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Magnoliae Cortex inhibits immediate-type allergic reactions

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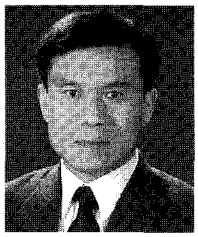
SUMMARY

The effect of aqueous extract of Magnoliae Cortex (Magnoliaceae) (MCAE) on the immediate-type allergic reaction was investigated. MCAE inhibited compound 48/80 induced systemic anaphylactic reaction in rats. MCAE (0.1 and 1 g/kg) also significantly inhibited local immunoglobulin E (IgE)-mediated passive cutaneous anaphylactic (PCA) reaction. MCAE (0.001 to 1 mg/ml) dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-dinitrophenyl (DNP) IgE. Moreover, MCAE (0.01 to 1 mg/ml) had a significant inhibitory effect on anti-DNP IgE-mediated tumor necrosis factor-α (TNF-α) production. These results indicate that MCAE inhibits immediate-type allergic reaction *in vivo* and *in vitro*.

Key words: Magnoliae Cortex; Immediate-type allergic reaction; Compound 48/80; Anti-DNP IgE; Histamine; Tumor necrosis factor-α

Magnoliae Cortex (Korean name; Hu-bak) is the dried bark of stem, root or branch of *Magnolia officinalis* Rehd. et Wils. or *Magnolia officinalis* Rehd. et Wils. var. *biloba* Rehd. et Wils. (Magnoliacae)(Chang and But, 1987). This crude drug has the pharmacological functions of antiallergic effect (type IV allergic reaction), myocardial protective effect, anti-inflammatory effect, antioxidative effect, central depressant effect (Watanabe *et al.*, 1983; Zhou and Xu, 1992; Wang *et al.*, 1995; Tsai *et al.*, 1996; Taniguchi *et al.*, 2000). The primary effector cell in immediate-type allergic reactions is the mast cells. Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells (Lagunoff *et al.*, 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of compound 48/80 induces almost a 90% release of histamine from mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Allansmith *et al.*, 1989). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). It has been established that the anti-IgE antibody induces passive cutaneous anaphylactic (PCA) reaction as a typical model for the immediate hypersensitivity (Saito and Nomura, 1989). Although mast cells store small amounts of cytokines in their granules (Gordon and Galli, 1990), these cells dramatically increase their production of tumor necrosis factor-α (TNF-α), IL-6, and other cytokines within 30 min after their surface FœRI are cross-linked with



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specific antigen (Plut *et al.*, 1989; Burd *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989; Galli *et al.*, 1991; Gurish *et al.*, 1991). Therefore, modulation of TNF-α production by mast cells should provide us with a useful therapeutic strategy for allergic disease.

In this study, we showed that MCAE inhibited compound 48/80-induced systemic anaphylactic reaction, anti-dinitrophenyl (DNP) IgE antibody-induced PCA, and histamine and TNF-α production from rat peritoneal mast cells (RPMC).

MATERIALS AND METHODS

Reagents and Animals

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). Murine TNF-α was obtained from R & D Systems Inc. (USA). The original stock of male Sprague-Dawley rats were purchased from Dae-Han Experimental Animal Center (Taejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five per cage in a laminar air flow room maintained under a temperature of 22±2°C and relative humidity of 55±5% throughout the study.

Preparation of MCAE

Magnoliae Cortex was purchased from the oriental drug store, Bohwa Dang (Jeonju, Korea). A voucher specimen (number WSP-96-10) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with distilled water at 70°C for 5 h (two times). The extract was filtered through a 0.45 μm filter, and the filtrate was lyophilized, and kept at −4°C. The yield of dried extract from starting crude materials was about 13.2%. 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 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). Rats were given an intraperitoneal injection of 0.008 g/kg body weigh (BW) of the mast cell degranulator, compound 48/80. MCAE was dissolved in saline and administered intraperitoneally, anally and orally ranging from 0.005 to 1 g/kg BW 1 h before the injection of compound 48/80 (n=10/group). In time dependent experiment, MCAE (1 g/kg BW) was administered intraperitoneally, anally and orally at 5 and 10 min after compound 48/80 injection (n=10/group). Mortality was monitored for 1 h after induction of anaphylactic shock.

Induction of PCA

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the rat's tail vein. The anti-DNP IgE and DNP-HSA were diluted in PBS. The rats were injected intradermally with 0.5 μg of anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each rat, 48 h later, received an injection of 1 mg of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. MCAE (0.001 to 1 g/kg BW) was orally administered 1 h before the challenge. Then 30 min after the challenge, the rats were sacrificed and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan) and the amount of dye was calculated with Evans blue measuring-line.

