Gallic Acid Inhibits Histamine Release and Pro-inflammatory Cytokine Production in Mast Cells

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The discovery of drugs for the treatment of inflammatory allergic diseases such as, asthma, allergic rhinitis, and sinusitis is a very important subject in human health. Gallic acid (3,4,5-trihydroxybenzoic acid), a polyphenyl natural products from gallnut and green tea, is known to have anti-oxidant, anti-inflammatory, anti-microbial, and radical scavenging activities. The aim of the present study was to elucidate whether gallic acid modulates the inflammatory allergic reaction and to study its possible mechanisms of action. Gallic acid attenuated compound 48/80- or immunoglobulin E (IgE)-induced histamine release from mast cells. The inhibitory effect of gallic acid on the histamine release was mediated by the modulation of cAMP and intracellular calcium. Gallic acid decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated pro-inflammatory cytokine gene expression and production such as TNF-α and IL-6 in human mast cells. The inhibitory effect of gallic acid on the pro-inflammatory cytokine was nuclear factor-κB and p38 mitogen-activated protein kinase dependent. In addition, gallic acid inhibited compound 48/80-induced systemic allergic reaction and IgE-mediated local allergic reaction. The inhibitory activity of gallic acid on the allergic reaction and histamine release was found to be similar with disodium cromoglicate. Our findings provide evidence that gallic acid inhibits mast cell-derived inflammatory allergic reactions by blocking histamine release and pro-inflammatory cytokine expression, and suggest the mechanisms of action. Furthermore, in vivo and in vitro anti-allergic effect of gallic acid suggests a possible therapeutic application of this agent in inflammatory allergic diseases.

Key Words: gallic acid; inflammatory allergic reaction; mast cell; histamine; inflammatory cytokine.

Gallic acid and its derivatives are polyphenyl natural products and particularly abundant in processed beverages such as red wine and green tea (Graham, 1992). It has a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial, and anti-cancer activities (Bachrach and Wang, 2002; Kroes et al., 1992; Kubo et al., 2001).

Mast cells, which are constituents of virtually all organs and tissue, are important mediators of allergic reaction. Immediate hypersensitivity (anaphylaxis) is mediated by histamine release in response to antigen cross-linking of immunoglobulin E (IgE) bound to FcεRI on mast cells. Mast cell activation causes the process of degranulation that results in the release of mediators, such as histamine and an array of inflammatory cytokines (Church and Levi-Schaffer, 1997; Metcalfe et al., 1981). 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 degranulation also can be elicited by the synthetic compound 48/80, and it has been used as a direct and convenient reagent to study the mechanism of allergic reaction (Ennis et al., 1980).

The signaling pathway leading to degranulation of mast cells after engagement of the FcεRI receptor has been extensively characterized (Beaven et al., 1984; Metcalfe et al., 1981). Activation of mast cells leads to phosphorylation of tyrosine kinase and mobilization of internal Ca2+. This is followed by activation of protein kinase C, mitogen-activated protein kinase (MAPKs), nuclear factor-κB (NF-κB), and releasing of inflammatory cytokines. 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-α (TNF-α), interleukin (IL)-6, IL-4, IL-13 and transforming growth factor-β (Bradding et al., 1993; Burd et al., 1989; Plaut et al., 1989). TNF-α and IL-6 from mast cells promotes leukocyte migration and inflammatory lesions.
Although these inflammatory cytokines possess beneficial effect on host defense, they could trigger pathological conditions when overexpressed. Therefore the reduction of TNF-α and IL-6 from mast cells is one of the key indicators of reduced inflammatory allergic symptoms.

MAPKs and transcription factor NF-κB have important activities as mediators of cellular responses to extracellular signals. Some of the MAPKs important to mammalian cells include extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38. p38 MAPK and NF-κB are thought to play an important role in the regulation of pro-inflammatory molecules on cellular responses, especially, TNF-α, IL-1β, and IL-6 (Azzolina et al., 2003; Baldassare et al., 1999; Beyaert et al., 1996).

