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4-arylcoumarin inhibits immediate-type allergy

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Abstract
This study was undertaken to investigate the effect of 4-arylcoumarin (5,7-dimethoxy-4-*p*-methoxyphenylcoumarin; MPC) on immediate-type allergic reaction and inflammatory cytokine secretion. MPC inhibited compound 48/80-induced systemic reactions in mice. When MPC was given as a pretreatment at concentrations ranging from 0.001 to 1 g/kg, the serum histamine levels induced by compound 48/80 were reduced in a dose-dependent manner. In addition, MPC also inhibited the passive cutaneous anaphylaxis activated by anti-dinitrophenyl (DNP) IgE antibody dose dependently. Furthermore, MPC decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor-α and interleukin-6 secretion in human mast cells. These results indicate that MPC may be beneficial in the treatment of immediate-type allergic reactions.

Keywords: *4-arylcoumarins, immediate-type allergic reaction, mast cells, tumor necrosis factor-α, interleukin-6.*

Introduction

Mast cells are important mediators of inflammatory responses such as allergy and hypersensitivity. Immediate-type hypersensitivity (anaphylaxis), an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to the Fcε receptor I (FcεRI) on mast cells. Mast cell activation causes the process of degranulation, which results in the releasing of mediators, such as histamine and an array of inflammatory cytokines (Metcalf et al. 1981; Lagunoff et al. 1983; Church et al. 1997). Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen et al. 1996). Mast cell activation is initiated upon interaction of multivalent antigen with its specific IgE antibody attached to the cell membrane via FcεRI (Metzger et al. 1986; Alber et al. 1991). Anti-dinitrophenyl (DNP) IgE antibody and

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antigen have been established to induce passive cutaneous anaphylaxis (PCA) reactions as a typical *in vitro* model for immediate hypersensitivity. Mast cell degranulation can also be elicited by non-immunologic stimulators, such as neuropeptides, basic compounds, complement components, and certain drugs (Lagunoff et al. 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent stimulators of 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 (Ennis et al. 1980). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators, such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines, such as tumor necrosis factor (TNF-α), interleukins (IL-6, IL-4, IL-13), and transforming growth factor-β (Burd et al. 1989; Plaut et al. 1989; Galli et al. 1991; Bradding et al. 1993). Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. In experimental allergic models, disodium cromoglycate (DSCG; a mast cell stabilizer) shows an obvious inhibitory effect on the immediate-type allergic reactions. This inhibitory effect is thought to be based on the inhibition of the mediator from mast cells (Thomson & Evans 1973; Wells & Mann 1983). As part of our continuing search for biologically active anti-allergenic agents from the endophytic actinomycetes, 5,7-dimethoxy-4-*p*-methoxyphenylcoumarin (Figure 1), the major active ingredients from the culture filtrate of *Streptomyces aureofaciens* CMUAc130 (Taechowisan et al. 2005) showed anti-inflammatory activity (Taechowisan et al. 2007). In the present study, we evaluated the effect of 5,7-dimethoxy-4-*p*-methoxyphenylcoumarin (MPC) on compound 48/80-induced systemic reaction and anti-dinitrophenyl (DNP) IgE antibody-induced local allergic reaction. Additionally, the effect of 5,7-dimethoxy-4-*p*-methoxyphenylcoumarin on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced TNF-α and IL-6 secretion in human mast cells (HMC-1) was also investigated.

Materials and methods

Preparation of 4-arylcoumarin

Streptomyces aureofaciens CMUAc130 was isolated from the root tissues of *Zingiber officinale* by the surface-sterilization technique (Taechowisan et al. 2003). Identification of the isolate to species level was based on morphological, cultural, physiological and

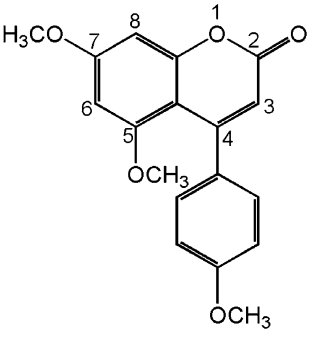

Figure 1. Chemical structure of 5,7-dimethoxy-4-*p*-methoxyphenylcoumarin.

biochemical characteristics, and 16S rDNA gene sequencing as described by Taechowisan and Lumyong (2003). Solid medium for sporulation used in this study was International *Streptomyces* Project Medium 4 (ISP-4) and the liquid medium used for fermentation was ISP-2 (Shirling & Gottlieb 1966). Large-scale fermentation, crude extraction and purification of the compounds were carried out following the methods of Taechowisan et al. (2005).

