$p<0.05$: significantly different from the saline value.

Table 6. Effect of AERC on compound 48/80-induced cAMP content in RPMC

AERC treatment (mg/ml)	Compound 48/80(5 μg/ml)	cAMP(p mol)
None(saline)	-	0.39±0.03
None(saline)	+	0.21±0.03
1.0	-	2.38±0.19*
1.0	+	1.86±0.16*

RPMC (2×10^5 cells/ml) were pretreated with AERC at 37°C. Each datum represents the mean±SEM of three independent experiments. *$P<0.05$, significantly different from the saline value.

the mast cell-dependent anaphylactic reaction in murine model, and suggest that the activity may be due to the inhibition of histamine release from the mast cells. Recent studies have shown that compound 48/80 is able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a,b). The evidence indicates that the protein is Gi-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990). Tasaka *et al.* (1986) reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the membrane permeability increase may be an essential trigger for release of the mediator from mast cells. The intracellular cAMP content of the mast cells, when incubated with AERC increased about 6-fold in comparison with that of basal cells. This result indicates that the inhibitory effect of AERC on degranulation of mast cells may be mediated through an increase in cAMP level. In conclusion, the results which we obtained suggest that AERC inhibited the compound 48/80-induced anaphylactic reaction *in vivo* and *in vitro* in a murine model. Therefore, further work should address the possibility that AERC may also be active in the inhibition of human mast cell degranulation and, therefore, in the treatment of human allergic disorders.

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