The objective of the present study was to assess the effect of gallic acid on the inflammatory allergic reaction. We also investigated the molecular mechanism responsible for the inhibitory effect of gallic acid on histamine releases and pro-inflammatory cytokine production in mast cells. In addition, we examined the effect of gallic acid on systemic and local allergic reaction to assess its anti-allergic effect in vivo. In the present study, we clearly demonstrated that gallic acid has an anti-allergic effect on in vivo and in vitro allergy models.

MATERIALS AND METHODS

Reagents. Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), o-phthaldialdehyde, phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, metrizamide, pyrrolidine dithiocarbamate (PDTC), disodium cromoglycate (DSCG), and gallic acid were purchased from Sigma (St. Louis, MO). SB 203580 was purchased from Calbiochem (La Jolla, CA). rTNF-α and rIL-6 were purchased from R&D Systems Inc. (Minneapolis, MN).

Animals. The original stock of male ICR mice and male Sprague-Dawley rats were purchased from Dae-Han Experimental Animal Center (Daejeon, Korea). The animals were housed 5 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. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Preparation of rat peritoneal mast cells (RPMC) and cell culture. RPMCs were isolated as previously described (Kim et al., 2005). In brief, the peritoneal cells were suspended in Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 12 mM NaHCO3, 2.7 mM KCl, 0.3 mM Na2HPO4, and 0.1% gelatin), layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged for 15 min at 400 x g. The cells that remained at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode buffer. 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 exclusion. Human mast cells (HMC-1) were cultured in Iscove’s medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated serum albumin (HSA), o-phthaldialdehyde, and antibiotics-free Iscove’s medium containing 8 μg/ml penicillin/streptomycin.

Histamine assay. Histamine content of RPMCs and serum was measured by the o-phthaldialdehyde spectrofluorometric procedure as previously described (Kim et al., 2005). RPMCs were preincubated with gallic acid, and then incubated for 10 min with compound 48/80 (5 μg/ml). RPMC suspensions (2 x 105 cells/ml) were also sensitize with anti-DNP IgE (10 μg/ml) for 16 h. The cells were preincubated with gallic acid at 37°C for 10 min prior to the challenge with DNP-HSA (1 μg/ml). The cells were separated from the released histamine by centrifugation at 400 g for 5 min at 4°C. The blood from the mice was centrifuged at 400 x g for 10 min and the serum was withdrawn to measure histamine content.

cAMP assay. The cAMP level was measured as previously described (Peachell et al., 1988). In brief, RPMCs were resuspended in prewarmed Tyrode buffer. An aliquot of cell was added to an equivalent volume of prewarmed buffer containing gallic acid. The reaction was allowed to proceed for indicated time and terminated by the addition of ice-cold acidified ethanol. The sample was reconstituted in assay buffer and cAMP level was determined by enzyme immunoassay using a commercial kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Intracellular Ca2+ measurements. Fura-2/AM (2 μM, Molecular Probes, Eugene, OR) was used to determine the intracellular calcium following the manufacturer’s protocol briefly described as following. RPMCs were preincubated with Fura-2/AM for 30 min at 37°C. After washing the dye from the cell surface, gallic acid was pretreated 10 min prior to the compound 48/80 treatment. The fluorescent intensity was recorded using a fluorescent plate reader (Molecular Devices, Sunnyvale, CA) at an excitation of 340 nm and an emission of 500 nm.

RNA extraction and semiquantitative RT-PCR. The total cellular RNA was isolated from the cells (1 x 10⁶ per well in 24-well plate) after stimulation of PMA plus A23187 with or without gallic acid using a TRI reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol. The first strand complementary DNA (cDNA) was synthesized using the Superscript II reverse transcriptase enzyme (Life Technologies, Grand Island, NY). A reverse-transcribe polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-6, and β-actin (internal control). The conditions for the reverse transcription and PCR steps were similar to those previously described (Kim and Sharma, 2004). The primer sets were chosen by the Primer3 program (Whitehead Institute, Cambridge, MA). The cycle number was optimized in order to ensure product accumulation in the exponential range. The amplified products were separated by electrophoresis on 2% agarose gel containing ethidium bromide, documented using a Kodak DC 290 digital camera, and digitized with UN-SCAN-IT software (Silk Scientific, Orem, UT).

