Natural Product Sciences
11(4) : 207-212 (2005)

Inhibitory Effect of *Tetragonia tetragonoides* Water Extract on the Production of TNF-α and Tryptase in Trypsin-Stimulated Human Mast Cells

Ok-Hwa Kang, Yeon-A Choi, Hye-Jung Park, Jin Tae, Chon-Sik Kang, Dong-Sung Lee, Ju-Ho Kim, and Young-Mi Lee*

Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Iksan, Jeonbuk 570-749, Korea

Abstract – *Tetragonia tetragonoides* (Aizoaceae) has been known as an anti-cancer agent. The activation of proteinase-activated receptor-2 (PAR-2) by trypsin appears to play a role in inflammation. In the present study, we examined the inhibitory effects of *Tetragonia tetragonoides* water extract (TTWE) on the production of tumor necrosis factor-α (TNF-α) and tryptase in trypsin-stimulated human leukemic mast cells (HMC-1) expressing PAR-2. HMC-1 cells were stimulated with trypsin in the presence or absence of TTWE (10, 100, and 1000 μg/ml). The level of TNF-α secretion from HMC-1 cells was measured by enzyme-linked immunosorbent assay (ELISA). TNF-α and tryptase mRNA expression were examined by reverse transcription-PCR. Also, extracellular signal-regulated kinase (ERK) activation was assessed by Western blot analysis. Trypsin activity was measured using the substrate Bz-DL-Arg-p-nitroanilide (BAPNA). It was observed that TNF-α secretion, tryptase mRNA and TNF-α mRNA expression in trypsin-stimulated HMC-1 cells were inhibited by pretreatment of TTWE (1000 μg/ml). Furthermore, the pretreatment of TTWE (1000 μg/ml) resulted in the reduction of ERK phosphorylation and trypsin activity. These results suggest that TTWE might have the inhibitory effects on the PAR-2-dependent inflammation processes and it is likely to function as PAR-2 antagonist.
Keywords – *Tetragonia tetragonoides*, Aizoaceae, trypsin, mast cells, tryptase, TNF-α, ERK

Introduction

Mast cells play an important role in the development of inflammation by releasing various mediators such as histamine, neutral protease, and proinflammatory cytokines (Metcalfe *et al.*, 1997). Human mast cells are well known to mediate the immediate type allergic reactions. In addition, there is increasing evidence that mast cells are involved in chronic inflammatory processes such as inflammatory bowel disease (IBD), autoimmune diseases, tissue remodeling, immune diseases, and fibrosis. It was reported that mast cell hyperplasia in the gut is a feature of IBD (Stoyanova and Gulubova, 2002).

The gastrointestinal tract is exposed physiologically to high levels of proteinases during diseases. Elevated colonic proteinase activity could contribute to the pathophysiology of ulcerative colitis (Bustos *et al.*, 1998). Trypsin is one of proteases derived from digestive glands and inflammatory cells by bacterial and viral pathogens and has been shown to activate protease-activated receptor-2 (PAR-2) and PAR-4 (Macfarlane *et al.*, 2001). It has been shown that the activation of PARs by trypsin induces nitric oxide-dependent vasodilation, extravasation of plasma proteins, infiltration of neutrophils, and colonic inflammation (Kawabata *et al.*, 1998; Cenac *et al.*, 2002) and that PAR-2 is highly expressed in the gastrointestinal tract (Bohm *et al.*, 1996; Xu *et al.*, 1998). Trypsin also stimulates inflammatory mediator release from peritoneal macrophages (Lundberg *et al.*, 2000). In particular, the intracolonic injection of PAR-2 agonists induced the inflammatory reactions characterized by granulocyte infiltration, increased wall thickness, tissue damage, and elevated T-helper cell type 1 cytokine (Cenac *et al.*, 2002). We previously demonstrated that trypsin induced TNF-α secretion by HMC-1 cells via extracellular signal-regulated kinase (ERK) signaling pathway (Kang *et al.*, 2003).