Structure elucidation of the compounds

The structure of the active compound has been identified using NMR and mass spectral data. The melting point of the compounds was determined on a Buchi-540 melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, IR spectra on a Perkin-Elmer 1 spectrometer, ^{1}H and ^{13}C NMR spectra on a Bruker DRX 500 spectrometer, and EI-MS and FAB-MS, respectively, on a Hewlett-Packard 5989 B and a Finnigan/Thermo Quest Mat 95 XL mass spectrometer.

Compound 48/80-induced systemic reaction

Male ICR mice were purchased from National Laboratory Animal Center (Mahidol University, Thailand). Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator, compound 48/80 (Sigma Chemical Co., USA). MPC was dissolved in saline and administered intraperitoneally (range: 0.001–1 g/kg BW) 1 h before the injection of compound 48/80 ($n = 10$/group). In the time-dependent experiment, MPC (1 g/kg) was administered intraperitoneally at 5, 10 and 20 min after injection of compound 48/80 ($n = 10$/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.

Passive cutaneous anaphylaxis reaction

The mice were injected intradermally with 0.5 μg of anti-DNP IgE (Sigma Chemical Co.) into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. After 48 h, each mouse was received an injection of 1 μg of DNP-human serum albumin (DNP-HAS) (Sigma Chemical Co.) in PBS containing 4% Evans blue (1:4) via the tail vein. MPC (0.01–1 g/kg BW) was intraperitoneally administered 1 h before the challenge. Some 30 min after the challenge, the mice were sacrificed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13) based on the previous report (Katayama et al. 1978). The absorbent intensity of the extraction was measured at 620 nm using a spectrophotometer.

Preparation of plasma and histamine determination

The blood was centrifuged at $400 \times g$ for 10 min. The plasma was withdrawn and histamine content was measured by the *o*-phthaldialdehyde (Sigma Chemical Co.)

spectrofluorometric procedure (Shore et al. 1959). The fluorescent intensity was measured at a 438-nm emission and a 353-nm excitation using a spectrofluorometer.

Assay of TNF-α and IL-6 secretion

TNF-α and IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Scuderi et al. 1986). HMC-1 cells were cultured with α-minimal essential medium (α-MEM; Sigma Chemical Co.) plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with 20 nM of phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co.) plus 1 μM of calcium ionophore A23187 (A23187) (Sigma Chemical Co.) for 6 h in the absence or presence of MPC. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF-α and IL-6, respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF-α and rIL-6 (R&D Systems Inc., USA) were added to serum previously determined to be negative to endogenous TNF-α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF-α and IL-6, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (Sigma Chemical Co.) tablets substrates. Optical density reading was made within 10 min of the addition of the substrate with a 405-nm filter.

Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 11.01 (SPSS Inc., Chicago, IL, USA). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. A p value ≤ 0.05 was used to indicate significance.

Results

MPC inhibits compound 48/80-induced systemic reaction

To assess the contribution of MPC in systemic reaction, an *in vivo* model of systemic anaphylaxis was used. Compound 48/80 (0.008 g/kg) was used as a model of induction of systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. Compound 48/80 induced fatal shock in 100% of animals. When PBS was administered intraperitoneally at concentrations ranging from 0.001 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced (Table I). In addition, the mortality of mice administered with MPC (1 g/kg) 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table II). In this same *in vivo* experiment, DSCG also had an inhibitory effect on compound 48/80-induced systemic reaction.

MPC inhibits compound 48/80-induced plasma histamine release

The effect of MPC on compound 48/80-induced plasma histamine release was investigated. MPC (0.001–1 g/kg BW) was administered 1 h before the compound

Table I. Effect of MPC on compound 48/80-induced systemic allergic reaction.