Assay of TNF-α and IL-6 secretion. The secretion of TNF-α and IL-6 was measured by modification of an enzyme-linked immunosorbent assay (ELISA). HMC-1 cells were stimulated with PMA (20 nM) plus A23187 (1 μM). 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 were added to the serum which was 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 or IL-6, and IL-6, 2.2’-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) tablet substrates. Optical density was read within 10 min of the addition of the substrate with a 405 nm filter.

Transient transfection and luciferase activity assay. For transient transfections, HMC-1 cells were seeded at 2 x 10⁵ in a 6-well plate 1 day before transient transfection. The expression vectors containing the dominant negative form of p38 kinase (DN-p38 kinase in PCMv5) (a gift from Dr. Kim, Chonbuk National University Medical School, Jeonbuk, Korea), or NF-κB luciferase reporter construct (pNF-κB-LUC, plasmid containing NF-κB binding site; STANTAGEN, Grand Island, NY) were transfected with serum and antibiotics-free Iscove’s medium containing 8 μl of Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). After 5 h of incubation, medium was replaced with Iscove’s medium containing 10% FBS and antibiotics. Cells were allowed to recover at 37°C for 20 h and subsequently were stimulated as
indicated. Cells were then used for the Western blot analysis. For luciferase activity assay, cell lysates were prepared and assayed for luciferase activity using Luciferase Assay System (Promega, Madison, WI), according to the manufacturer’s instructions.

Western blot analysis. HMC-1 cells (3 × 10^6 in a 6-well plate) were washed with PBS and resuspended in lysis buffer. Samples of protein were electrophoresed using 8–12% SDS-PAGE, as previously described (Kim and Sharma, 2004), and then transferred to nitrocellulose membrane. The p38 MAPK, ERK, and JNK activation was determined using anti-phospho-p38, -ERK, and -JNK antibodies (Cell Signaling, Beverly, MA). The nucleus and cytosolic p65 NF-κB, and IkBα was assayed using anti-NF-κB (p65) and anti-IkBα antibodies (Santa Cruz Biotech, Santa Cruz, CA). Immunodetection was done using an enhanced chemiluminescence detection kit (Amersham).

Electrophoretic mobility shift assay (EMSA). Nuclear protein (10 μg) was incubated for 20 min at room temperature with 20 μg of BSA, 2 μg of poly (dI-dC) from Pharmacia (Uppsala, Sweden), 2 μg of buffer C (20 mM HEPES, 20% glycerol, 100 mM KCl, 0.5 mM PMSF), 4 μg of buffer F (20% ficoll-400, 100 mM HEPES, 300 mM KCl, 10 mM DTT, 0.5 mM PMSF), and 20,000 cpm of a 32P-labeled probe encoding the NF-κB consensus sequence (5′-GAT CTC AGA GGG GAC TTT CCG AGA AGA-3′) or activated protein-1 (AP-1) oligonucleotide (5′-GAT CTG CAT GAG TCA GAC ACA CA-3′) in a final volume of 20 μl. DNA-protein complexes were resolved at 120 V for 2 h in a 5% polyacrylamide gel, dried, and visualized.

Compound 48/80-induced systemic allergic reaction. Mice were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80. Gallic acid was dissolved in saline and intraperitoneally administered 1 h before the 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 to measure serum histamine content.

Passive cutaneous anaphylaxis (PCA). An IgE-dependent cutaneous reaction was examined as previously described (Kim et al., 2004). The PCA reaction was generated by sensitizing skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the mouse tail vein. The anti-DNP IgE antibody and DNP-HSA were diluted in PBS. The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of 2 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-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. Gallic acid was intraperitoneally administered 1 h before the challenge. Thirty minutes after the challenge, the mice were killed 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). The intensity of absorbance was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

Statistical analysis. Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one-way ANOVA followed by Duncan’s Multiple Range test. A value of p < 0.05 was used to indicate significant differences.