Tetragonia tetragonoides (Aizoaceae) has been used for treatment of gastric cancer in East Asian countries. Recent study suggested that *Tetragonia tetragonoides* (Aizoaceae) has been shown to effectively inhibit the ulcer formation induced by sedative drugs (Okuyama and Yamazaki, 1983). *Tetragonia tetragonoides* contains a number of compounds. Two of cerebrosides have the anti-ulcerogenic activity (Fenwick, 1988; Cambie and Ash,

*Author for correspondence
Fax: +82-63-855-6807; E-mail: ymlee@wonkwang.ac.kr

1994) and the polysaccharides isolated from the leaves also have anti-inflammatory effects (Kato *et al.*, 1985).

In the present study, we investigated whether TTWE inhibit the PAR-2-dependent inflammatory responses in HMC-1 cells. We wish to report that TTWE inhibited the expression of inflammatory mediators, such as TNF-á and tryptase, in trypsin-stimulated HMC-1, and the inhibitory effects of TTWE could be caused by the blockage of ERK pathway as well as the reduction of trypsin activity.

Materials and Methods

Materials – Human trypsin, substrate Bz-DL-Arg-p-nitroanilide (BAPNA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) were purchased from Sigma (St. Louis, MO). Anti-ERK antibody and anti-phospho-ERK antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-IgG-horseradish peroxidase (HRP) conjugate was from DAKO (High Wycomde, Bucks,UK). Anti-tumor necrosis factor (TNF)-a antibody and recombinant human TNF-α were obtained from R&D Systems (Minneapolis, MN).

Plant materials and extract preparations – Herba of *Tetragonia tetragonoides* was purchased from an Oriental drug store, Daehak Hanyakkuk (Iksan, Korea) and identified by professor Dong-Yeul Kwon at the College of pharmacy, Wonkwang University. Voucher specimen (No. 03-10-10) has been deposited in the Herbarium at the College of Pharmacy, Wonkwang University. TTWE was prepared by decocting for 2 h in distilled water (100 g/l). The extract was filtered by a 0.45 μm filter, lyophilized, and kept at 4°C. The dried extract was dissolved in phosphate-buffered saline (PBS) for use.

Cell culture – The human leukemic mast cell line HMC-1 (kindly provided by Dr. Y. Kitamura; Osaka University Medical School, Osaka, Japan) was cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 IU/ml penicillin, 50 μg/ml streptomycin, and 1.2 mM α-thioglycerol at 37°C under 5% CO_2 in air.

Cell viability – The cell viability was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) assay. HMC-1 cells were seeded at 5×10^4/ml densities in 96-well plates (Nunc, Denmark) in 100 μl cell suspension per well. Each group had three wells with a non-treated group as control. TTWE (10, 100, and 1000 μg/ml) was added to each well and incubated for 48 h at 37°C, 5% CO_2. MTT (5 mg/ml) 10 μl was added to each well and then cells were cultured for another 4 h. The supernatant was discarded and 100 μl of dimethyl sulphoxide (DMSO) was added to each well. When the formazan crystals were added, MTT forms clearly visible purple crystals of MTT-formazan. The optical density read at 540 nm. The optical density of MTT-formazan formed in control (untreated) cells was taken as 100% of viability.

Enzyme-linked immunosorbent assay for TNF-α – Cells were seed at 5×10^5 cells per well in 24 well tissue culture plates and pretreated with various concentration of TTWE (10, 100, and 1000 μg/ml) 30 min before trypsin (100 nM) stimulation. Eight hours after trypsin stimulation, TNF-α concentrations in the supernatant were measured as commercial instruction (Pharmingen assay, SanDiego, CA). Briefly, ELISA plates (Falcon, Becton Dickinson Labware, USA) were coated overnight at 4°C with anti-human TNF-α antibody diluted in coating buffer (0.1 M carbonate, pH 9.5) and then washed four times with PBS containing 0.05% tween 20 (PBS-T). Non-specific protein binding sites were blocked with assay diluent (PBS containing 10% FBS, pH 7.0) for at least 1 h, and 100 μl of each sample or TNF-α standards diluted in assay diluent were applied to wells. After incubation for 2 h, 100 μl of working detector (biotinylated anti-TNF-α monoclonal antibody and avidin-HRP reagent) was added and incubated for 1 h. Consequently, 100 μl of substrate solution (tetramethylbenzidine (TMB) and H_2O_2) was added to wells and incubated for 30 min in the dark before stopping the reaction by 50 μl stop solution (2N H_2SO_4) and absorbance was read at 450 nm. All subsequent steps took place at room temperature, and all standards and samples were assayed in triplicate.

