

# Inhibitory Effects of Curcuminoids on Passive Cutaneous Anaphylaxis Reaction and Scratching Behavior in Mice

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To understand anti-allergic effect of *Curcuma aromatica* (family Zingiberaceae), which inhibited passive cutaneous anaphylaxis (PCA) reaction in preliminary study, we isolated its main constituents, curcumin, demethoxycurcumin and bisdemethoxycurcumin, and investigated their inhibitory effects against PCA reaction and scratching behavior. These curcuminoids inhibited the PCA reaction induced by the IgE-antigen complex (IAC) and the scratching behavior induced by compound 48/80. These curcuminoids also inhibited degranulation, protein expression of TNF- $\alpha$  and IL-4, and transcription factor NF- $\kappa$ B activation in IAC-induced RBL-2H3 cells. Of these curcuminoids, curcumin exhibited the most potent inhibition, followed by demethoxycurcumin and bisdemethoxycurcumin. These findings suggest that curcuminoids, particularly curcumin, can improve the symptoms of allergic diseases, such as anaphylaxis and itching.

**Key words:** Allergy, Curcuminoids, Curcumin, Anaphylaxis, IgE

## Selected by Editors

## INTRODUCTION

Mast cells and basophils are well-known critical participants in various biological processes of allergic diseases (Stevens et al., 1989). These cells express surface membrane receptors, with high affinity and specificity for IgE. The interaction of antigen-bound IgE in surface membrane receptors causes the release of histamine, prostaglandins, leukotrienes and cytokines (Mitre et al., 2006). These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally, cytokine-induced reactions cause tissue inflammation. These allergic diseases are now rapidly increasing chronic health problem in most countries (Wuthrich, 1989). Anti-allergic agents, such as anti-histamines, steroids and immunosuppressants, have been used against allergic diseases, such

as allergic rhinitis, atopic dermatitis, asthma and food allergies (Sakuma et al., 2001; Schafer-Korting et al., 1996; Simons, 1992), but improving these diseases is very difficult. Therefore, herbal medicines have been advanced for allergic diseases, and their effectiveness has received increasing attention (Bielory, 2004).

*Curcuma* sp. including *Curcuma longa* and *Curcuma aromatica*, which contains curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin as main constituents, exhibits useful pharmacological effects such as anti-inflammatory, anti-tumor, and immunological activities (Antony et al., 1999; Ozaki, 1990). The biological activities, such as radical scavenging, anti-inflammation, anti-tumor, anti-allergic and neuroprotective effects, of its representative constituent curcumin have been investigated (Antony et al., 1999; Dairam et al., 2007; Kuttan et al., 1987; Matsuda et al., 2004; Ram et al., 2003). Matsuda et al. (2007) reported inhibitory effect of curcumin and bisdemethoxycurcumin in degranulation and TNF- $\alpha$  and IL-4 protein expression in RBL-2H3 cells. Nevertheless, the antiallergic activity of curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin, remains to be thoroughly studied *in vivo*.

Therefore, curcuminoids, curcumin, demethoxycur-

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cumin and bisdemethoxycurcumin, were isolated from the rhizome of *Curcuma aromatica* found to inhibit the mouse passive cutaneous anaphylaxis (PCA) reaction induced by the antigen-IgE complex and their inhibitory activity against scratching behaviors and PCA reaction investigated *in vivo*.

## MATERIALS AND METHODS

### Materials

Dulbecco's modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide, cremophor EL and compound 48/80 were purchased from Sigma Co.

Curcumin (purity, >95%), demethoxycurcumin (purity, >95%) and bisdemethoxycurcumin (purity, >95%) (Fig. 1) from the rhizome of *Curcuma aromatica* were isolated according to the previous reports (Dairam et al., 2007).

### Animals

The male ICR and BALB/c mice (20-25 g) were supplied by the Orient Experimental Animal Breeding Center. All animals were housed in wire cages at 20-22°C and 50  $\pm$  10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center) and allowed water ad libitum. All procedures relating to the animals and their care conformed to the international guidelines 'Principles of Laboratory Animals Care' (NIH publication no. 85-23, revised 1985).