Treatment (g/kg BW)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)	
		MPC	DSCG
None (saline)	+	100	100
0.001	+	100	100
0.005	+	100	100
0.01	+	80	100
0.05	+	50	70
0.1	+	30	40
0.5	+	10	0
1	+	0	0
1	−	0	0

Groups of mice ($n = 10$/group) were intraperitoneally pretreated with 200 μl of saline or MPC or DSCG. Various doses of MPC or DSCG were given 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

48/80 injection. MPC (0.05–1 g/kg) and DSCG (1 g/kg) significantly inhibited compound 48/80-induced plasma histamine release (Figure 2).

MPC inhibits the IgE-mediated local allergic reaction

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions and, in part, mediated by histamine in the blood stream (Mican et al. 1992). Local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Intraperitoneal administration of MPC (0.1–1 g/kg) and DSCG (1 g/kg) showed a marked inhibition rate in PCA reaction (Figure 3).

MPC inhibits pro-inflammatory cytokine secretion in HMC-1 cells

We examined whether MPC could regulate pro-inflammatory cytokines, such as TNF-α and IL-6 in HMC-1 cells. HMC-1 cell line is a useful cell for studying cytokine activation pathway (Sillaber et al. 1993; Moller et al. 1998). Stimulation of HMC-1 cells with PMA plus A23187 induced the secretion of both cytokines. However, pretreatment with MPC decreased PMA plus A23187-induced TNF-α and IL-6 secretion (Figures 4 and 5).

Table II. Time-dependent effect of MPC on compound 48/80-induced systemic allergic reaction.

Treatment (g/kg BW)	Time (min)	Compound 48/80 (0.008 g/kg BW)	Mortality (%)	
			MPC	DSCG
None (saline)		+	100	100
1	5	+	30	0
1	10	+	70	50
1	20	+	100	100

Groups of mice ($n = 10$/group) were intraperitoneally pretreated with 200 μl of saline or MPC or DSCG. MPC or DSCG (1 g/kg) was given at 5, 10, and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

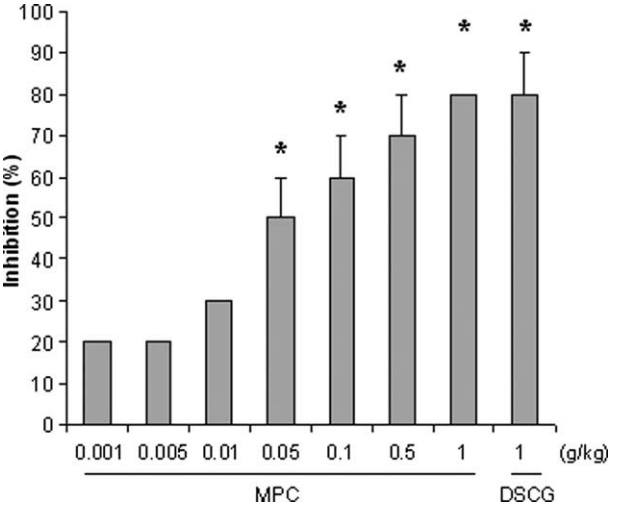

Figure 2. Effect of MPC on compound 48/80-induced plasma histamine release. Groups of mice ($n = 10$ group) were intraperitoneally pretreated with 200 μl of saline or MPC or DSCG. MPC or DSCG was given 1 h before the intraperitoneal injection of compound 48/80. Each date represents the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the saline value.

Discussion

Several reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Many studies have shown that compound 48/80 and other polybasic compounds are able to activate G-proteins (Mousli et al. 1990a,b; Chahdi et al. 2000). Tasaka et al. (1986) reported that compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. These

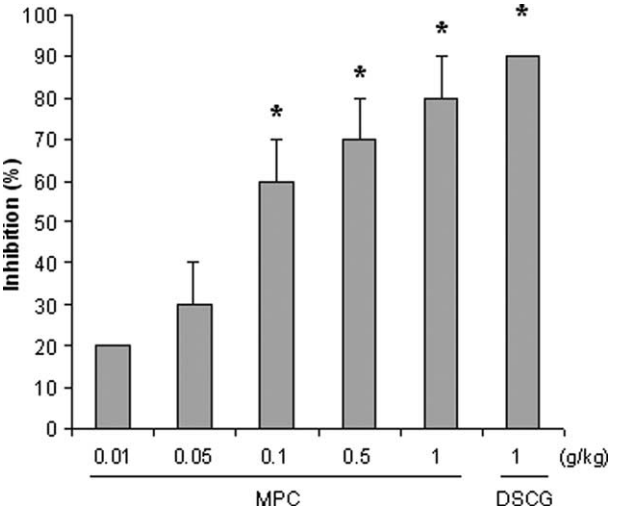

Figure 3. Effect of MPC on the PCA reaction. MPC or DSCG was intraperitoneally administered 1 h prior to the challenge with antigen. Each date represents the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the saline value.

