

Curcumin inhibits compound 48/80 induced systemic anaphylaxis

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Abstract: Curcumin, the active component of turmeric, is a polyphenolic phytochemical with anti-tumor, anti-inflammatory, anti-oxidative and anti-allergic properties. Mast cells participate in allergic inflammation by virtue of their ability of being activated to allergens and lead to the release of number of biologically active mediators including histamine, prostaglandins, leukotrienes, various cytokines etc. In this report, we have investigated effects of curcumin on non-immunological stimulations like Compound 48/80 induced systemic anaphylaxis. *In vitro* experiments have confirmed non-toxicity of curcumin (50 μ M) as assessed by MTT test but 100 μ M dose was found toxic. Curcumin (50 μ M) inhibited Compound 48/80 induced mouse peritoneal mast cell (MPMC) degranulation and histamine release in dose-dependent manner. Therefore, it is worth to study effect of curcumin on non-immunological stimulations as most often it occurs without IgE involvement. Whether it has mast cell membrane stabilizing activity or some other signaling mechanisms are involved underlying its potential could be explored further.

Keywords: Compound 48/80, Curcumin, Histamine, Mast Cells, Systemic Anaphylaxis

1. Introduction

Mast cells are bone marrow-derived effector cells of the immune system, found abundantly in connective tissue, skin, mucosal membranes, and tissues which interface with the external environment. Mast cells are the primary effectors involved in allergic or immediate hypersensitivity responses. Mast cell activation by both IgE-dependent and IgE-independent stimuli initiate degranulation which results in the fusion of cytoplasmic granule membranes with plasma membranes. Mast cells participate in airway inflammation by IgE-receptor (Fc ϵ RI) cross-linking with exposed antigen, secreting variety of pro-inflammatory mediators including histamine, serotonin, eicosanoides and platelet activating factors as well as various cytokines and chemokines [1-3]. Mast cell degranulations can also be elicited by basic secretagogues. Histamine, a major component of mast cell granules, exerts many effects related to the immediate-phase of allergic inflammation including vasodilation, increased vascular permeability, tissue edema, contraction of bronchial and intestinal smooth muscle, and increased mucus production [4]. Compound 48/80 is known to be a potent inducer of

degranulation, responsible for the release of histamine and other chemical mediators associated with anaphylactic symptoms, and the activation of mast cells. The most potent secretagogues include the synthetic compound 48/80 and polymer of basic amino acids [5]. Compared with the natural process, a higher concentration of compound 48/80 induces almost 90% of histamine release from mast cells. It can be used as a direct reagent to investigate the mechanism of allergy and anaphylaxis [6]. Local allergen challenge in patients with bronchial asthma [7], allergic conjunctivitis [8] and atopic dermatitis [9] has been shown to result in rapid elevation of Prostaglandin D2 (PGD2) level in nasal and bronchial lavage fluids (BALF), tears and skin chamber fluids.

Curcumin, a phytochemical present in turmeric, rhizome of *Curcuma longa* has been shown to have a wide variety of pharmacological activity including have anti-tumor, anti-inflammatory, anti-oxidative, and anti-allergic effects [10-12]. In addition, previous studies demonstrated that curcumin had an inhibitory effect on histamine release from mast cells triggered by IgE, calcium ionophore A 23187, or concanavalin A [13-15]. Studies have suggested that curcumin has downregulated Th2 response and reduced lung inflammations in latex sensitized mice suggesting

possible role of curcumin in controlling allergic disorders [16]. Studies demonstrate that known mast cell stabilizer, DSCG has mast cell stabilizing activity after non-immunological stimulations [17]. However, no report till date has shown suppressive effect of curcumin on compound 48/80-induced systemic anaphylaxis.

2.1. Materials

Curcumin, compound 48/80, disodium cromoglycate (DSCG), bovine serum albumin (BSA), and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO, USA). For all *in vitro* experiments, curcumin was dissolved in dimethylsulfoxide (DMSO) and freshly diluted in Phosphate buffered buffer (before use).