### Measurement of PCA reaction

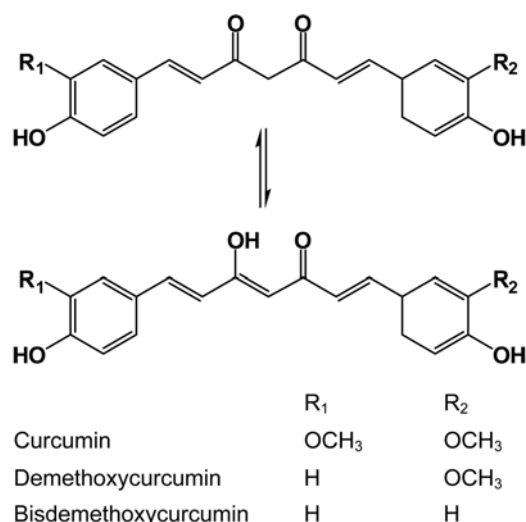


**Fig. 1.** Structure of curcuminoids isolated from *Curcuma aromatica*

An IgE-dependent cutaneous reaction was measured according to the previous method of Choo et al. (2003). The male ICR mice were intradermally injected, with 10  $\mu$ g of anti-DNP IgE, into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each mouse received an injection of 200  $\mu$ L of 3% Evans blue in PBS, containing 200  $\mu$ g of DNP-HSA, *via* the tail vein. The test agents (10 or 50 mg/kg of curcumin, demethoxycurcumin or bisdemethoxycurcumin suspended in 2% cremophor EL) were orally administered 1 h prior to the DNP-HSA injection. Thirty min after the DNP-HSA injection, the mice were sacrificed, their dorsal skins were removed and the pigmented area was measured. After extraction with 1 mL 1.0 M KOH and 4 mL of a mixture of acetone and 0.2 M phosphoric acid (13:5), the amount of dye was determined colorimetrically at 620 nm.

### Measurement of scratching behavioral frequency

Male BALB/c mice were placed in acrylic cages (22 x 22 x 24 cm) for about 10 min to become acclimatized. The behavioral experiments were performed according to the method of Sugimoto et al. (1998). The rostral part of the skin on the back of the mice was clipped, and 50  $\mu$ g/50  $\mu$ L of compound 48/80 (dissolved in saline) intradermally injected into each mouse. Control mice received a saline injection in the place of the compound 48/80. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and the scratching behaviors recorded using an 8-mm video camera (SV-K80, Samsung) under unmanned conditions. Scratching of the injected site with the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches per second, and a series of these behaviors was counted as one incident of scratching over a 60 min period. The test agents (10 or 50 mg/kg of curcumin, demethoxycurcumin or bisdemethoxycurcumin suspended in 2% cremophor EL) were orally administered 1 h before the scratching agent.

### Assay of degranulation of RBL-2H3 cells stimulated by IgE-antigen complex

The inhibitory activity of test agents against the release of  $\beta$ -hexosaminidase from RBL-2H3 cells was evaluated according to Choo et al. (2003). The RBL-2H3 cells were grown in DMEM supplemented with 10% fetal bovine serum and L-glutamine and split every 3 days. Before the experiment, the cells were

dispensed into 24 well plates at a concentration of  $5 \times 10^5$  cells per well, using a medium containing 0.5  $\mu\text{g}/\text{mL}$  of mouse monoclonal IgE, and were incubated overnight at 37°C in 5%  $\text{CO}_2$  to sensitize the cells. The cells were washed with 500  $\mu\text{L}$  of siraganian buffer (pH 7.2) containing NaCl 119 mM, KCl 5 mM,  $\text{MgCl}_2$  0.4 mM, piperazine- $\text{N,N}'$ -bis(2-ethanesulfonic acid) 25 mM, NaOH 40 mM) and incubated in 160  $\mu\text{L}$  of siraganian buffer containing glucose 5.6 mM,  $\text{CaCl}_2$  1 mM, and 0.1% BSA for an additional 10 min at 37°C. The cells were then exposed to 40  $\mu\text{L}$  of the test agents (curcumin, demethoxycurcumin or bisdemethoxycurcumin dissolved in 1% dimethylsulfoxide) for 20 min, followed by a treatment with 20  $\mu\text{L}$  of the antigen (DNP-HSA, 1  $\mu\text{g}/\text{mL}$ ) for 10 min at 37°C to activate the cells and evoke allergic reactions (degranulation). The reaction was quenched by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at  $800 \times g$  for 10 min, and 25  $\mu\text{L}$  aliquots of the supernatant were transferred to 96 well plates and incubated with 25  $\mu\text{L}$  of the substrate (p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide 1 mM) for 1 h at 37°C. The reaction was stopped by adding 200  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  0.1 M. The absorbance was measured using a ELISA microplate reader at a wavelength of 405 nm.