RNA extraction and reverse-transcription PCR (RT-PCR) for TNF-α and tryptase – TTWE-pretreated HMC-1 cells were stimulated with trypsin (100 nM) for 2 h. Total RNA was isolated from the harvested cells using the easy-BLUE^{TM} RNA extraction kit (iNtRON Biotech, Korea). The concentration of total RNA in the final elute can be determined by spectrophotometry. Total RNA (5 μg) was converted to cDNA by reverse transcriptase at 37°C for 90 min using first-strand cDNA synthesis kit (Amersham Pharmacia Biotech). PCR amplication was performed as follows: tryptase (94°C for 45 sec, 50°C for 1 min, 72°C for 1 min: 30 cycles), TNF-α (94°C for 1 min, 60°C for 1 min, 74°C for 1 min: 30 cycles), GAPDH (94°C for 1 min; 60°C for 2 min; 72°C for 1 min: 30 cycles). Primers used in this study were as follows: tryptase (forward primer 5'-AGGATGCTGAAT-CTGCTGCTGCTG-3'and reverse primer 5'-TCACGG-CTTTTTGGGGACATAGTG-3': 831 bp), (Vanderslice *et*

al., 1990) TNF-α (forward primer 5'-CAAAGTAGAC-CTGCCCAGAC-3' and reverse primer 5'-GACCTC-TCTCTAATCAGCCC-3': 490bp), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward primer 5'-CCATGTT-CGTCATGGGTGTGAACCA-3' and reverse primer 5'-GCCAGTAGAGGCAGGGATGATGTTC-3': 251 bp) (Lee *et al.*, 2000) Final PCR products were separated on 1% agarose gels and photographed under UV light.

Western blot analysis for ERK – TTWE-pretreated HMC-1 cells were stimulated with trypsin (100 nM) for 15 min. The cells were lysed with ice-cold lysis buffer (iNtRON Biotech, Korea). Western blot analysis was performed according to a standard procedure. Lysates (50 μg of protein) was separated by SDS-PAGE with 12% acrylamide gel and transferred on PVDF membrane (Millipore). After blocking with 5% skim milk, membranes were blotted with anti-human-phospho-ERK for 12 h at 4°C. HRP-conjugated antibody against rabbit IgG was used as a secondary antibody. Finally, Epitopes on proteins recognized specifically by antibodies were visualized by using enhanced chemiluminescence (ECL) detection kit (Amersham, Milan). After stripping, the membranes were reprobed with anti-ERK antibody as respective loading controls.

Trypsin activity assay – Trypsin activity was assessed by the amidolytic ability of trypsin on a substrate BAPNA (Smith *et al.*, 1984). Samples were added to each tube containing 2.85 mL of 0.1 M Tris-HCl (pH 8.0) and 1M glycerol, and the assay was started with the addition of 20 mM substrate in DMSO. After 30 min incubation at 37°C, the reactions were stopped by the addition of 50 μl of glacial acetic acid, and the absorbance was measured at 405 nm with spectrophotometer and compared to those given by standard curves of p-nitroanilide to determine the concentration of product released. Inhibition percentages of trypsin activity were calculated using the following equation:

$$\% \text{ of inhibition} = \frac{(A-B)}{A} \times 100$$

where A is a trypsin activity without TTWE and B is a trypsin activity with TTWE.