RESULTS

Effect of Gallic Acid on Compound 48/80- or IgE-Mediated Histamine Release from RPMC

We first evaluated the ability of gallic acid to inhibit compound 48/80-induced or IgE-mediated histamine release from RPMC. Very low levels of histamine were detectable in unstimulated cells; however, RPMC released a high level of histamine when stimulated with compound 48/80 (5 μg/ml) or when anti-DNP IgE sensitized RPMC were challenged with DNP-HSA (Fig. 1). Gallic acid dose-dependently inhibited compound 48/80-induced histamine release (83.3% inhibition at 1 μM and 93.3% inhibition at 10 μM). In addition, gallic acid inhibited IgE-mediated histamine release in a dose-dependent manner (70.0% inhibition at 1 μM and 83.3% inhibition at 10 μM). The concentration and duration of gallic acid treatment used in these studies had no significant effect on the viability of RPMC (data not shown).

Effect of Gallic Acid on cAMP and Intracellular Calcium in RPMC

To investigate the mechanisms of gallic acid on the reduction of histamine release, we assayed the cAMP levels and intracellular calcium. When RPMC were incubated with gallic acid (10 μM), the cAMP content increased at 15–60 s and decreased to basal levels at 120 s (Fig. 2A). Calcium movements across membranes of mast cells are critical to histamine release (Beaven and Metzger, 1993). To further investigate the mechanisms of gallic acid on the reduction of histamine release, we assayed the intracellular calcium. Figure 2B shows the stimulation of intracellular calcium when the RPMC are treated with compound 48/80 (2 μg/ml). Preincubation of gallic acid (10 μM) with RPMC decreased the intracellular calcium level induced by compound 48/80.

Effect of Gallic Acid on Pro-Inflammatory Cytokine Expressions in Human Mast Cells (HMC-1)

We examined whether gallic acid 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). Stimulation of HMC-1 cells with PMA (20 nM) plus A23187 (1 lM) during 4 h induced the gene expression of both cytokines. Gallic acid (0.1–10 lM) dose-dependently inhibited TNF-α and IL-6 gene expression induced by PMA plus A23187 (Fig. 3A). In contrast to TNF-α and IL-6, the level of β-actin mRNA expression remained the same under these conditions. To confirm the correlation of mRNA expression with protein production, we measured the TNF-α and IL-6 secretion by ELISA (Fig. 3B).

Stimulation of HMC-1 cells with PMA (20 nM) plus A23187 (1 lM) during 16 h induced the secretion of both cytokines. Treatment with gallic acid (10 lM) blocked TNF-α and IL-6 secretion induced by PMA plus A23187 in HMC-1 cells (68.4% inhibition in TNF-α and 49.8% inhibition in IL-6) (Fig. 3B).

**Effect of Gallic Acid on NF-κB Activation**

NF-κB is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory responses. To investigate the intracellular mechanism responsible for the inhibitory effect of gallic acid on TNF-α and IL-6 expression, we examined the effect of gallic acid on NF-κB activity using Western blot and EMSA. Stimulation of HMC-1 cells with PMA plus A23187 for 2 h induced the degradation of IκBα and nuclear translocation of p65 NF-κB. Gallic acid inhibited the PMA plus A23187-induced degradation of IκBα and nuclear translocation of p65 NF-κB (Fig. 4A).

In order to investigate whether NF-κB/DNA binding was inhibited by gallic acid, we performed EMSA. We used PDTC, a potent inhibitor of NF-κB as a positive control. Cells pretreated with either gallic acid (10 lM) or PDTC (50 lM) were subsequently stimulated by PMA plus A23187, and the effect of gallic acid and PDTC on binding activity of transcription factors was examined. Treatment of PMA plus A23187 caused a significant increase in the DNA binding activity of NF-κB within 4 h (Fig. 4B). In the presence of gallic acid, PMA plus A23187-induced NF-κB/DNA binding was markedly suppressed. Because AP-1 is also involved in the expression of inflammatory cytokines, we examined the effect of gallic acid on DNA binding activity of AP-1. Although AP-1 binding was substantially up-regulated by PMA plus A23187, this AP-1 binding was not inhibited by gallic acid or PDTC.
To further confirm the inhibitory effect of gallic acid on NF-κB activation, we examined the effect of gallic acid on the NF-κB-dependent gene reporter assay. HMC-1 cells were transiently transfected with a NF-κB-luciferase reporter construct or the empty vector. Exposure of cells to PMA plus A23187 increased the luciferase activity in the cells transfected with the NF-κB-luciferase reporter construct (Fig. 4C). Gallic acid significantly reduced the PMA plus A23187-induced luciferase activity. Taken together with the data showing the inhibition of TNF-α and IL-6 production with the inhibitor of NF-κB, PDTC (Fig. 5D), NF-κB is involved in PMA plus A23187-induced TNF-α and IL-6 expression in HMC-1 cells.