Preparation of 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 seconds. The peritoneal cavity was carefully opened and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. After that, 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 ml of Tyrode buffer B were layered on 2 ml of metrizamide (22.5 w/v%) 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 ml Tyrode buffer A. 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.

Compound 48/80-induced or anti-DNP IgE-mediated histamine release

Purified RPMC were resuspended in Tyrode buffer A for the treatment of compound 48/80. RPMC suspensions (2×10^5 cells/ml) were preincubated for 10 min at 37°C before the addition of compound 48/80 (5 μg/ml). The cells were preincubated with the MCAE (0.001 to 1 mg/ml), and then incubated (10 min) with the compound 48/80. RPMC suspensions (2×10^5 cells/ml) were also sensitized with anti-DNP IgE (10 μg/ml) for 6 h. The cells were preincubated with the MCAE at 37°C for 10 min prior to the challenge with DNP-HAS (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

Assay of histamine release

The amount of histamine was measured by the o-phthaldialdehyde 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 PC, Japan).

Assay of TNF-α production

TNF-α production was measured with the quantitative sandwich enzyme immunoassay technique, using a commercial kit (R&D Systems, U.S.A.). RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of MCAE (0.001 to 1 μg/ml) before the challenge DNP-HAS (0.1 μg/ml). TNF-α production was measured by ELISA. The ELISA was performed by coating 4-well plates with murine polyclonal antibody with specificity for murine TNF-α Standard, controls, and samples are pipetted into the wells and any mouse TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 μl) is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop solution (100 μl) is added. The intensity of the color measured is in proportion to the amount of mouse TNF-α bound in the initial step. Optical density readings were made on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter. The sample values are then read off the standard curves.

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

MCAE inhibits compound 48/80-induced systemic anaphylactic reaction

Initially, to determine the effect of MCAE in systemic anaphylactic reaction, we used compound 48/80 (0.008 g/kg BW) as a systemic fatal anaphylaxis inducer. After the intraperitoneal injection of compound 48/80, the rats were monitored for 1 h, after which the mortality rate was determined. As shown in Table 1, an administration of 200 μl saline as a control induced a fatal shock in 100% of each group. When the MCAE was intraperitoneally, anally and orally administered at concentrations ranging from 0.005 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. In addition, the mortality of rats administered intraperitoneally, anally and orally with MCAE (1 g/kg) 5 min and 10 min after compound 48/80 injection increased time-dependently (n=10/group) (Table 2).

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

MCAE treatment (g/Kg BW)	Compoud 48/80 (0.008 g/Kg BW)	Mortality(%)		
		Intraperitoneally	Anally	Orally
None (saline)	+	100	100	100
0.005	+	100	100	100
0.01	+	90	100	100
0.05	+	60	100	100
0.1	+	10	70	80
0.5	+	0	0	70
1	+	0	0	20
1	–	0	0	0

Groups of rats (n=10/group) were intraperitoneally, anally and orally pretreated with 200 μl saline or MCAE. MCAE was given at various doses 1 h before the compound 48/80 injection. The compound 48/80 solution was intraperitoneally given to the group of rats. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead rats×100/total number of experimental rats.

Table 2. Time-dependent effect of MCAE on compound 48/80-induced systemic anaphylactic reaction

MCAE treatment (g/Kg BW)	Compound 48/80 (0.008g/Kg BW)	Mortality (%)	
		5 min	10 min
Intraperitoneally			
None(saline)	+	100	100
1	+	0	20
1	–	0	0
Anally			
None (saline)	+	100	100
1	+	20	50
1	–	0	0
Orally			
None (saline)	+	100	100
1	+	60	80
1	–	0	0

Groups of rats (n=10/group) were intraperitoneally, anally and orally pretreated with 200 μl saline or MCAE. MCAE 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 rats. Mortality (%) within 1 h following compound 48/80 injection was represented as the number of dead rats×100/total number of experimental rats.

Table 3. Effect of MCAE on the 48 h PCA

MCAE treatment (g/kg BW)	Anti-DNP IgE plus DNP-HSA	Amount of dye (μg/site)
None (saline)	+	6.81±0.71
0.001	+	5.46±0.49
0.01	+	4.73±0.42
0.1	+	3.37±0.40[a]
1	+	2.68±0.24[a]

MCAE was administered orally 1 h prior to the challenge with antigen. Each datum represents the mean±SEM of three independent experiments. [a]P<0.05; significantly different from the saline value.

