Natural Product Sciences
7(4) : 95~101 (2001)

Effect of *Terminalia chebula* on Immediate Hypersensitivity Reaction in Mice and Rats

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Abstract – We investigated the effect of aqueous extract of *Terminalia chebula* (Combretaceae) (TCAE) on the immediate hypersensitivity reaction *in vivo* and *in vitro*. TCAE (0.01 to 1 g/kg) dose-dependently inhibited compound 48/80 induced systemic anaphylaxis in mice. When TCAE was pretreated at concentrations ranging from 0.01 to 1 g/kg, the plasma histamine levels were reduced in a dose-dependent manner. TCAE (0.1 and 1 g/kg) significantly inhibited local immunoglobulin E (IgE)-mediated passive cutaneous anaphylactic reaction. TCAE (0.001 to 1 mg/ml) also dose-dependently inhibited the histamine release from rat peritoneal mast cells (RPMC) activated by compound 48/80 or anti-dinitrophenyl (DNP) IgE. TCAE (0.01 to 1 mg/ml) had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production from RPMC. These results indicate that TCAE inhibits immediate hypersensitivity reaction *in vivo* and *in vitro*.

Key words – *Terminalia chebula,* Immediate hypersensitivity reaction, Compound 48/80, anti-DNP IgE, Tumor necrosis factor-α.

Introduction

The medicinal *Terminalia* fruit is the dried ripe fruit of *Terminalia chebula* Retz. or *Terminalia chebula* Retz. var. *tomentella* Kurt (Combretaceae). It has been used in protracted diarrhea with hematochezia and prolapse of the rectum, chronic cough with sore throat and hoarseness of voice (Zhu, 1998; Jixian, 1997). Hypersensitivity may be classified into four types. One of these, type I hypersensitivity (immediate hypersensitivity reaction), popularly known as allergy, is a major clinical problem in human. It has been found that the histamine release from mast cells is an essential step in the pathological process of a type I hypersensitivity (Ishizaka *et al.*, 1977). Compound 48/80 is one of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). It is a mixture of polymers synthesized by condensing *N*-methyl-*p*-methoxyphenyl ethylamine with formaldehyde (Baltzly *et al.*, 1949), and its hypotensive effect, resulting from histamine release, was shown by Paton (Paton *et al.*, 1951). 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). 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 anaphylaxis (PCA) reactions as a typical model for the immediate hypersensitivity (Saito *et al.*, 1989). Although mast cells store small amounts of cytokines in their granules (Gorden *et al.*, 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 FcεRI are cross-linked with specific antigen (Plaut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989; Burd *et al.*, 1989; Gurish *et al.*, 1991; Galli *et al.*, 1991). Therefore, modulation of TNF-α production by mast cells should provide us with a useful therapeutic strategy for allergic disease. Shin *et al.* reported that *Terminalia chebula* methanol extract inhibited systemic and local anaphylaxis (Shin *et al.*, 2001). However, the effect of the *Terminalia chebula* water extract on immediate hypersensitivity reaction has not been studied.

In the present study, we showed that TCAE

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inhibited compound 48/80-induced systemic anaphylaxis, anti-dinitrophenyl (DNP) IgE antibody-induced PCA, and histamine and TNF-α production from rat peritoneal mast cells (RPMC).

Materials and Methods

Reagents – 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).

Animals – The original stock of male ICR mice and male SD 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 to ten 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 TCAE – The fruits of *Terminalia chebula* were purchased from the Oriental drug store, Bohwa Dang (Chonju, Korea). A voucher specimen 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 Whatman No.1 filter paper, and the filtrate was lyophilized, and kept at −4°C. The yield of dried extract from starting crude materials was about 11.9%. 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 anaphylaxis – 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 body weight (BW) of the mast cell degranulator, compound 48/80. TCAE was dissolved in saline and administered by intraperitoneal injection from 0.005 to 1 g/kg BW 1 h before the injection of compound 48/80 (n = 10/group). In time dependent experiment, TCAE (1 g/kg BW) was injected intraperitoneally at 5 and 10 min after compound 48/80 injection (n = 10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse.