2.2. Methods --- Compound 48/80-Induced Systemic Anaphylactic Reactions

Mice (n=5/group) received an intraperitoneal injection of compound 48/80 (1, 4 and 8 mg/kg, i.p) as described previously [15-16]. Curcumin (5, 10 and 20 mg/kg, i.p) was administered an hour prior to administration of compound 48/80. Survival rate was monitored for an hour after induction of anaphylactic shock.

2.3. Compound 48/80 Induced Systemic Anaphylaxis and Histamine Release in Serum

To investigate effect of Compound 48/80 on systemic anaphylaxis and mortality, histamine release was measured in the serum of mice as discussed [18] with slight modification. Histamine release in supernatants of peritoneal mast cells after degranulation induced by Compound 48/80 *in vitro* was also detected. Briefly 90 μ l of serum /supernatant was mixed with 90 μ l of 0.1M HCl, 36 μ l of 1M NaOH and 9 μ l of O-phthalaldehyde (OPD, Sigma) at room temperature. After 7 min, reaction was stopped by adding 18 μ l of 3M HCl. Samples were transferred to a flat bottom 96 well microplates (black). Fluorescence intensity was measured using a 360nm excitation filter and a 460 nm emission filter. The values are expressed as net histamine release and percent inhibition.

2.4. Study of Mast Cell Degranulation: Isolation of Peritoneal Mast Cells, its Purification by BSA Layering Method and Compound 48/80 Induced Histamine Release

Mast cells were isolated from peritoneal exudates after intraperitoneal injection of 10 ml of HEPES-Tyrode buffer into the peritoneal cavity of mice. The peritoneal fluid was aspirated and centrifuged at 3000 rpm for 10 minutes at 4^oC. Cells were washed in PBS twice for purification. The cell suspension was layered on 38% BSA and pure population of mast cells were obtained as described [19]. MPMC preparation was approximately 95% pure as assessed by toluidine blue staining and at least 98% of these cells were assessed viable by trypan blue dye exclusion test. Purified

MPMCs (1×10^6 cells/ml) were resuspended in HEPES-Tyrode buffer 136 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 11 mM NaHCO₃, 0.6 mM NaH₂PO₄, 2.75 mM MgCl₂, 5.4 mM HEPES, 1.0 mg/mL BSA, 1.0 mg/mL glucose, 0.1 mg/mL heparin, pH 7.4) and were incubated with compound 48/80 (4 μ g/ml) with and without curcumin (10, 20 and 50 μ M) and supernatants were used to assay histamine release from peritoneal mast cells .

2.5. Measurement of Toxicity of Curcumin by MTT (3-(4, 5-Dimethylthiazolyl)-2, 5-Diphenyltetrazolium Bromide): MPMC Viability Assay

To test the viability of MPMCs, the 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed as previously described [20]. Briefly, MPMCs (2×10^5 cells/well) were incubated with various concentrations (10~100 μ M) of curcumin at 37^oC for 2 hours. After addition of MTT (100 μ g in 100 μ L saline), MPMCs were incubated at 37^oC for 1hour. MPMC viability was compared with H₂O₂ (a known apoptosis inducer) induced cells. Absorbance was measured at 570 nm by spectrophotometer (Biotek, USA).

2.6. Statistical Analysis

Experiments were repeated three times with consistent results. Unless otherwise stated, data are expressed as mean \pm SEM for the number of experiments. Statistical evaluation of the results to determine difference between all groups was performed using one way analysis of variance (ANOVA), followed by student unpaired t test. Results with p values less than 0.05 were considered significant.

3. Results

3.1. Curcumin Inhibits Compound 48/80-Induced Systemic Anaphylaxis and Histamine Release

Lower doses of Compound 48/80 (1 and 4mg/kg, i.p.) was unable to cause mortality while higher dose (8mg/kg, i.p.) has killed all mice after an hour of 48/80 injection. Curcumin dose-dependently inhibited compound 48/80 induced mortality (Table 1) and histamine release in serum. DSCG (50mg/kg, i.p.) is known to have mast cell stabilizing activity but curcumin (20mg/kg, i.p.) was found better in attenuating histamine release (Fig 1a & 1b).