Statistical analysis – The results were expressed as mean ± S.E. for a number of experiments. Statistical significance was compared between each treated group and control by the Student's *t*-test. Each experiment was repeated at least three times and yielded comparable results. Values with $^*p < 0.05$ were considered significant.

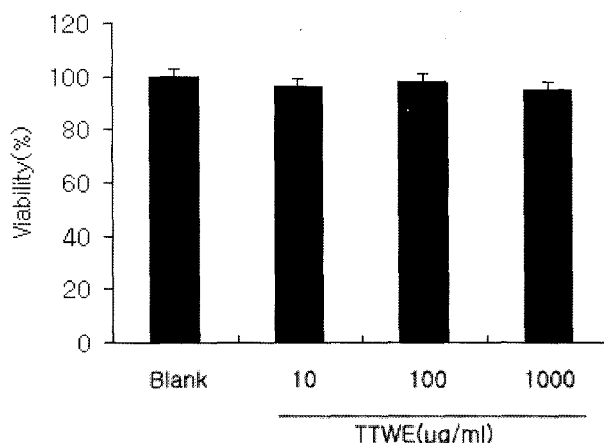

Fig. 1. Effect of *Tetragonia tetragonoides* water extract (TTWE) on cell viability of HMC-1 cells The cells were incubated with various concentration of TTWE (10, 100, and 1000 μg/ml). Cell viability was evaluated by MTT assay (expressed as percent of control). Values are mean ± S.E. of three independent experiments.

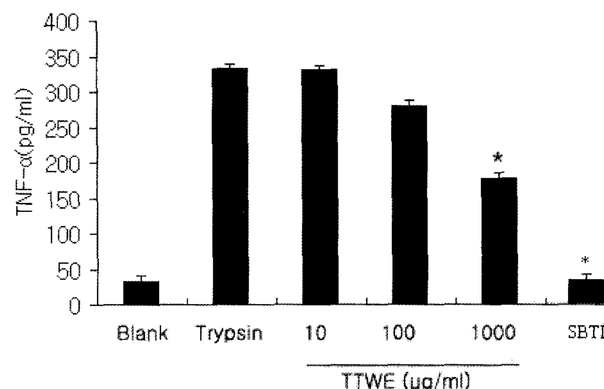

Fig. 2. Effect of *Tetragonia tetragonoides* water extract (TTWE) on TNF-α secretion in trypsin-stimulated HMC-1 cells The cells (5×10^5 cells/well) were pre-incubated with three concentrations of TTWE (10, 100, and 1000 μg/ml) before stimulation with trypsin (100 nM) for 8 h. TNF-α levels in supernatant were measured by ELISA. SBTI: Soybean trypsin inhibitor. Values are the mean ± S.E. of duplicate determinations from three separate experiments ($^*p < 0.05$).

Results

Effect of TTWE on cell viability of HMC-1 cells – To examine the direct cytotoxic effect of TTWE, cell viability was assessed by MTT assay. HMC-1 cells were incubated with various concentrations of TTWE (10, 100, and 1000 μg/ml) for 48 h. HMC-1 cells showed viability (> 96%) even 1000 μg/ml (Fig. 1).

Effect of TTWE on trypsin-induced TNF-α secretion – To study the effect of TTWE on trypsin-induced TNF-α secretion from HMC-1 cells, HMC-1 cells were pretreated with various concentrations of TTWE (10, 100, and 1000