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. TCAE (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.* (Katayama *et al.*, 1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

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

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.* (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.

Inhibition of 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 TCAE (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 TCAE 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 inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = (A-B)/A\times100$$

A: Histamine release without TCAE
B: Histamine release with TCAE

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 TCAE (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 immunobilized 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

Effect of TCAE on compound 48/80-induced systemic anaphylaxis – To assess the contribution of TCAE in systemic anaphylaxis, we first used the *in vivo* model of systemic anaphylaxis. 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 mice were monitored for 1

Table 1. Effect of TCAE on compound 48/80-induced systemic anaphylaxis

TCAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)
None(saline)	+	100
0.005	+	100
0.01	+	90
0.05	+	30
0.1	+	10
0.5	+	0
1	+	0
1	–	0

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

Table 2. Time-dependent effect of TCAE on compound 48/80-induced systemic anaphylaxis

TCAE treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality(%)	
		5 min after	10 min after
None(saline)	+	100	100
1	+	0	0
1	–	0	0

Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μl saline or TCAE. TCAE 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 h following compound 48/80 injection was represented as the number of dead mice×100/total number of experimental mice.

h, after which the mortality rate was determined. As shown in Table 1, an intraperitoneal injection of 200 μl saline as a control induced a fatal shock in 100% of mice. When mice were pretreated with TCAE at concentrations ranging from 0.005 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was reduced dose-dependently. We investigated the time-dependent effect of TCAE on systemic anaphylaxis. The mortality of mice injected intraperitoneally with TCAE (1 g/kg) 5 min and 10 min after compound 48/80 injection was 0%. (Table 2).

Effect of TCAE on compound 48/80-induced plasma histamine release – The ability of TCAE to influence compound 48/80-induced plasma histamine release was investigated. TCAE was given from 0.005 to 1 g/kg BW 1 h before (n = 10/group) compound 48/80 injection. The correlation results with those of the mortality test were shown when their plasma histamine contents were measured (Fig 1). The inhibition rate of histamine by TCAE was significant at doses of 0.5 and 1 g/kg.

Effect of TCAE on anti-DNP IgE-induced 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 procedures, local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge. Oral administration of TCAE (0.1 and 1 g/kg) showed a marked inhibition rate in PCA reaction (Fig. 2).

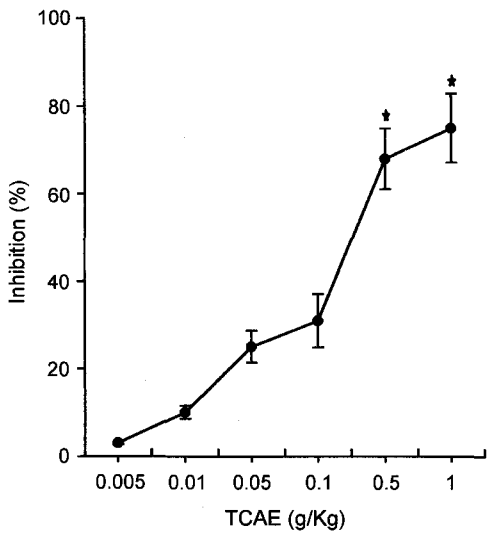

Fig. 1. Effect of TCAE on compound 48/80-induced plasma histamine release. Groups of mice were intraperitoneally pretreated with 200 μl saline or TCAE. TCAE was given with various doses 1 h before the compound 48/80 injection. Each value is the mean± SEM of three independent experiments. *p<0.05; significantly different from the saline value.