### Enzyme-linked immunosorbent assay (ELISA)

RBL-2H3 cells were performed by the method of Choo et al. (2005). Briefly, the previously cultured RBL-2H3 cells ( $5 \times 10^5$  cells) were treated with 0.5  $\mu\text{g}/\text{mL}$  of mouse monoclonal IgE for sensitization of cells. The cells (1.8 mL) were exposed to 0.2 mL of test agents for 1 h (for immunoblot) or 4 h for ELISA, followed by the treatment with 0.2 mL of DNP-HSA (1  $\mu\text{g}/\text{mL}$ ) for 40 min at 37°C. The immunoblot for NF- $\kappa\text{B}$  (pp65 and p65) was performed to the method of Lee et al. (2008). The supernatant (50  $\mu\text{L}$ ) was transferred into 96-well (ELISA) plates and then IL-4 and TNF- $\alpha$  concentrations were determined using commercial ELISA Kits (Pierce Biotechnology) (Matsuda et al., 2003).

### Statistical analysis

All data were expressed as the mean  $\pm$  standard deviation, with statistical significance analyzed using one-way ANOVA followed by a Student-Newman-Keuls test.

## RESULTS

In the preliminary study, 80% methanol extract of the rhizome of *Curcuma aromatica* was found to inhibit the mouse PCA reaction induced by IgE-antigen complex. Therefore, its main constituents, cur-

cumin, demethoxycurcumin and bisdemethoxycurcumin, from 80% methanol extract of the rhizome of *Curcuma aromatica* were isolated, and its PCA reaction-inhibitory effect in mice measured (Table I). The PCA reaction was induced by an injection of IgE and antigen, with these curcuminoids administered orally 1 h prior to the challenge with antigen. The curcuminoids potently inhibited the PCA reaction. Of these curcuminoids, curcumin exhibited the most potent inhibition. Curcumin at doses of 10 and 50 mg/kg inhibited the PCA reaction by 31% and 47%, respectively. However, their inhibitory effects were weak compared with that of azelastine. These curcuminoids also inhibited the scratching behavior induced by compound 48/80. Curcumin at doses of 10 and 50 mg/kg also potently reduced the scratching behavior frequency by 42% and 65%, respectively. The inhibitory effect of curcumin at a dose of 50 mg/kg was comparable with that of azelastine at a dose of 10 mg/kg.

To understand the anti-allergic mechanism of the curcuminoids, its degranulation-inhibitory activities against RBL-2H3 cells were measured (Fig. 2). The curcuminoids inhibited the degranulation of RBL-2H3 cells induced by the IgE-antigen complex; its  $\text{IC}_{50}$  values were 33, 39 and  $> 50$   $\mu\text{M}$ , respectively, and that of azelastine was 28  $\mu\text{M}$ . When the inhibitory effects of the curcuminoids on the protein expression of a proinflammatory cytokine TNF- $\alpha$  and an IgE-switch-

**Table I.** Effect of curcuminoids on the mouse passive cutaneous anaphylaxis reaction induced by IgE-antigen complex and scratching behavior induced by compound 48/80

| Agent                 | Dose (mg/kg) | Inhibition (%)            |                                  |
|-----------------------|--------------|---------------------------|----------------------------------|
|                       |              | PCA reaction <sup>a</sup> | Scratching behavior <sup>b</sup> |
| Curcumin              | 10           | 31 $\pm$ 6 <sup>d</sup>   | 42 $\pm$ 8 <sup>f</sup>          |
|                       | 50           | 47 $\pm$ 8 <sup>e</sup>   | 65 $\pm$ 10 <sup>g</sup>         |
| Demethoxy-curcumin    | 10           | 16 $\pm$ 3 <sup>c</sup>   | 17 $\pm$ 1 <sup>e</sup>          |
|                       | 50           | 41 $\pm$ 8 <sup>d,e</sup> | 27 $\pm$ 4 <sup>c</sup>          |
| Bisdemethoxy-curcumin | 10           | 14 $\pm$ 5 <sup>c</sup>   | 10 $\pm$ 1 <sup>c</sup>          |
|                       | 50           | 28 $\pm$ 5 <sup>d</sup>   | 14 $\pm$ 1 <sup>d</sup>          |
| Azelastine            | 10           | 76 $\pm$ 5 <sup>f</sup>   | 72 $\pm$ 5 <sup>g</sup>          |

<sup>a</sup>The amounts of extravasated Evan blue from the dorsal skin (1  $\times$  1 cm) of the control stimulated with the IgE-antigen complex and vehicle-treated groups were 25  $\pm$  3 and 11  $\pm$  2  $\mu\text{g}$ , respectively.

<sup>b</sup>Scratching behavior number frequency of the normal group, which was treated with saline alone, and the control group, which was treated with compound 48/80, for 1 h were 3  $\pm$  1 and 239  $\pm$  25, respectively.

Inhibition values indicate the mean  $\pm$  S.D. (n = 5).

