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Cichorium Intybus inhibits mast cell-mediated immediate-type allergic reactions

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Running title: *Cichorium intybus* inhibits allergic reaction

SUMMARY

We investigated the effect of aqueous extract of *Cichorium intybus* (CIAE) on mast cell-mediated immediate type allergic reactions. CIAE dose-dependently inhibited systemic anaphylactic reaction induced by compound 48/80 in mice. Especially, CIAE inhibited compound 48/80-induced anaphylactic reaction 100% with the dose of 1000 mg/kg. CIAE 1000 mg/kg also significantly inhibited local anaphylactic reaction activated by anti-dinitrophenyl (DNP) IgE. When mice were pretreated with CIAE at a concentration ranging from 0.1 to 1000 mg/kg, the plasma histamine levels were reduced in a dose-dependent manner. CIAE (1 to 1000 g/ml) dose-dependently inhibited histamine release from the rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-DNP IgE. These results indicate that CIAE inhibits mast cell-mediated immediate-type allergic reactions.

Key words: *Cichorium intybus*; Mast cell-mediated immediate type allergic reactions, Peritoneal mast cells

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INTRODUCTION

As part of our continuing search for biologically active antiallergic agents from medicinal sources, *Cichorium intybus* (Compositae) was analysed. Mast cells are widely distributed throughout vascularized tissues and certain epithelia. 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). The most potent secretagogues include the synthetic compound 48/80 and polymers of basic amino acids (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 anaphylactic reaction (Allansmith *et al.*, 1989). Cross-linking of FcRI on mast cells with IgE and specific antigen causes rapid cell degranulation, releasing histamine, proteoglycans, and neutral proteases (Nakatani *et al.*, 1994). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) as a typical model for the mast cell mediated immediate hypersensitivity (Saito and Nomura, 1989).

In the present study, we showed that aqueous

extract of Cichorium intybus (CIAE) inhibited both compound 48/80-induced systemic allergic reaction and anti-dinitrophenyl (DNP) IgE antibody-induced PCA reaction

MATERIALS AND METHODS

Materials

Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), metrizamide and forskolin were purchased from Sigma Chemical Co. (St. Louis, MO).

Systemic allergic reaction

Mice were given i.p. injection of 8 mg/kg. CIAE was dissolved in saline and administered by i.p. 1 h before the injection of compound 48/80. Mortality was monitored for 1 h after induction of anaphylactic reaction. After the mortality test, blood was obtained from each mouse's heart.

PCA

An IgE-dependent cutaneous reaction was generated by sensitizing the skin with anti-DNP IgE followed 48 h later with DNP-HSA. Rats were injected with anti-DNP IgE (0.5 μg/site, i.d.) into each of 4 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each rat received an injection of 1 mg of DNP-HSA in phosphate buffered saline (PBS) containing 4% evans blue (1:4) via the tail vein 48 h later. CIAE was i.p. administered 1 h before the challenge. Thirty min after the challenge, the rats were sacrificed, and the dorsal skin was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 1.0 N KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbant intensity of the extraction was measured at 620 nm in a spectrofluorometer, and the amount of dye was calculated with the evans blue measuring-line.

Preparation of plasma and histamine determination

The blood was centrifuged at 400 × g for 10 min. The plasma was withdrawn and the histamine content was measured by the o-phthalaldehyde spectroflurometric procedure of Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer.

Preparation of rat peritoneal mast cells (RPMC)

RPMC were isolated as previously described (Kim *et al.*, 1998). In brief, rats were anesthetized by ether, and injected with 20 ml of Tyrode buffer B (NaCl, glucose, $NaHCO_3$, KCl, NaH_2PO_4) containing 0.1% gelatin (Sigma Chemical Co.), 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 ml Tyrode buffer B were layered on 2 ml of 22.5% w/v metrizamide (density, 1.120 g/ml, Sigma Chemical Co.) 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 (NaCl, glucose, KCl, $CaCl_2$, $MgCl_2$) containing 0.1% bovine serum

albumin (Sigma Chemical Co.). 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 preincu- bated for 10 min at 37°C before the addition of compound 48/80 (1 μg/ml). The cells were preincubated with the CIAE preparations for 10 min, and then incubated (10 min) with compound 48/80. RPMC (2×10^5 cells/ml) were sensitized with 10 μg/ml anti-DNP IgE for 2 h and preincubated with CIAE at 37°C for 10 min prior to the challenge with 1 μg/ml DNP-HSA. 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.

Assay of histamine release

The inhibition percentage of histamine release was calculated using the following equation:

% Inhibition = (Histamine release without CIAE Histamine release with CIAE)×100 /Histamine release without CIAE

Statistical analysis

All values are presented as means and their standard deviations (SD). Data were analysed by Dunnet's Method after the application of the one-way ANOVA. Results with $P < 0.05$ were considered statistically significant.