Effect of Gallic Acid on p38 MAPK Activation

MAPK pathways play a crucial role in the regulation of pro-inflammatory molecules on cellular responses (Arbabi and Maier, 2002; Beyaert et al., 1996). Previously we documented that PMA plus A23187 activates all three types of MAPKs such as p38, JNK, and ERK at 15–30 min in HMC-1 cells (Kim et al., 2005). To evaluate the mechanisms of effect of gallic acid on the pro-inflammatory cytokine expression, we examined the effect of gallic acid on the activation of MAPKs. Gallic acid (10 μM) attenuated the PMA plus A23187-induced p38 MAPK activation but did not affect the phosphorylation of JNK and ERK (Fig. 5A). To confirm the idea that modulation of p38 MAPK activity is important for the expression of TNF-α and IL-6 in our system, HMC-1 cells were transiently transfected with dominant negative form of p38 MAPK (DN-p38). To determine if expression of DN-p38 constructs affects TNF-α and IL-6 secretion, transfected cells were stimulated with PMA plus A23187 and cultured for 16 h. Compared to transfection with the control vector, pCMV5, p38 variant attenuated PMA plus A23187-induced TNF-α and IL-6 secretion (44.8% inhibition in TNF-α and 37.8% inhibition in IL-6). Expression of DN-p38 was confirmed by Western blot (Fig. 5B).

To further strengthen the relation of p38 MAPK and pro-inflammatory cytokines expressions in our system, we next investigated the effects of pharmacological agents that modulate p38 MAPK activity. Treatment of cells with the selective p38 inhibitor, SB 203580 (5 μM), blocked the PMA plus A23187-induced TNF-α and IL-6 production assayed by Western blot (Fig. 5C). The secretion of TNF-α and IL-6 induced by PMA plus A23187 was also blocked by SB 203580 (Fig. 5D). These data indicate that p38 MAPK activation contribute to the regulation of TNF-α and IL-6. Both pharmacologic agents (SB 203580 and PDTC) used in this experiment did not show cytotoxicity (data not shown).
Effect of Gallic Acid on the Compound 48/80-Induced Systemic Allergic Reaction

The in vivo model of systemic immediate-type allergic reaction (anaphylaxis) was used in order to assess the impact of gallic acid on immediate-type allergic reaction. Compound 48/80 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 (8 mg/kg, BW) induced fatal shock in 100% of animals. When gallic acid was intraperitoneally administered at concentrations ranging from 1 to 50 mg/kg (BW) for 1 h, the mortality induced by compound 48/80 was dose-dependently reduced (Table 1). We compared the anti-anaphylactic effect of gallic acid with DSCG, a known anti-allergic drug. Gallic acid completely inhibited the compound 48/80-induced fatal shock at 50 mg/kg (BW), while DSCG inhibited the compound 48/80-induced fatal shock at 100 mg/kg (BW). The effect of gallic acid on the compound 48/80-induced serum histamine release was investigated as well. Gallic acid and DSCG were administered at concentrations ranging from 1 to 100 mg/kg (BW) 1 h prior to (n = 10/group) the injection of compound 48/80. Injection of compound 48/80 markedly caused an increase in serum histamine release which was inhibited by gallic acid treatment in a dose-dependent manner (63% inhibition at 10 mg/kg and 74.3% inhibition at 100 mg/kg) (Fig. 6A). While the inhibition of serum histamine release by DSCG was 60.1% at 100 mg/kg.

