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Role of Gallic Acid in Inflammatory Allergic Process

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The aim of the present study was to elucidate whether gallic acid could modulate the inflammatory allergic reaction and to study its mechanism of action Gallic acid inhibited 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 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, and the inhibitory effect of gallic acid was on dependent nuclear factor-κB and p38 mitogen-activated protein kinase. Our findings provide evidence that gallic acid inhibits mast cell-derived inflammatory allergic reaction by blocking histamine release and pro-inflammatory cytokine expression.

Key Words: Gallic acid, Inflammatory allergic reaction, Mast cell, Histamine, Inflammatory cytokine

INTRODUCTION

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 (Metcalfe et al, 1981; Church & Levi-Schaffer, 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 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 (Metcalfe et al, 1981; Beaven et al, 1984). Activation of mast cells leads to phosphorylation of tyrosine kinase and mobilization of internal Ca^{2+}. This is followed by activation of protein kinase C, mitogen- cti-vated protein kinase (MAPKs), nuclear factor-κB (NF-κB), and release 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 (Burd et al, 1989; Plaut et al, 1989; Bradding et al, 1993). TNF-α and IL-6 from mast cells promotes leukocyte migration and inflammatory lesions (Walsh et al, 1991; Mican et al, 1992; Hide et al, 1997). 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 cell is one of the key indicators of reduced inflammatory allergic symptom.

MAPKs and transcription factor NF-κB have important activities as mediators of cellular responses to extracellular signals. Some of the MAPKs, which are 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 (Beyaert et al, 1996; Baldassare et al, 1999; Azzolina et al, 2003).

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 (Kroes et al, 1992; Kubo et al, 2001; Bachrach & Wang, 2002).

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

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ABBREVIATIONS: RPMC, rat peritoneal mast cells; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; EMSA, electrophoretic mobility shift assay; PDTC, pyrrolidine dithiocarbamate; PMA, phorbol 12-myristate 13-acetate; HMC-1, human mast cells.

pro-inflammatory cytokine production in mast cells. In the present study, we clearly demonstrated that gallic acid has an anti-allergic effect on in vitro allergy models.

METHODS

Preparation of rat peritoneal mast cells (RPMC) and cell culture

RPMC 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 $NaHCO_3$, 2.7 mM KCl, 0.3 mM NaH_2PO_4 and 0.1% gelatin), layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged for 15 min at 400 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 trypane blue exclusion. Human mast cells (HMC-1) were cultured in Iscove's medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated FBS and 40 U/ml penicillin/streptomycin.

Histamine assay

Histamine content of RPMC and serum was measured by the o-phthaldialdehyde spectrofluorometric procedure as previously described (Kim et al, 2005). RPMC were preincubated with gallic acid, and then incubated for 10 min with compound 48/80 (5 μg/ml). RPMC suspensions (2×10^5 cells/ml) were also sensitized 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 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, RPMC were resuspended in prewarmed Tyrode buffer, and an aliquot of cells 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 Ca^{2+}

Fura-2/AM (2 μM, Molecular probes, Eugene, OR) was used to determine the intracellular calcium, by following the manufacturer's protocol. Briefly described, RPMC were preincubated with Fura-2/AM for 30 min at 37°C. After washing off the dye from the cell surface, the cells were pretreated with gallic acid 10 min before 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 (1×10^6/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-transcriptase 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 & Sharma, 2004). The primer sets were chosen by the Primer3 program (Whithead 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 sequentially exposed to biotinylated 2,2'-azino-bis (3-ethylbenzthiazoline-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 2×10^6 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 were subsequently 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 & Sharma, 2004), and then transferred to nitrocellulose membrane. The p38 MAPK, ERK, and JNK activation was determined using anti-phopspho-p38, -ERK, and -JNK antibodies, respectively (Cell Signaling, Beverly, MA). The nucleus and cytosolic p65 NF-κB, and IκBα were assayed using anti-NF-κB (p65) and anti-IκBα antibodies respectively (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 ^{32}P-labeled probe encoding the κB consensus sequence (5'-GAT CTC AGA GGG GAC TTT CCG AGA AGA-3') or 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.

Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one way analysis of variance 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 anti-DNP IgE sensitized RPMC were challenged with DNP-HSA (Fig. 1). Gallic acid dose-dependently inhibited the 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 sec and decreased to basal levels at 120 sec (Fig. 2A). Calcium movements across membranes of mast cells are critical to histamine release (Beaven & Metzger, 1993). Therefore, to further investigate the mechanisms of gallic acid on the reduction of histamine release, we assayed the intracellular calcium concentration. Fig. 2B shows the increase of intracellular calcium concentration when the RPMC were treated with compound 48/80 (2 μg/ml). Preincubation of RPMC with gallic acid (10 μM) decreased the intracellular calcium level induced by compound 48/80.

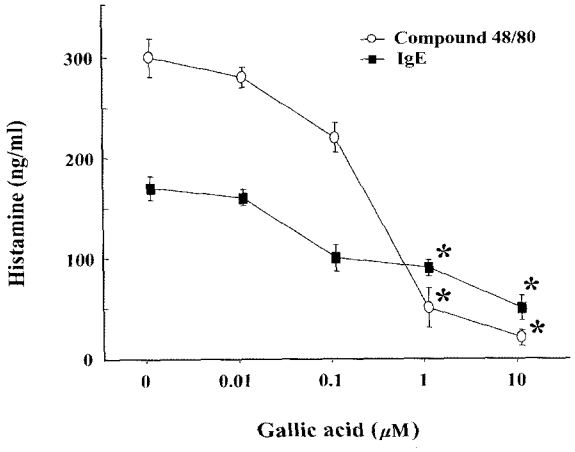

Fig. 1. Effect of gallic acid on compound 48/80-induced or IgE-mediated histamine release from RPMC. The cells (2×10^5 cells/ml) were preincubated with gallic acid at 37°C for 10 min prior to incubation with either compound 48/80 (naïve RPMC) or DNP-HSA (anti-DNP IgE sensitized RPMC). Each data represent mean± SEM of 3 independent experiments. *$p<0.05$ (significantly different from the compound 48/80 or IgE value).

Effect of gallic acid on pro-inflammatory cytokine expressions in human mast cells (HMC-1)

Since HMC-1 cell line is a useful cell for studying cytokine activation pathway (Sillaber et al, 1993), we examined whether gallic acid could regulate pro-inflammatory cytokines such as TNF-α and IL-6 in HMC-1 cells. Stimulation of HMC-1 cells with PMA (20 nM) plus A23187 (1 μM) during 4 h induced the gene expression of both cytokines. However, as shown in Fig. 3A, gallic acid (0.1~10 μM) dose-dependently inhibited TNF-α and IL-6 gene expression induced by PMA plus A23187. 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 biosynthesis, 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 μM) during 16 h induced the secretion of both cytokines. Treatment with gallic acid (10 μM) 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).