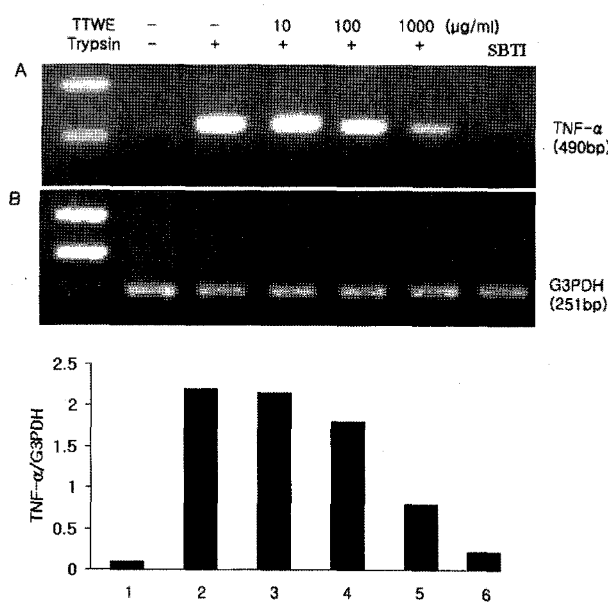

Fig. 3. Effect of *Tetragonia tetragonoides* water extract (TTWE) on TNF-α mRNA expression in trypsin-stimulated HMC-1 cells The cells were pre-incubated with three concentrations of TTWE (10, 100, and 1000 μg/ml) before stimulation with trypsin (100 nM) for 2 h. Total RNA (5 μg) was converted to cDNA by reverse transcriptase. GAPDH mRNA was carried out in parallel to confirm equivalency of cDNA preparation (B). Size marker is ëDNA/HaeIII. SBTI: Soybean trypsin inhibitor.

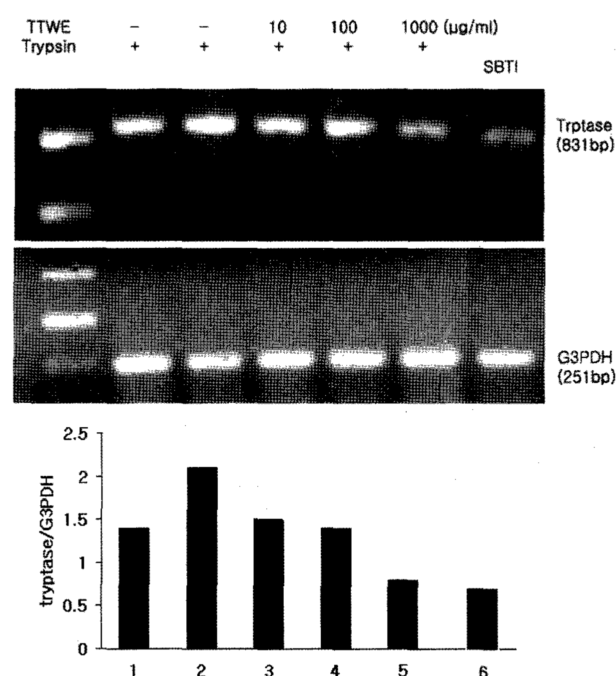

Fig. 4. Effect of *Tetragonia tetragonoides* water extract (TTWE) on tryptase mRNA expression in trypsin-stimulated HMC-1 cells The cells were pre-incubated with three concentrations of TTWE (10, 100, and 1000 μg/ml) before stimulation with trypsin (100 nM) for 2 h. Total RNA (5 μg) was converted to cDNA by reverse transcriptase. GAPDH mRNA was carried out in parallel to confirm equivalency of cDNA preparation (B). Size marker is λDNA/HaeIII. SBTI: Soybean trypsin inhibitor.

μg/ml) for 30 min and then stimulated with trypsin (100 nM) for 8 h. The levels of TNF-α secreted from HMC-1 cells were measured by ELISA. Treatment of trypsin appears to induce a 10 fold increase in TNF-α secretion compared to trypsin-nontreated cells. TTWE significantly inhibited TNF-α secretion induced by trypsin in high concentration (1000 μg/ml) but little in low concentration (Fig. 2). TTWE showed 47% inhibition for TNF-α in concentration of 1000 μg/ml. This result demonstrates that TTWE could downregulate TNF-α secretion in trypsin-stimulated HMC-1 cells.