MCAE inhibits PCA

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reaction (Wershil *et al.*, 1987). As described in this experimental procedure, local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge. Oral administration of MCAE (0.1 and 1 g/kg) showed a marked inhibition rate in PCA reaction (Table 3).

Table 4. Effect of MCAE on compound 48/80-induced histamine release from RPMC

MCAE treatment (mg/ml)	Compound 48/80 (5 μg /ml)	Amount of histamine (μg/ml)
None (saline)	+	0.164±0.019
0.001	+	0.156±0.014
0.01	+	0.110±0.009[a]
0.1	+	0.052±0.006[a]
1	+	0.043±0.003[a]

The cells (2×10^5 cells/ml) were preincubated with MCAE at 37°C for 10 min prior to incubation with compound 48/80. Each datum represents the mean±SEM of three independent experiments. [a]$P<0.05$; significantly different from the saline value.

Table 5. Effect of MCAE on IgE-mediated histamine release from RPMC

MCAE treatment (mg/ml)	Anti-DNP IgE plus DNP-HSA	Amount of histamine (μg/ml)
None (saline)	+	0.107±0.091
0.001	+	0.105±0.011
0.01	+	0.069±0.008
0.1	+	0.043±0.004[a]
1	+	0.032±0.004[a]

The cells (2×10^5 cells/ml) were preincubated with MCAE at 37°C for 10 min prior to challenge with DNP-HSA. Each datum represents the mean±SEM of three independent experiments. [a]$P<0.05$; significantly different from the saline value.

MCAE inhibits compound 48/80-induced or anti-DNP IgE-mediated histamine release

The inhibitory effect of MCAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC are shown in Table 4 and Table 5. MCAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001 to 1 mg/ml. Especially, MCAE significantly inhibited the compound 48/80-induced histamine release at the concentrations of 0.01 to 1 mg/ml. MCAE significantly inhibited IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/ml.

MCAE inhibits TNF-α production

Finally, we investigated the ability of MCAE to influence anti-DNP IgE-mediated TNF-α production in RPMC. MCAE significantly inhibited TNF-α production at concentrations of 0.01 to 1 mg/ml (Table 6).

DISCUSSION

The results of this study demonstrated that MCAE pretreatment profoundly inhibited compound 48/80-induced systemic anaphylactic reaction and anti-DNP IgE-induced PCA. MCAE inhibited the

Table 6. Effect of MCAE on anti-DNP IgE-mediated TNF-α production in RPMC

MCAE treatment (mg/ml)	Anti-DNP IgE plus DNP-HSA	TNF-α production (pg/ml)
None (saline)		69.5±5.8
None (saline)	+	209.1±18.7
0.001	+	187.0±18.2
0.01	+	118.2±12.3[a]
0.1	+	102.6±9.8[a]
1	+	91.9±9.4[a]

RPMC (3×10^5 cells/ml) were sensitized with anti-DNP IgE (1 μg/ml) and incubated for 18 h in the absence or presence of MCAE before the challenge with DNP-HAS (0.1 μg/ml). Each datum represents the mean±SEM of three independent experiments. [a]$P<0.05$; significantly different from the saline value.

compound 48/80 or anti-DNP IgE-mediated histamine release from RPMC. MCAE also inhibited the compound 48/80-mediated histamice release in plasma (data not shown). Therefore, we simply speculate that these results indicate that anaphylactic degranulation of mast cells is inhibited by MCAE. There is no doubt that stimulation of mast cells with compound 48/80 or anti-DNP IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b). The evidence indicates that the protein is G inhibitory-like and that the activation is inhibited by benzalkonium chloride (Bueb *et al.*, 1990).

In spite of the increasing evidence of the role of several other mediators (Rafferty and Holgate, 1989; Rimmer and Church, 1990), histamine is still regarded as the principal mediator of antigen-induced skin reactions. In addition, intradermal and intranasal application of chemical mediators and chemical mediator releasers increase vascular permeability in a manner similar to that of allergic models (Inagaki *et al.*, 1989; Inagaki *et al.*, 1990). The MCAE administered rats are protected from IgE-mediated local anaphylaxis. This finding suggests that MCAE might be useful in the treatment of allergic skin reactions. Our data demonstrated that MCAE inhibited anti-DNP IgE-induced TNF-α production from RPMC. The effect of MCAE on mast cell cytokine production *in vivo* and the relative importance of mast cells as source of TNF-α during inflammatory and immune responses are important areas for future studies. In conclusion, these results provide evidence that MCAE may be beneficial in the treatment of allergic diseases.

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