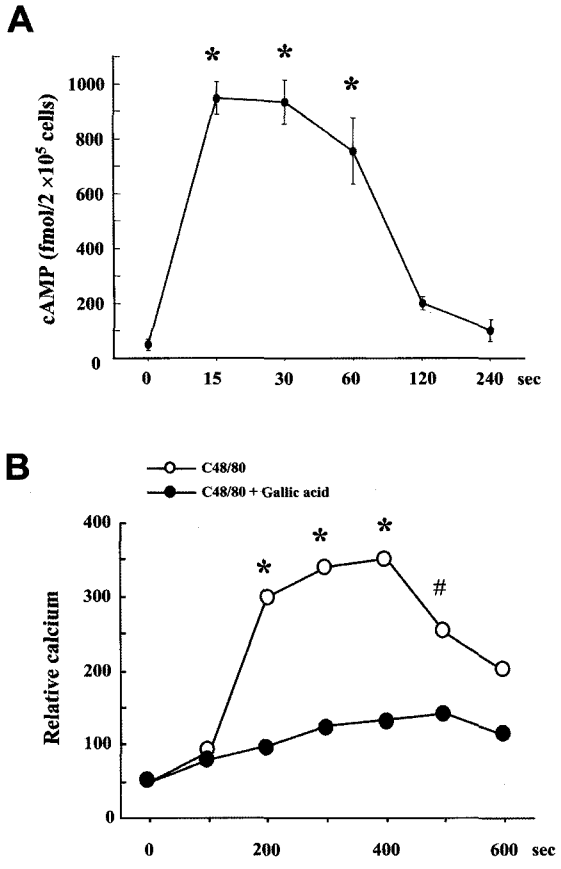

Fig. 2. Effect of gallic acid on cAMP and intracellular calcium in RPMC. (A) RPMC were treated with gallic acid (10 μM) at 37°C. (B) RPMC were preincubated with gallic acid (10 μM) for 10 min before adding compound 48/80, and then another 10 min with compound 48/80 (2 μg/ml). Each data represent mean±SEM of 3 independent experiments. *Statistically significant from the control at $p<0.05$. #Significantly different from the compound 48/80 value at $p<0.05$. C48/80, compound 48/80.

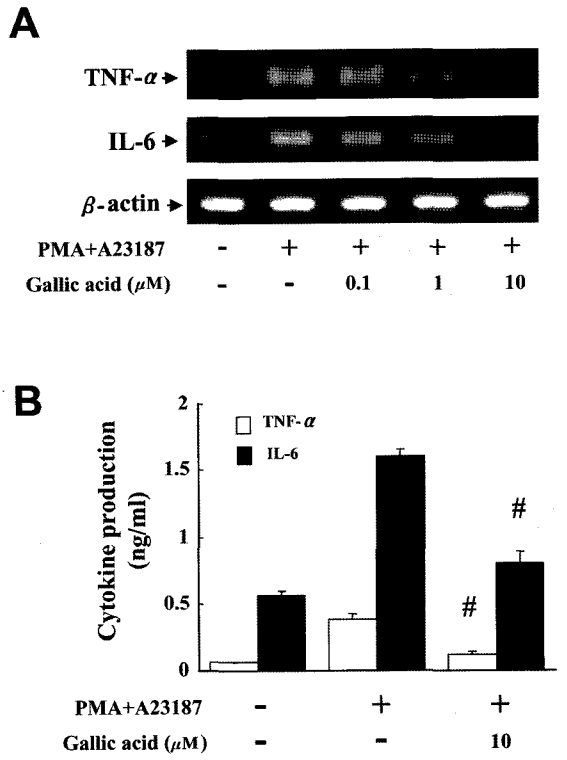

Fig. 3. Effect of gallic acid on pro-inflammatory cytokine in HMC-1 cells. (A) HMC-1 cells were treated with indicated concentration of gallic acid for 30 min before stimulated with PMA (20 nM) plus A23187 (1 μM) for 4 h. Total RNA was isolated, and TNF-α and IL-6 mRNA expression was determined by RT-PCR. (B) The secretion of TNF-α and IL-6 was measured by ELISA, as described in Materials and methods. #Significantly different from the compound 48/80 value at $p<0.05$.