Effect of TTWE on trypsin-induced TNF-α and tryptase mRNA expression – To study the effect of TTWE on TNF-α synthesis in trypsin-stimulated HMC-1 cells, TTWE-pretreated HMC-1 cells were stimulated with trypsin for 2 h. TNF-α and tryptase mRNA expression levels in intracellular of HMC-1 cells activated by trypsin were determined by RT-PCR. Treatment of trypsin led to an increase in TNF-α and tryptase mRNA expression in HMC-1 cells. Pretreatment of TTWE (1000 μg/ml) attenuated TNF-α mRNA expression similar to control level (Fig. 3). In addition, TTWE (1000 μg/ml) inhibited the expression of tryptase which was constitively transcripted in mast cells (Fig. 4). These results indicate that TTWE modulates TNF-α and tryptase synthesis in trypsin-stimulated HMC-1 cells.

Effect of TTWE on trypsin-induced ERK phosphorylation – To examine the effect of TTWE on trypsin-induced ERK phosphorylation, TTWE-pretreated HMC-1 cells were stimulated with trypsin for 15 min. Major immunoreactive band was identified with phosphorylated ERK by Western blot analysis and the level of phosphorylation was described as the relative ratio of band density to that of whole ERK (Fig. 5). Trypsin (100 nM) stimulation (Fig. 5, lane 2) induced 2.5-fold increase in the phosphorylation of ERK compared to that of trypsin-nontreated cells (Fig. 5, lane 1). TTWE pretreatment (1000 μg/ml) resulted in the blockade of trypsin-induced ERK phosphorylation, without affecting the levels of whole ERK (Fig. 5, lane 4, 5, and 6). The result suggests that the inhibitory effect of TTWE might be caused through the suppression of ERK activation pathway.

Effect of TTWE on trypsin activity – There is a possibility that the inhibitory effect of TTWE was caused by the direct blockage of the trypsin activity. Thus, the trypsin activity assay was performed using BAPNA substrate. Trypsin (20 μg/ml) was incubated in the presence

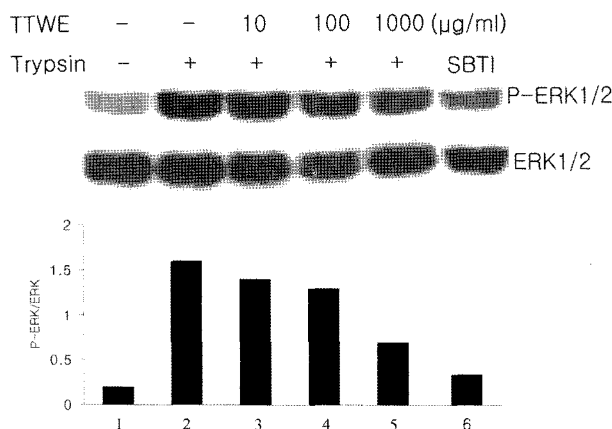

Fig. 5. Effect of *Tetragonia tetragonoides* water extract (TTWE) on ERK1/2 phosphorylation in trypsin-stimulated HMC-1 cells HMC-1 cells (5×10^6 cells/ml) pretreated with TTWE (10, 100, and 1000 μg/ml) were cultured for 15 min with trypsin (100 nM), and ERK1/2 phosphorylation were analyzed by Western blot using specific antibodies against ERK1/2 and phospho-ERK1/2. SBTI: Soybean trypsin inhibitor.

Table 1. Inhibitory effect of *Tetragonia tetragonoides* water extract (TTWE) on trypsin activity

Treatment		Inhibition (%)
SBTI (mM)	0.1	11.0 ± 0.8
	1	82.0 ± 4.8
	10	94.0 ± 5.1
TTWE (μg/ml)	10	1.3 ± 0.2
	100	11.2 ± 0.5
	1000	32.0 ± 0.9*

SBTI : Soybean trypsin inhibitor, Trypsin concentration is 20 μg/ml. Values are the mean ± S.E. of duplicate determinations from three separate experiments (*$p < 0.05$).

or absence of TTWE. TTWE inhibited trypsin activity in a concentration-dependent manner and showed 32% inhibition in trypsin activity in concentration of 1000 μg/ml. In contrast, SBTI used as a positive control almost inhibited the trypsin activity in the concentration of 10 μM (Table 1). These result suggests that TTWE might inhibit the production of TNF-α and tryptase through both blockage of ERK signaling pathway and inhibition of trypsin activity.