Effect of Gallic Acid on the IgE-Mediated PCA Reaction

Another way to test the anaphylactic reactions is to induce PCA. A local extravasation was induced by a local injection of IgE followed by an antigenic challenge. The administration of gallic acid (1–100 mg/kg) showed a marked inhibition in the PCA reaction (49.2% inhibition at 10 mg/kg and 65.8% inhibition at 100 mg/kg) (Fig. 6B). The inhibition of PCA reaction by DSCG was 56.3% at 100 mg/kg.
Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, and various cytokines from mast cells (Kemp and Lockey, 2002). Using in vitro and in vivo models, we show that gallic acid reduces mast cell-derived inflammatory allergic responses. In the present study, we clearly demonstrated that gallic acid decreased compound 48/80 or IgE-induced histamine release and PMA plus calcium ionophore-mediated pro-inflammatory cytokine expression. Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of the signal transduction pathway, which leads to histamine release. In the present study, we compared the effect of gallic acid with the clinically available anti-allergic drug, DSCG, which is known as a mast cell stabilizer (Sieghart et al., 1978). We observed that gallic acid has similar anti-allergic activities than that of DSCG. In addition, gallic acid administered to mice results in protection from IgE-mediated PCA, one of the most important in vivo models of anaphylaxis in local allergic reaction. This finding suggests that gallic acid might be useful in the treatment of allergic skin reactions.

DISCUSSION

Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, and various cytokines from mast cells (Kemp and Lockey, 2002). Using in vitro and in vivo models, we show that gallic acid reduces mast cell-derived inflammatory allergic responses. In the present study, we clearly demonstrated that gallic acid decreased compound 48/80 or IgE-induced histamine release and PMA plus calcium ionophore-mediated pro-inflammatory cytokine expression.

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cAMP and intracellular calcium pathways are critical to the degranulation of mast cells. Agents that stimulate an intracellular cAMP level have been shown to reduce mast cell degranulation. An increase of cAMP is believed to precede the inhibition of histamine release from mast cells in response to stimulation of IgE receptors or compound 48/80 (Kaliner and Austen, 1974; Tasaka et al., 1986; Weston and Peachell, 1998). In addition, calcium movements across membranes of mast cells represent a major target for effective anti-allergic drugs, as these are essential events linking stimulation to secretion (Beaven et al., 1984). The transduction pathways modulating cAMP and intracellular calcium are modified by ADP-ribosylates G-protein binding protein (Alfonso et al., 2000). The release of histamine is known to be depressed by an increase in intracellular cAMP content due to the activation of adenylate cyclase or inhibition of cAMP phosphodiesterase (Makino et al., 1987). The intracellular cAMP content of the mast cells, when incubated with gallic acid, increased in comparison with that of basal cells. These results suggest that the effects of gallic acid on the allergic reaction may be associated with an increase in the intracellular cAMP content of the mast cells as a result of an inhibition of the cAMP phosphodiesterase. The mode of action of gallic acid is likely related the prevention of calcium release from the calcium store of mast cells due to elevation of the intracellular cAMP level by inhibition of the cAMP phosphodiesterase. The fact that cAMP elevating drugs such as gallic acid inhibit calcium release from the intracellular calcium store suggests the regulatory role of cAMP in histamine release. Our results showing an enhancement of cAMP and attenuation of intracellular calcium in mast cells following gallic acid treatment are consistent with other reports. According from these observations, we strongly speculate that increased cAMP and

### TABLE 1

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<th>Dose (mg/kg)</th>
<th>Compound 48/80 (8 mg/kg)</th>
<th>Mortality (%)</th>
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<tr>
<td>None (saline)</td>
<td>+</td>
<td>100</td>
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<tr>
<td>Gallic acid 1</td>
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<td>100</td>
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*Note. Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μl of saline or drugs at various doses 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. DSCG, disodium cromoglycate.*

![Effect of gallic acid on serum histamine release and PCA reactions.](image-url)

(A) Groups of mice (n = 10/group) were intraperitoneally treated with 200 μl of saline or drugs at various doses 1 h before the intraperitoneal injection of the compound 48/80 (8 mg/kg). The blood was obtained from the heart of each mouse and histamine content was measured by spectrofluorometry. (B) Drugs were administered 1 h prior to the challenge with antigen. Each amounts of dye was extracted as described in Materials and methods and measured by spectrophotometry. *Statistical significant at p < 0.05. BW, body weight.*
decreased intracellular calcium might be involved in the inhibitory effect of gallic acid on histamine release.