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 mechanism involved in 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 both 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, by using PDTC, a potent inhibitor of NF-κB as a positive control, we performed EMSA. Cells pretreated with either gallic acid (10 μM) or PDTC (50 μM) were subsequently stimulated by PMA plus A23187, and the effect of gallic acid and PDTC on binding activity of transcription factors was examined. Fig. 4B was shown that treatment of PMA plus A23187 caused a significant increase in the DNA binding activity of NF-κB within 4 h. 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. Thus, HMC-1 cells were transiently transfected with a NF-κB-luciferase reporter construct or the empty vector. Exposure of the cells, transfected with the NF-κB-luciferase reporter construct, to PMA plus A23187 increased the luciferase activity (Fig. 4C). Gallic acid significantly reduced the PMA plus A23187-induced luciferase activity. The above result together with the data showing the inhibition of TNF-α and IL-6 production with the inhibitor of NF-κB, PDTC (Fig. 5D), indicate that 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

MAPKs pathways play a crucial role in the regulation of pro-inflammatory molecules on cellular responses (Be-

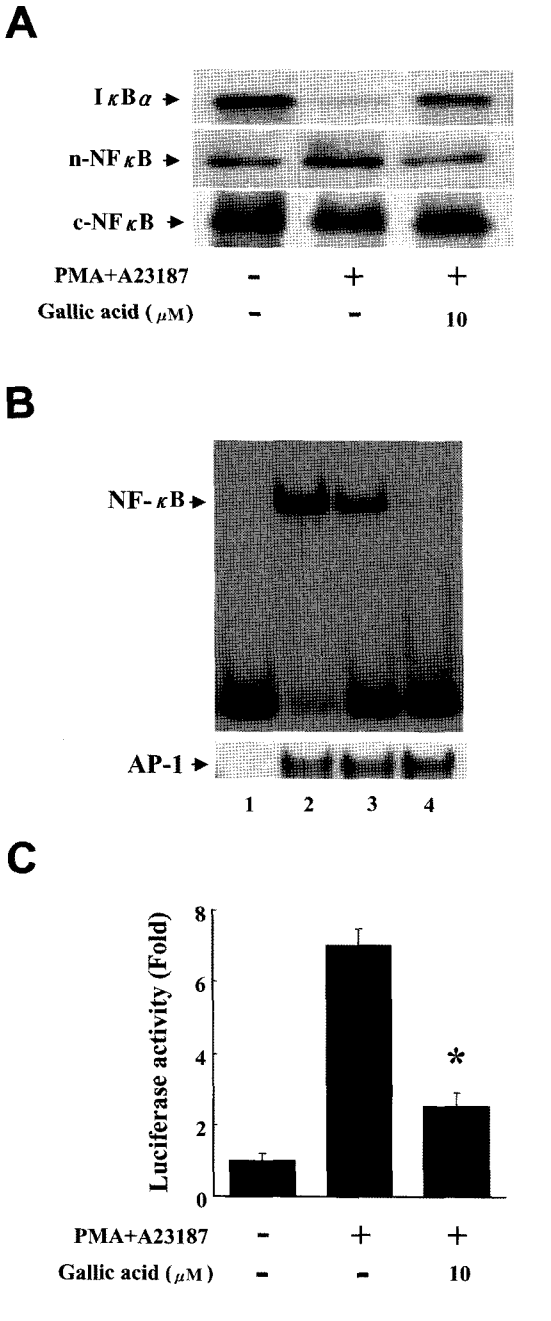

Fig. 4. Effect of gallic acid on activation of NF-κB. HMC-1 cells were pretreated with gallic acid (10 μM) for 30 min prior to PMA (20 nM) plus A23187 (1 μM) stimulation. (A) IκBα degradation and NF-κB translocation were assayed by Western blot (n-NF-κB, nucleus NF-κB; c-NF-κB, cytoplasmic NF-κB). (B) Nuclear extracts prepared and incubated with ^{32}P-labeled oligonucleotides corresponding to NF-κB and AP-1 was analyzed by EMSA. 1, No treatment; 2, PMA plus A23187; 3, PMA plus A23187 plus gallic acid; 4, PMA plus A23187 plus PDTC (50 μM). (C) Cells were transiently transfected with NF-κB-luciferase reporter construct or empty vector. Then, the cells were incubated with PMA plus A23187 with or without gallic acid. NF-κB-dependent transcriptional activity was determined by luciferase activity assay. These data are representative of three independent experiments. *Statistically significant from the PMA plus A23187 value at $p < 0.05$.