Discussion

Recent studies have suggested that mast cells might play a role in the pathogenesis of IBD (Raithel *et al.*, 2001, Stoyanova and Gulubova, 2002). The tissue of patients with IBD reveals an increase of mast cell number and accumulation of the mast cell-derived mediators, such as histamine, cytokines, serine proteases, leukotrienes, and prostaglandins. Our previous study have showed that the levels of tryptase and tumor necrosis factor (TNF)-α in intestinal mast cells were significantly elevated in UC tissues compared to normal tissues (Kim *et al.*, 2003). We have also reported that TNF-α secretion in mast cells might be induced by trypsin treatment, which lead the activation of PAR-2 and PAR-4 (Kang *et al.*, 2003). Therefore, screening of trypsin inhibitors, PAR-2 and PAR-4 antagonists may be a pharmacological strategy for the treatment of intestinal inflammation. It has been reported that SBTI, a trypsin inhibitor, significantly inhibited the inflammatory reaction induced by the implantation of dry sponges in rats (Damas *et al.*, 1990). SBTI also inhibited collagenase-induced oedema in the rat paw (Souza *et al.*, 1995). In this study, SBTI (10 μM) and TTWE (1000 μg/ml) showed 94% and 32% inhibition on the trypsin activity, respectively. Furthermore, SBTI and TTWE significantly inhibited trypsin-induced TNF-α and tryptase production in HMC-1 cells.

To examine mechanism on inhibition of trypsin-induced TNF-α and tryptase production, ERK phosphorylation was investigated. Trypsin induced the ERK phosphorylation but not JNK or p38 MAP kinase (Kang *et al.*, 2003). In the present study, we have observed that TTWE reduced the production of TNF-α and tryptase in trypsin-stimulated mast cells through inhibition of ERK phosphorylation.

Taken together, TTWE might inhibit the production of tryptase and TNF-α in mast cells through inhibition of both trypsin activity and ERK pathway. However, to provide a rationale for use as anti-inflammatory agents, the effect of TTWE should be investigated in *in vivo* experimental models of trypsin-dependent intestinal inflammation.

Acknowledgements

This work was supported by grants from Wonkwang University in 2004.

References

Bohm, S.K., Kong, W., Bromme, D., Smeekens, S.P., Anderson, D.C., Connolly, A., Kahn, M., Nelken, N.A., Coughlin, S.R., Payan, D.G., and Bunnett, N.W., Molecular cloning, expression and potential functions of the human proteinase-activated receptor-2. *Biochem. J.* **314,** 1009-1016 (1996).

Bustos, D., Negri, G., De Paula, J.A., Di Carlo, M., Yapur, V., Facente, A., and De Paula, A. Colonic proteinases: increased activity in patients with ulcerative colitis. *Medicina* (B Aires), **58,** 262-264 (1998).

Cambie, R.C. and Ash, J., Fijian Medicinal Plants, CSIRO:

Melbourne, Australia (1994).

Cenac, N., Coelho, A.M., Nguyen, C., Compton, S., Andrade-Gordon, P., MacNaughton, W.K., Wallace, J.L., Hollenberg, M.D., Bunnett, N.W., Garcia-Villar, R., Bueno, L., and Vergnolle, N., Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am. J. Pathol.* **161**, 1903-1915 (1998).

Damas J., Bourdon V., Remacle-Volon G., Adam A., Proteinase inhibitors, kinins and the inflammatory reaction induced by sponge implantation in rats. *Eur. J. Pharmacol.* **175**, 341-346 (1990).