<sup>c,d,e,f,g</sup>Items with the same letter in each column were not significantly different ( $p < 0.05$ ).

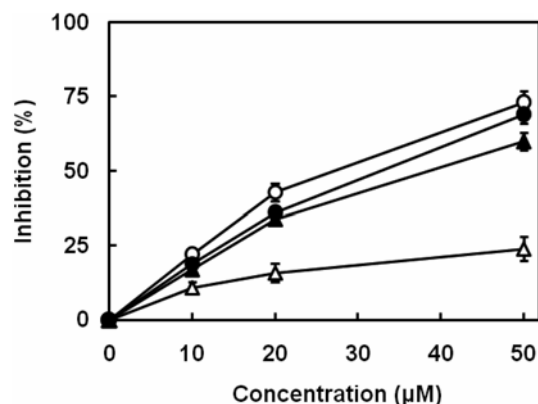


**Fig. 2.** Effect of curcuminoids on the degranulation of RBL-2H3 cells induced by IgE-antigen complex. RBL-2H3 cells, which were grown in DMEM supplemented with 10% fetal bovine serum and L-glutamine, were dispensed into 24 well plates, at a concentration of  $5 \times 10^5$  cells per well, and sensitized using 0.5  $\mu\text{g/mL}$  of mouse monoclonal IgE. They were then washed with 500  $\mu\text{L}$  of siraganian buffer, exposed curcuminoids, curcumin (●), demethoxycurcumin (▲), bisdemethoxycurcumin (△), and azelastine (○) at concentrations of 10, 20 and 50  $\mu\text{M}$  dissolved in 0.2% DMSO for 20 min, and treated with 20  $\mu\text{L}$  of antigen (DNP-HSA, 1  $\mu\text{g/mL}$ ) for 10 min at 37°C. The release of  $\beta$ -hexosaminidase from RBL-2H3 cells was measured according to the method of Choo et al. (2003).

ing cytokine IL-4 in RBL-2H3 cells induced by IgE-antigen complex were measured using an ELISA assay, curcumin exhibited the most potent inhibition. Curcumin at a concentration of 20  $\mu\text{M}$  was found to inhibit the expression of TNF- $\alpha$  and IL-4 by 71% and 58%, respectively (Fig. 3), although its inhibitory effects were comparable to that of betamethasone at a concentration of 10  $\mu\text{M}$ . These curcuminoids also inhibited transcription factor NF- $\kappa\text{B}$  activation. Of these curcuminoids, curcumin exhibited the most potent inhibition.

## DISCUSSION

Steroids, anti-histamines and immunosuppressants have been used clinically for inflammatory skin disorders, atopic and chronic dermatitis (Schafer-Korting et al., 1996; Baroody and Naclerio, 2000; Ruhlmann and Nordheim, 1997). Nevertheless, these agents show the side effect, such intense skin atrophy, sneezing and neprotoxicity. Therefore, new agents for clinical uses should be developed from herbal medicines.

*Curcuma aromatica* has been reported to exhibit anti-allergic and anti-inflammatory effects (Antony et al., 1999; Ozaki, 1990). Its main constituent curcumin inhibited the degranulation of RBL-2H3 cells induced by IAC. Curcumin also exhibited anti-asthmatic effects. However, the inhibitory effects of curcumin and its