yaert et al, 1996; Arbabi & Maier, 2002). We recently reported 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 elucidate 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. Fig. 5A was shown that gallic acid (10 μM) attenuated the PMA plus A23187-induced p38 MAPK activation, but did not affect the phosphorylation of JNK and ERK. To confirm the fact 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 examine whether the expression of DN-p38 constructs affects TNF-α and IL-6 secretion, the 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). The expression of DN-p38 was confirmed by Western blot (Fig. 5B).

To further confirm the relation between 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 the cells with SB 203580 (5 μM), p38 specific inhibitor, blocked the PMA plus A23187-induced TNF-α and IL-6 synthesis, 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 contributes 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).

DISCUSSION

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

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 & Austen, 1974; Tasaka et al, 1986; Weston & Peachell, 1998). In addition, calcium movements across membranes of mast cells represent a major target for effective anti-allergic drugs, since they are essential events linking stimulation to secretion (Beaven et al, 1984). The transduction pathways which modulate cAMP and intracellular calcium are modified by ADP-rybosylates G-protein binding protein (Alfonso et al, 2000). The release of histamine is known to be depressed by an increase of intracellular cAMP content, because of 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 com-

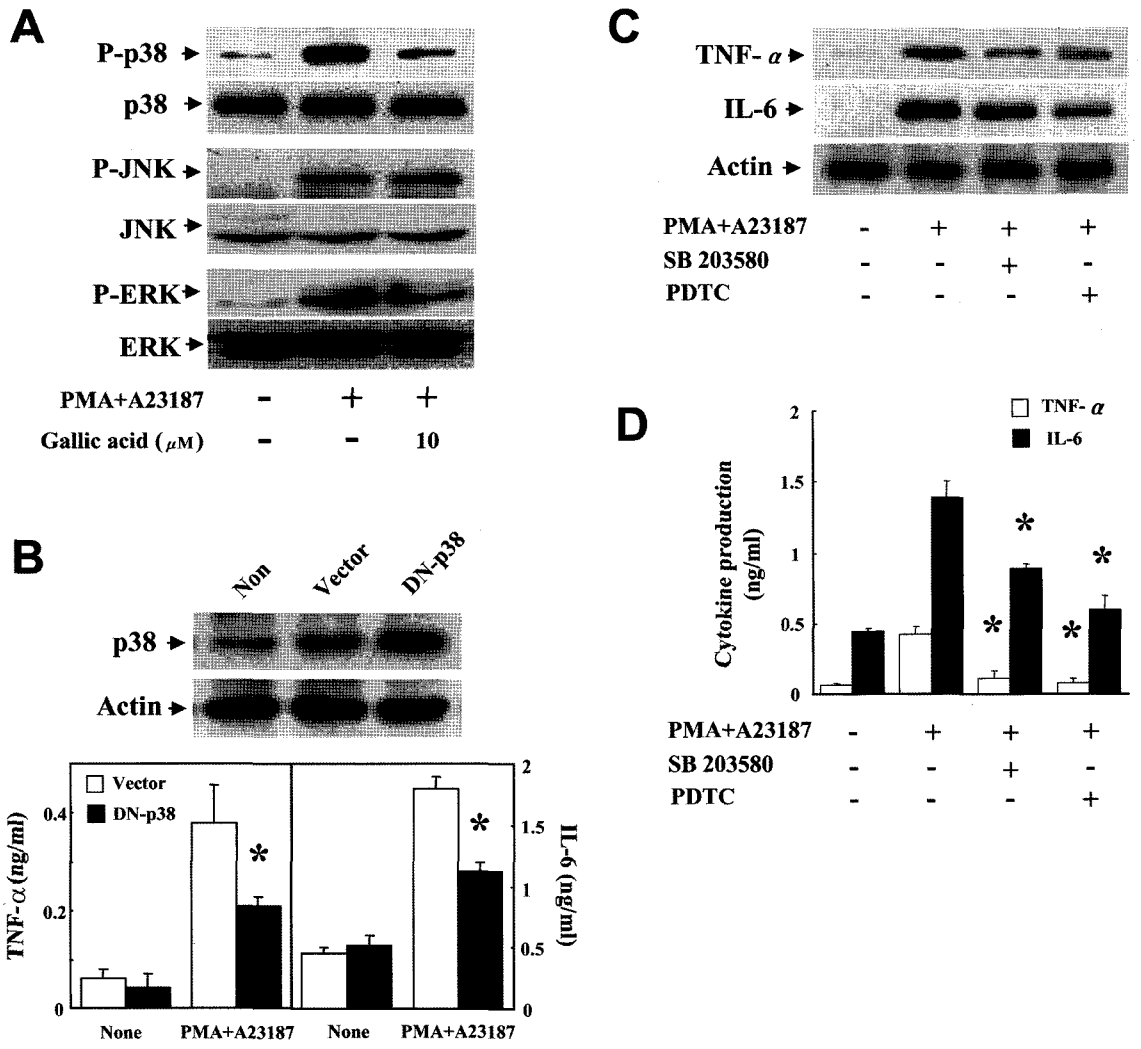

Fig. 5. Effect of gallic acid on p38 MAPK in HMC-1 cells. (A) HMC-1 cells were pretreated with gallic acid (10 μM) for 30 min prior to PMA (20 nM) plus A23187 (1 μM) stimulation, and the phosphorylation of p38, JNK, and ERK was assayed by Western blot. (B) HMC-1 cells were transfected with either control plasmid or DN-p38/pCMV. The production of p38 MAPK was then determined by Western blot. After transfection of control vector or DN-p38, cells were stimulated with PMA (20 nM) plus A23187 (1 μM), and then the secretion of TNF-α and IL-6 from HMC-1 cells was measured by ELISA. *Statistically significant from the control vector