Fenwick, G.R., Bracken (*Pteridium aquilinum*)-toxic effects and toxic constituents. *J. Sci. Food Agric.* **46**, 147-173 (1998).

Kang, O.H., Jeong, H.J., Kim, D.K., Choi, S.C., Kim, T.H., Nah, Y.H., Kim, H.M., and Lee, Y.M., Trypsin induces tumour necrosis factor-alpha secretion from a human leukemic mast cell line. *Cell Biochem. Funct.* **21**, 161-167 (2003).

Kato, M., Takeda, T., Ogihara, Y., Shimizu, M., Nomura, T., and Tomita, Y., Studies in the structure of the polysaccharides from *Tetragonia tetragonioides*. *Chem. Pharm. Bull. Jpn.* **33**, 3675-3680 (1985).

Kawabata, A., Kuroda, R., Minami, T., Kataoka, K., and Taneda, M., Increased vascular permeability by a specific agonist of protease-activated receptor-2 in rat hindpaw. *Br. J. Pharmacol.* **125,** 419-422 (1998).

Kim, J.A., Choi, S.C., Yun, K.J., Kim, D.K., Han, M.K., Seo, G.S., Yeom, J.J., Kim, T.H., Nah, Y.H., and Lee, Y.M., Expression of protease-activated receptor 2 in ulcerative colitis. *Inflamm. Bowel. Dis.* **9**, 224-229 (2003).

Lee, Y.B., Schrader, J.W., and Kim, S.U., p38 map kinase regulates TNF-αlpha production in human astrocytes and microglia by multiple mechanisms. *Cytokine* **12**, 874-880 (2000).

Lundberg, A.H., Eubanks, J.W.3rd; Henry, J., Sabek, O., Kotb, M., Gaber, L., Norby-Teglund, A., and Gaber, A.O., Trypsin stimulates production of cytokines from peritoneal macrophages in vitro and in vivo. *Pancreas* **21**, 41-51 (2000).

Macfarlane, S.R., Seatter, M.J., Kanke, T., Hunter, G.D., and Plevin, R., Proteinase-activated receptors. *Pharmacol. Rev.* **53,** 245-282 (2001).

Metcalfe, D.D., Baram, D., and Mekori, Y.A., Mast cells. *Physiol. Rev.* **77,** 1033-1079 (1997).

Okuyama, E. and Yamazaki, M., The principles of *Tetratogonia tetragonoides* having anti-ulcerogenic activity. II. Isolation and structure of cerebrosides. *Chem. Pharm. Bull.* **31,** 2209-2219 (1983).

Raithel, M., Winterkamp, S., Pacurar, A., Ulrich, P., Hochberger, J., and Hahn, E.G., Release of mast cell tryptase from human colorectal mucosa in inflammatory bowel disease. *Scand. J. Gastroenterol.* **36**, 174-179 (2001).

Smith, T.J., Hougland, M.W., and Johnson, D.A., Human lung tryptase. Purification and characterization. *J. Biol. Chem.* **259,** 11046-11051 (1984).

Souza Pinto, J.C., Remacle-Volon, G., Sampaio, C.A., and Damas, J. Collagenase-induced oedema in the rat paw and the kinin system. *Eur. J. Pharmacol.*, **274,** 101-107 (1995).

Stoyanova, I.I., and Gulubova, M.V., Mast cells and inflammatory mediators in chronic ulcerative colitis. *Acta Histochem.* **104,** 185-192 (2002).

Vanderslice, P., Ballinger, S.M., Tam, E.K., Goldstein, S.M., Craik, C.S., and Caughey, G.H., Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family. *Proc. Natl. Acad. Sci. USA* **87**, 3811-3815 (1990).

Xu, W.F., Andersen, H., Whitmore, T.E., Presnell, S.R., Yee, D.P., Ching, A., Gilbert, T., Davie, E.W., and Foster, D.C., Cloning and characterization of human protease-activated receptor 4. *Proc. Natl. Acad. Sci. USA* **95**, 6642-6646 (1998).

(Accepted November 18, 2005)