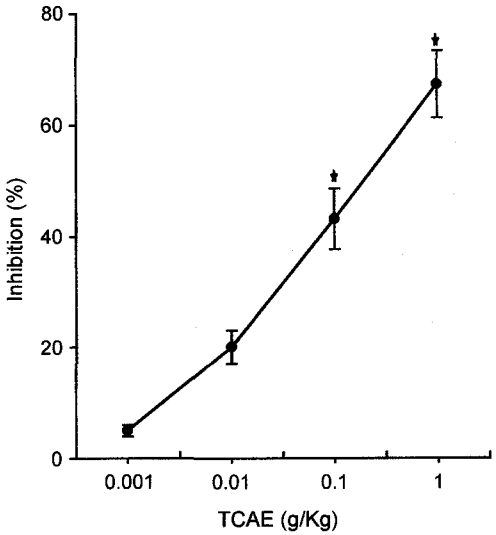

Fig. 2. Effect of TCAE on 48 h PCA. TCAE was administered orally 1 h prior to the challenge with antigen. Each value is the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

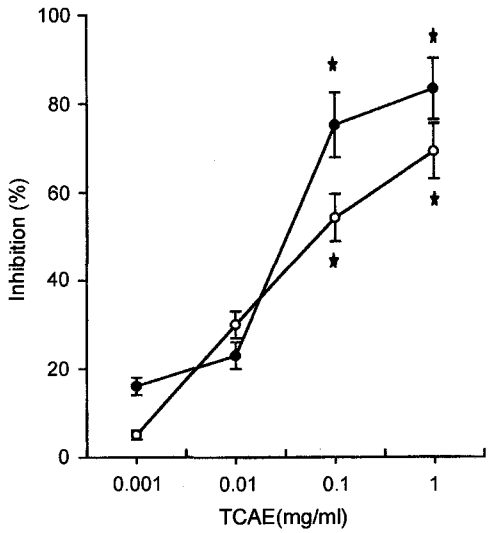

Fig. 3. Effect of TCAE on compound 48/80-induced (●) or IgE-mediated (○) histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with TCAE at 37°C for 10 min prior to incubation with compound 48/80 or challenge with DNP-HAS. Each value is the mean±SEM of three independent experiments. *p<0.05; significantly different from the saline value.

Table 3. Effect of TCAE on anti-DNP IgE-induced TNF-α production in RPMC

TCAE 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	+	197.0±20.3
0.01	+	88.6± 9.9*
0.1	+	89.7± 8.6*
1	+	72.3± 8.0*

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 TCAE before the challenge with DNP-HAS (0.1 μg/ml). The data represents the meanSEM of three independent experiments. *$p<0.05$: significantly different from the saline value.

Effect of TCAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC – The inhibitory effect of TCAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC are shown in Fig. 3. TCAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001 to 1 mg/ml. Especially, TCAE significantly inhibited the compound 48/80-induced or IgE-mediated histamine release at the concentrations of 0.1 and 1 mg/ml.

Effect of TCAE on anti-DNP IgE-induced TNF-α production from RPMC – Finally, we investigated the ability of TCAE to influence anti-DNP IgE-induced TNF-α production in RPMC. TCAE significantly inhibited TNF-α production at concentrations from 0.01 to 1 mg/ml (Table 3). No significant cytotoxicity of TCAE on the culture was observed in the concentrations used in the experiments, as assessed by trypan blue uptake.

Discussion

The results obtained in the present study provide evidence that TCAE inhibits both compound 48/80-induced and anti-DNP IgE-induced anaphylactic reactions. TCAE also inhibited the compound 48/80 or anti-DNP IgE-mediated histamine release from RPMC. Therefore, we simply speculate that these results indicate that anaphylactic degranulation of mast cells is inhibited by TCAE. 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 *et al.*, 1989; Rimmer *et al.*, 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 TCAE administered rats are protected from IgE-mediated local anaphylaxis. This finding suggests that TCAE might be useful in the treatment of allergic skin reactions. Our data demonstrated that TCAE inhibited anti-DNP IgE-induced TNF-α production from RPMC. The effect of TCAE 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, the results obtained proved that TCAE inhibited the mast cell-mediated immediate hypersensitivity *in vivo* and *in vitro* in a murine model. Therefore, Further work should address the possibility that TCAE may also be active in the inhibition of human mast cell degranulation.

Acknowledgements

This work was supported by Woosuk University.

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(Accepted November 6, 2001)