transfection at $p<0.05$. HMC-1 cells were pretreated with pharmacologic inhibitor of p38 MAPK (SB 203580) and NF-κB (PDTC) for 30 min prior to PMA (20 nM) plus A23187 (1 μM) stimulation, and production and secretion of TNF-α and IL-6 were measured by Western blot (C) and ELISA (D), respectively. *Statistically significant from the PMA plus A23187 value at $p<0.05$.

parison 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 to the prevention of calcium release from the calcium store of mast cells due to elevation of the intracellular cAMP level by inhibiting 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, which showed an enhancement of cAMP and attenuation of intracellular calcium in mast cells following gallic acid treatment, are consistent with other reports. According from these other observations, we strongly speculate that increased cAMP and decreased intracellular calcium are 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 observations indicate that reduction of TNF-α and IL-6 from mast cells is one of the key indicators of reduced allergic symptoms. Several reports show the relation between 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 the present study, gallic acid decreased the elevated intracellular calcium levels and inhibited the gene expression and protein neosynthesis 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 genes 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 & 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 (Shapiro & Dinarello 1995; Manthey et al, 1998). 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 inhibits p38 MAPK activation and downstream TNF-α and IL-6 production.

Gallic acid, one of polyphenolic compounds, has been reported to decreases histamine release from rat basophilic leukemia cells and suppresses pro-inflammatory cytokine production in murine peritoneal macrophages (Matsuo et al, 1997; Kwon et al, 2004). In addition, gallic acid related polyphenol compounds, epigallocatechin-3-gallate and theaflavin-3,3'-digallate, inhibit the activation of MAPK (Chung et al, 2001). These data support our findings which showed the inhibitory effects of gallic acid on 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 be used to prevent or treat mast cell-mediated allergic diseases.

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