3.2. Curcumin Inhibits Compound 48/80-Induced MPMC Degranulation and Histamine Release

Here we have evaluated effect of curcumin on compound 48/80-induced MPMC degranulation. Normal MPMCs were generally oval in shape and contained many fine granules surrounding a prominent nucleus. After stimulation with compound 48/80 for 10 minutes, MPMCs were characterised by cell swelling and degranulation. When MPMCs were incubated with curcumin alone,

MPMCs were similar to those seen in normal samples where it was not induced by Compound 48/80. Pre-treatment of curcumin inhibited the degranulation of MPMCs stimulated with compound 48/80, and cell size appeared to be somewhat larger than the control. Number of degranulated cells were counted under high power (40X) of light microscope (Table 2 & Fig 2). Histamine release was dose-dependently inhibited by curcumin (10-50 μ M, Fig 3).

3.3. Curcumin Has No Cytotoxicity on MPMCs at 10~50 μ M

MTT conversion assay was used to determine the viability of MPMCs treated with curcumin. MPMCs were almost 100% viable after exposure to 10~50 μ M of curcumin for 2 hours. However, 100 μ M, curcumin found toxic (Fig 4).

4. Discussion

This is the first study to evaluate the effect of curcumin on systemic anaphylaxis induced by compound 48/80. Present data demonstrate that curcumin suppresses both compound 48/80-induced Systemic anaphylaxis and mast cell degranulation *in vitro*. Several reports have shown that compound 48/80 increases the permeability of the lipid bilayer membrane of mast cells by causing perturbation of the membrane [21]. These findings indicate that the increase in cell membrane permeability may be an essential trigger for the release of mediators from mast cells. Thus, it is reasonable to presume that curcumin might inhibit histamine release by attenuating the permeability of MPMC membranes by preventing compound 48/80-induced membrane perturbation. However, we found no evidence in our study to substantiate this assumption. In order to reveal the mechanisms involved in histamine release inhibition by curcumin, additional research is required.

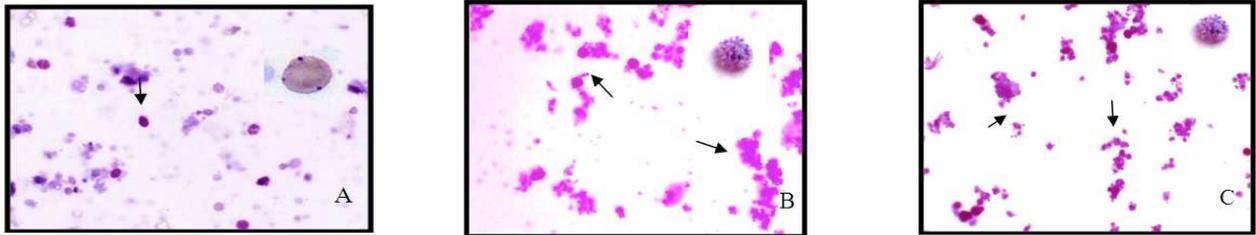
Although there are a few differences among the experimental conditions in studies concerning anti-allergic activities of curcumin, our results confirm those of Suzuki and co-workers and Lee and co-workers [13, 14]. It is well-recognized that compound 48/80 can induce a mast cell-dependent, non-specific anaphylactoid reaction. The mechanism of anaphylactoid response triggered by compound 48/80 is due to the massive release of vasoactive amines, such as histamine, from mast cells and basophils [22]. As noted, histamine is a typical mediator that causes various pathophysiologic events in acute allergic reactions [23]. Thus, it is established that curcumin inhibits mast cell-mediated anaphylactoid responses by suppressing histamine release from MPMCs. Compound 48/80 is known to activate mast cell secretory processes by increasing the rate of GTP γ S binding to G-proteins [24]; in turn, the activation of G-proteins can trigger intracellular signaling events such as activation of phospholipase, protein kinase C (PKC) and Ca²⁺ signaling which ultimately results in the release of histamine from these cells. Inositol 1,4,5-triphosphate then causes the movement

of Ca²⁺ from the endoplasmic reticulum, triggering store-operated Ca²⁺ entry through specialized Ca²⁺ release-activated calcium channels [25]. Degranulation of mast cells is involved in the synergistic activation of PKC and the increase in intracellular calcium concentration. The increase in intracellular Ca²⁺ induces the movement of granules to the plasma membrane followed by the degranulation of mast cells or basophils and activated formation of inflammatory mediators such as prostaglandins and leukotrienes.