RESULTS

To assess the contribution of CIAE in mast cell-mediated anaphylactic reaction, we first used the in vivo model of systemic allergic reaction. We used compound 48/80 as a systemic fatal allergic reaction inducer. After the i.p. injection of compound 48/80 (8 mg/kg), the mice were monitored for 1 h, after which the mortality rate was determined Injection of compound 48/80 plus saline as a control induced fatal shock in 100% of each group. When the CIAE (0.1 to 1000 mg/kg) was pretreated for 1 h, the mortality with compound 48/80 was dose-dependently reduced. Especially, CIAE inhibited compound 48/80-induced allergic reaction 100% with the dose of 1000 mg/kg (Table. 1).

A way to test local allergic reaction is to induce PCA. As described in the experimental procedures, local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Administration of CIAE (100 to 1000 mg/kg) showed a marked inhibition rate in PCA reaction (Table. 2).

The ability of CIAE to influence compound 48/80-induced plasma histamine release was investigated. CIAE was given from 0.1 to 1000 mg/kg 1 h before the compound 48/80 injection. While plasma levels of histamine were markedly elevated after the compound 48/80 injection in all groups of mice, the mice injected with CIAE showed a significant reduction in plasma histamine levels (Teble. 3).

The inhibitory effects of CIAE on compound 48/80-induced or IgE-mediated histamine release from RPMC are shown in Table. 4. CIAE dose-dependently inhibited the compound 48/80-induced or IgE-mediated histamine release at concentrations from 1 to 1000 g/ml. CIAE inhibited the histamine release significantly with the doses of 100 and 1000 g/ml.

Table 1. Effect of CIAE on compound 48/80-induced systemic allergic reaction

Treatment	Dose (mg/kg)	Mortality (%)
Saline	-	100
CIAE	0.1	100
	1	97.5±7.5
	10	80±10
	100	57.5±7.5
	1000	0

Groups of mice were i.p. pretreated with 200 μl saline or CIAE (at various doses) 1 h before (n = 10/group) the i.p. injection of compound 48/80. The compound 48/80 solution was i.p. given to the group of mice. Mortality (%) within 1 h following compound 48/80 injection was represented as No. of dead mice 100/total No. of experimental mice. Each datum represents the mean ± SD of five independent experiments. *P < 0.05; significantly different from the saline value.

Table 2. Effect of CIAE on the 48-h PCA in rats.

Treatment	Dose (mg/kg)	Inhibition (%)
Saline	-	0±0.3
CIAE	0.1	2±3.1
	1	3±7
	10	11±8
	100	48±5.1
	1000	62±14.6

CIAE was administered i.p. 1 h prior to the challenge with antigen. All values are expressed as a percentage of the control. Each datum represents the mean ± SD of four independent experiments. *P < 0.05; significantly different from the saline value.

Table 3. Effect of CIAE on compound 48/80-induced plasma histamine release.

Treatment	Dose (mg/kg)	Inhibition (%)
Saline	0	
CIAE	0.1	8±6
	1	13±4
	10	27±6
	100	43±12
	1000	74±13

Groups of mice were i.p. pretreated with 200 μl saline or CIAE (at various doses) 1 h before (n = 7/group) the compound 48/80 injection. All values are expressed as a percentage of the control. Each datum represents the mean ± SD of three independent experiments. *P < 0.05; significantly different from the saline value.

Table 4. Effect of CIAE on compound 48/80-induced or IgE-mediated histamine release from RPMC

Treatment	Dose(g/kg)	Inhibition (%)	
		Compound 48/80	Anti-DNP IgE
Saline	-	-	-
Caffeine	1	1.1±.0.1	4.1±1
	10	5.5±0.3	9.3±4.5
	100	41.1±2.3*	26.5±3.7*
	1000	49.3±4.15*	36.8±2.6*

RPMC (2×10^5 cells/ml) were preincubated with CIAE at 37°C for 10 min prior to incubation with compound 48/80 or DNP-HSA. All values are expressed as a percentage of the control. Each datum represents the mean ± SD of three independent experiments. *P < 0.05; significantly different from the saline value.

DISCUSSION

We have demonstrated that CIAE pretreatment profoundly affected compound 48/80-induced systemic allergic reaction and anti-DNP IgE-induced local allergic reaction. CIAE inhibited the release of histamine induced by specific antigens as well as nonspecific mechanisms from mast cells. We simply speculate that these results indicate that mast cell mediated immediate type allergic reactions are inhibited by CIAE. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1999a; Mousli et al., 1999a). 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 the release of the mediator from mast cells. CIAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. This is supported by a previous report that benzalkonium chloride and another selective antagonists inhibit the histamine release induced by compound 48/80 (Piotrowski *et al.*, 1984).

In conclusion, the results obtained proved that CIAE inhibited the IgE-mediated allergic reaction in vivo and in vitro in a murine model. It would be interesting to test CIAE on human cells, such as human basophils or human pulmonary mast cells, in light of important differences observed among different species.

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