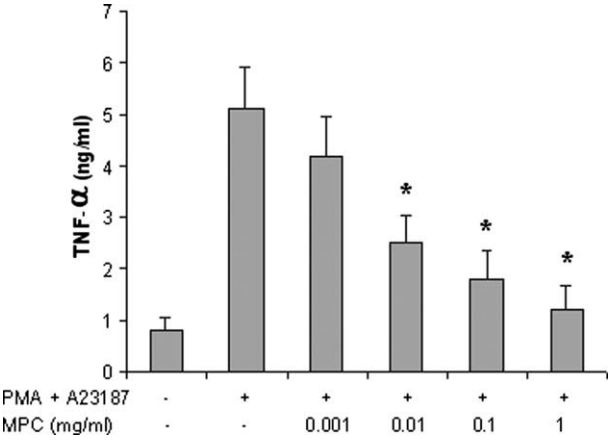

Figure 4. Effect of MPC on the TNF-α secretion. PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of MPC. TNF-α secreted into the medium is presented as the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the PMA + A23187 value.

reports indicate that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents with a membrane-stabilizing action may be desirable. The results of our study demonstrated that MPC has anti-allergic properties. MPC and DSCG inhibited compound 48/80-induced systemic reaction and anti-DNP IgE-mediated local allergic reaction. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by MPC. Taken together, we could speculate that MPC might stabilize the lipid bilayer membrane, thus preventing the perturbation induced by compound 48/80. In addition, mice administered MPC are protected from anti-DNP IgE-mediated PCA, one of the most important *in vivo* models of anaphylaxis in

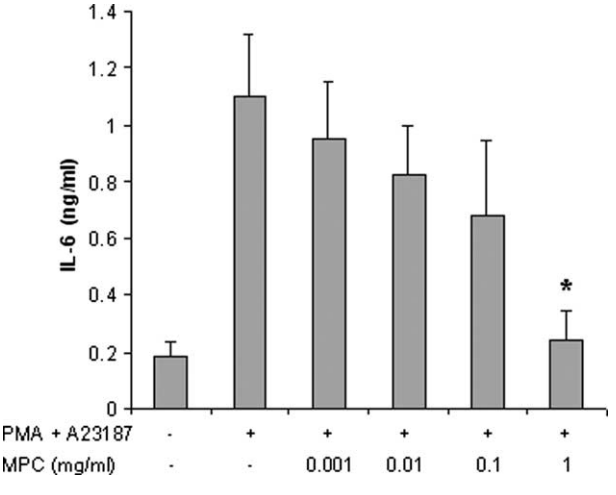

Figure 5. Effect of MPC on the IL-6 secretion. PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of MPC. IL-6 secreted into the medium is presented as the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the PMA + A23187 value.

local allergic reaction. This finding suggests that MPC might be useful in the treatment of allergic skin reactions. Mast cell-derived cytokines, especially TNF-α and IL-6 have a critical biological role in the allergic reaction. Mast cells are a principal source of TNF-α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the anti-TNF-α antibody (Walsh et al. 1991). IL-6 is also produced from mast cells and its local accumulation is associated with a PCA reaction (Mican et al. 1992). These reports indicate that reduction of pro-inflammatory cytokines from mast cell is a one of the key indicator of reduced allergic symptom. In the present study, MPC inhibited the secretion of TNF-α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the anti-allergic effect of MPC results from its reduction of TNF-α and IL-6 release from mast cells.

In conclusion, the results obtained in the present study provide evidence that MPC contributes importantly to the prevention or treatment of mast cell-mediated allergic diseases. In addition, it suggests that MPC may contain compounds with actions that inhibit mast cell-mediated allergic reactions *in vivo* and *in vitro*. The effort to identify active components from MPC in the immediate-type allergic reaction is ongoing in our laboratory.

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