The spectrum of cytokines produced by HMC-1 cells with PMA plus A23187 stimulation supports the well-recognized role of mast cells in immediate hypersensitivity. TNF-α and IL-6 play a major role in triggering and sustaining the inflammatory allergic response in mast cells. TNF-α promotes inflammation, leukocyte infiltration, granuloma formation and tissue fibrosis and is thought to be an initiator of cytokine related inflammatory states by stimulating cytokine production (Hide et al., 1997; Jeong et al., 2002b). Mast cells are a principal source of TNF-α in human dermis, and degranulation 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 PCA reaction (Mican et al., 1992). These reports indicate that reduction of TNF-α and IL-6 from mast cell is one of the key indicators of reduced allergic symptoms. Several reports show the relation of intracellular calcium and inflammatory cytokine production from mast cells (Jeong et al., 2002a; Tanaka et al., 2005). Depletion of intracellular calcium with various calcium blockers inhibits IgE-induced TNF-α and IL-6 production in mast cells. Furthermore, NF-κB pathway mediates these effects. In our present study, gallic acid decreased the elevated intracellular calcium levels and inhibited the gene expression and protein production of TNF-α and IL-6 in mast cells. These data suggest that inhibition of intracellular calcium level by gallic acid modulates inflammatory cytokine production.

To evaluate the mechanisms of effect of gallic acid on TNF-α and IL-6 expression, we examined the effect of gallic acid on NF-κB activation. Expression of TNF-α and IL-6 gene is dependent on the activation of transcription factor NF-κB (Collart et al., 1990). Activation of NF-κB requires phosphorylation and proteolytic degradation of the inhibitory protein IκBα, an endogenous inhibitor that binds to NF-κB in the cytoplasm (Azzolina et al., 2003). In PMA plus A23187-stimulated mast cells, gallic acid decreased the degradation of IκBα and nuclear translocation of p65 NF-κB. Gallic acid specifically inhibited DNA binding of NF-κB but not of AP-1. In addition, PDTC, a potent inhibitor of NF-κB, reduced PMA plus A23187-induced TNF-α and IL-6 production. These data demonstrate that gallic acid attenuates the activation of NF-κB and downstream TNF-α and IL-6 production.

The MAPK cascade is one of the important signaling pathways in immune responses (Arbabi and Maier, 2002). The expression of TNF-α and IL-6 is regulated by MAPKs. The exact signaling pathways among three types of MAPKs such as p38, ERK, and JNK, are still unclear; however, p38 MAPK is thought to play an important role in regulation of inflammatory responses. Activation of p38 MAPK is essential for the expression of the pro-inflammatory cytokines (Manthey et al., 1998; Shapiro and Dinarello, 1995). In our present study, PMA plus A23187 simultaneously activated all three MAPKs in HMC-1 cells. Among the MAPKs, gallic acid specifically inhibited the activation of p38 MAPK but not of ERK or JNK. Furthermore, the transfection of DN-p38 and the specific p38 MAPK inhibitor, SB 203580, decreased TNF-α and IL-6 production. These data suggest that gallic acid has the inhibitory activity on p38 MAPK activation and downstream TNF-α and IL-6 production.

Gallic acid, one of the polyphenolic compounds, has been reported to decreases histamine release from rat basophilic leukemia cells and suppresses pro-inflammatory cytokine production in murine peritoneal macrophages (Kwon et al., 2004; Matsuo et al., 1997). In addition, gallic acid related polyphenol compound, epigallocatechin-3-gallate and theaflavin-3,3’-digallate inhibit the activation of MAPK (Chung et al., 2001). These accumulated data support our findings showing the inhibitory effects of gallic acid to inflammatory allergic responses. The results presented in this report give an insight into the mechanism responsible for anti-allergic activity of gallic acid and evidence that gallic acid could contribute to the prevention or treatment of mast cell-mediated allergic diseases.

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REFERENCES