In the present study, curcumin has inhibited Compound 48/80 induced mast cell degranulation thereby histamine release in dose-dependent manner. Recently Anaphylactoid response induced by compound 48/80 has also been reported [26] with similar mechanism. Earlier studies on Curcumin [12] suggest that most of the compounds develop anti-allergic activities through mechanisms related to both anti-oxidative and non anti-oxidation activities. As reported earlier that curcumin has no toxic effects. Since reduction of MTT can occur only in metabolically active cells and the level of activity is a measure of the viability of cells. Upto 50 μ M, curcumin is non-toxic to cells *in vitro*. Investigations suggest that curcumin has inhibited Compound 48/80 induced histamine release from MPMCs in dose-dependent manner. Curcumin (20mg/kg, i.p.) may prove as potential mast cell stabilizer and comparable to standard drug disodium cromoglycate (DSCG, 50mg/kg, i.p.). Present study demonstrate that curcumin has beneficial effects in the prevention of non-immunologically induced mast cell-mediated diseases.

Table 1. Inhibitory effect of curcumin on compound 48/80 induced Systemic anaphylaxis.

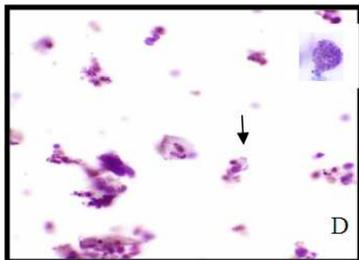
Group (5 mice each)	Drug Curcumin /DSCG	Compound 48/80 dose (i.p.)	Mortality rate (%)
I	(-)	1mg/kg	No mortality
II	(-)	4mg/kg	No mortality
III	(-)	8mg/kg	100
IV	Vehicle (DMSO)	8mg/kg	100
V	Curcumin (5mg/kg, i.p)	8mg/kg	100
VI	Curcumin (10mg/kg, i.p)	8mg/kg	20
VII	Curcumin (20mg/kg, i.p)	8mg/kg	No mortality
VIII	DSCG (50mg/kg, i.p.)	8mg/kg	20



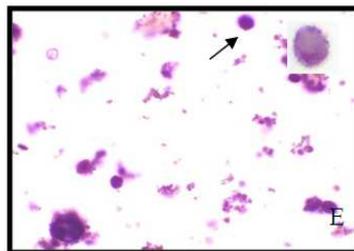
A : Normal Cells with no change in the morphology being round in shape with fine granules and regular surface

B: Cells stimulated with 4 µg/ml of compound 48/80 where most of the cells are degranulating

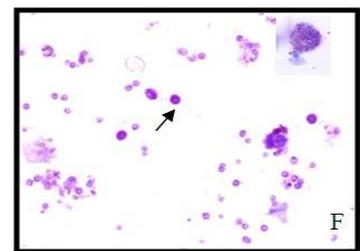
C: Cells pretreated with DMSO (vehicle) showing same structural changes like compound 48/80 induced degranulation



D: Cells pretreated with 10 µM of Curcumin and then stimulated with 4 µg/ml of compound 48/80 where lesser number of degranulating cells



E: Cells pretreated with 20 µM of Curcumin and then stimulated with 4 µg/ml of compound 48/80 where very few cells are degranulating



F: Cells pretreated with 50 µM Curcumin and minimum degranulation was observed. Arrow indicates mast Cells. (Morphology of the mast cells in particular group is shown in the inset; X 400 magnification).