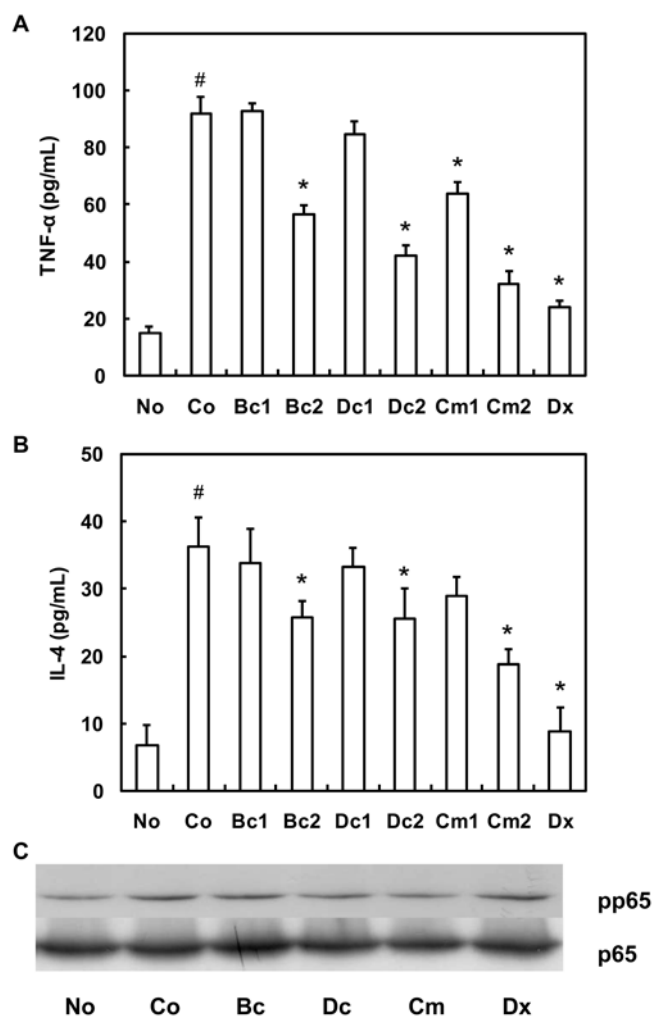


**Fig. 3.** Effect of curcuminoids on TNF- $\alpha$  (A) and IL-4 protein expression (B) and NF- $\kappa\text{B}$  activation (C) in RBL-2H3 cells induced by IgE-antigen complex. RBL-2H3 cells ( $5 \times 10^5$  cells) were treated with 0.5  $\mu\text{g/mL}$  of mouse monoclonal IgE, exposed to 0.2 mL of test agents for 1 h (for immunoblot) or for 4 h [for enzyme-linked immunoassay (ELISA)], followed by the treatment with 0.2 mL of dinitrophenol-human serum albumin (1  $\mu\text{g/mL}$ ) for 4 h (protein expression) at 37°C and then ELISA for IL-4 and TNF- $\alpha$  and immunoblot for NF- $\kappa\text{B}$  (pp65 and p65) were performed. The concentrations of test agents were as follows: Co, vehicle alone; Cm, 20  $\mu\text{M}$  curcumin; Dc, 20  $\mu\text{M}$  demethoxycurcumin; Bc, 20  $\mu\text{M}$  bisdemethoxycurcumin; Dx, 10  $\mu\text{M}$  dexamethasone in immunoblot analysis; and C, vehicle alone; Cm1, 10  $\mu\text{M}$  curcumin; Cm2, 20  $\mu\text{M}$  curcumin; Dc1, 10  $\mu\text{M}$  demethoxycurcumin; Dc2, 20  $\mu\text{M}$  demethoxycurcumin; Bc1, 10  $\mu\text{M}$  bisdemethoxycurcumin; Bc2, 20  $\mu\text{M}$  bisdemethoxycurcumin; Dx, 10  $\mu\text{M}$  dexamethasone in ELISA. Normal group (No) was treated with vehicle alone instead of agent and IgE-antigen complex. Values represent the mean  $\pm$  S.D. (N = 3). #Significantly different vs. normal group (No) ( $^{\#}p < 0.05$ ). \*Significantly different vs. the control group (Co) ( $^*p < 0.05$ ).

derivatives against PCA and itching reactions have not been investigated *in vivo*. In the present study,

curcuminoids, isolated from *Curcuma aromatica*, were found to inhibit the PCA reaction induced by IAC as well as compound 48/80-induced scratching behavior in mice. Curcuminoids were also found to inhibit the degranulation of IAC-induced RBL-2H3 cells, like the previously reported (Ozaki, 1990). Curcuminoids also inhibited the protein expressions of some cytokines, TNF- $\alpha$  and IL-4, in RBL-2H3 cells, like the previously reported (Matsuda et al., 2004). Matsuda et al. (2004) reported that curcumin showed potent inhibition against degranulation of RBL-2H3 cells and the cytokine expression, although both curcumin and bisdemethoxycurcumin inhibited them. In the present study, curcumin also exhibited the most potent inhibition, followed by demethoxycurcumin and bisdemethoxycurcumin. The curcumin also inhibited transcription factor NF- $\kappa$ B most potently resulting in the reduced expression of a proinflammatory cytokine TNF- $\alpha$ . These results were supported by the previous reports that curcumin, isolated from *Curcuma* species, potently inhibited NF- $\kappa$ B activation and exhibited anti-inflammatory effects (Kuttan et al., 1987; Matsuda et al., 2004; Ram et al., 2003; Shishodia et al., 2007). These findings suggest that the methoxy group of curcuminoids may be important in anti-allergic activities, such as PCA and itching reactions.

Based on these findings, the inhibitory effect of curcuminoids against the PCA reaction and scratching behavior may be due to the inhibition of mast cell degranulation and cytokine expressions of TNF- $\alpha$  and IL-4 and *Curcuma aromatica*, particularly curcumin, may improve the IgE-induced allergic diseases.

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