Fig 2. Light microscopic photographs showing morphological changes in Compound 48/80 induced MPMC degranulation and inhibitory effect of curcumin and DSCG

Table 2. Effect of curcumin on Compound48/80 induced mast cell degranulation in vitro

Compound 48/80(4µg/ml)	Curcumin(µM)	% mast cell degranulation
+	-	99±1.2
+	DMSO	99±2.0
+	10	50±2.3
+	20	30±2.5
+	50	20±3.0

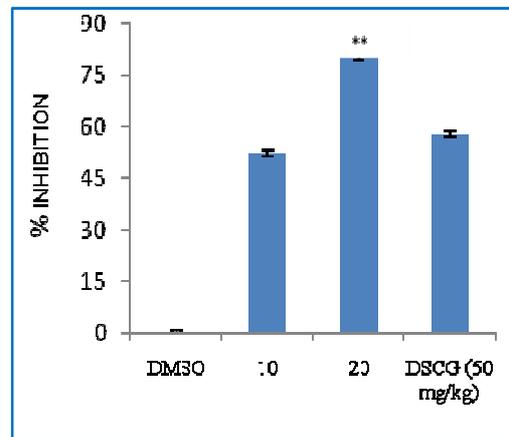


Fig.1b. Curcumin (10-20 mg/kg, ip) inhibited histamine release after systemic anaphylaxis in dose-dependent manner and better effective than DSCG. Values represent the mean ±SEM of five different experiment. **p*<0.05 significantly different from normal group ***p*<0.05 significantly different from C48/80 group.

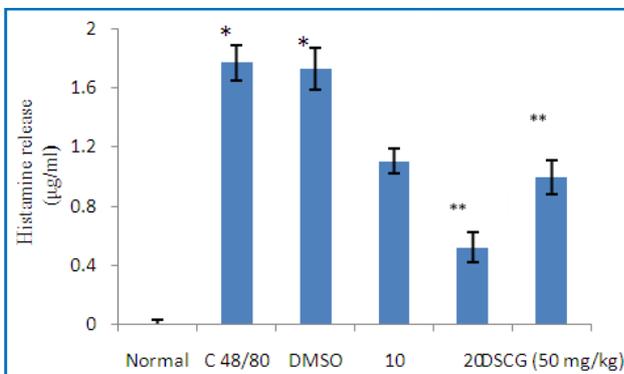


Fig.1a. Effect of curcumin on histamine release during systemic anaphylaxis induced by compound 48/80. Histamine release was inhibited in curcumin treated groups .

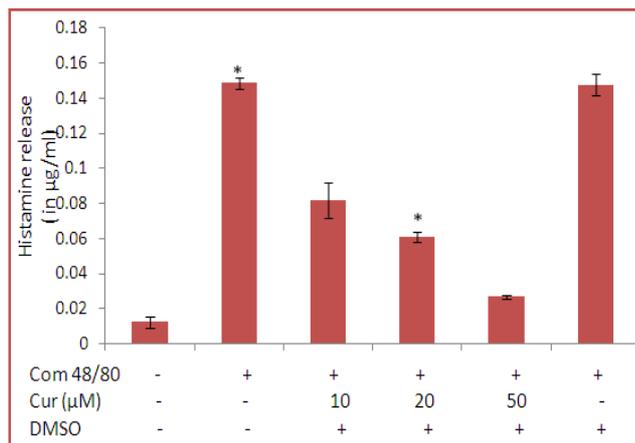


Fig 3. Effect of curcumin on histamine release in MPMCs induced by compound 48/80. Curcumin dose-dependently inhibited compound 48/80 induced histamine release (* $P < 0.05$).

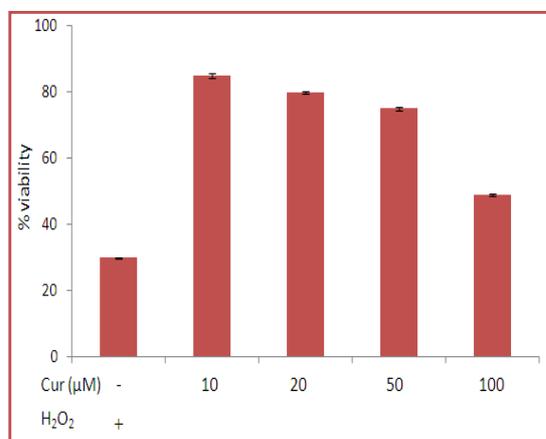


Fig 4. Effect of Curcumin on MPMCs viability. Curcumin was not toxic at 10-50µM but found toxic at 100µM as determined by MTT assay. Minimum viability was compared with H₂O₂ (an apoptosis inducer) and taken as negative control. Each value represents mean \pm S.E.M of five independent experiments.

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