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Inhibition of *Nelumbo nucifera* Stamens-derived Kaempferol on FcεRI-mediated GATA-1 Expression

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The transcription factor, GATA-1, plays an important role in the FcεRI α chain expression in mast cells and basophils. This study was conducted to investigate the downregulation of the transcription factor GATA-1 by kaempferol isolated from *Nelumbo nucifera* stamens in FcεRI-mediated allergic reactions. Kaempferol inhibited FcεRI-mediated histamine release. Western blotting analysis and RT-PCR showed that the protein and mRNA expression of GATA-1 was suppressed by kaempferol in a dose-dependent manner. These results suggest that kaempferol may inactivate basophils by downregulating the FcεRI α chain expression via the inhibition of the GATA-1 expression.

Keywords: **Kaempferol, *Nelumbo nucifera* stamens, FcεRI, GATA-1, KU812F cells**

Introduction

The development of hematopoietic cells is regulated by the cooperated reaction of various transcriptional factors [1, 2]. GATA proteins, including GATA-1, GATA-2 and GATA-3, consist of highly preserved zinc finger DNA binding domains, and play crucial roles in immune systems. Among the GATA proteins, GATA-1 largely regulates hematopoietic cells fate, and is critical for the differentiation of erythroid cells, eosinophils, mast cells and basophils, as well as cell specific gene regulation [3–6]. Mast cells and basophils are widely recognized as effector cells in IgE-dependent immediate allergic reaction. These proteins are characterized by expression of FcεRI, which is composed of one α, one β, and two γ subunits on the surface of effector cells in antigen/IgE-mediated hypersensitivity, the α chain is a specific factor of FcεRI and mostly stretches out into the extracellular area and directly binds to the Fc portion of the IgE with high affinity. The FcεRI α chain expression is modulated by multiple transcriptional factors such as GATA-1, PU-1 and Elf-1 in effector cells [7, 8]. Cross-binding of high affinity IgE receptor, FcεRI α chain, and inflammatory mediators involving histamine and β-hexosaminidase are released in immunologically activated mast cells and basophils, and result in allergic disorders involving asthma, atopic dermatitis, and allergic rhinitis [9–12].

Nelumbo nucifera Gaertn is a Nympaheceae family as perennial aquatic plant, commonly known as the lotus, and that is widely distributed throughout Eastern Asia. This plant has long been used in traditional medicines for the prevention of diarrhea, gastritis, insomnia, nervous prostration, and as a hemostatic [13–16]. *N. nucifera* stamens are flavonoid-rich, and have various physiological activities involving antioxidant, anti-inflammatory, and antidiabetic effects [17–19]. We previously showed that kaempferol suppressed expression of FcεRI via inhibition of extracellular regulated kinases (ERK)-1 activation [20, 21]. However, the regulation of GATA-1 by kaempferol in FcεRI-mediated allergic reactions has not been investi-

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gated. In present study, we investigated whether kaempferol exerted suppressive effects on GATA-1 activities in FcεRI-mediated human basophils, KU812F cells for the first time.

Materials and Methods

Isolation of kaempferol from *Nelumbo nucifera* stamens

Kaempferol was extracted and isolated from *N. nucifera* stamens as described by Lim *et al.* (Fig. 1) [19]. Kaempferol was kept in -20℃, and was solubilized in DMSO.

Cell culture and treatment

The KU812F cells were got from the ATCC (USA), and maintained as previously described [20]. Cells were treated with various concentration of kaempferol in serum-free medium, and were induced with 10 μg/ml of CRA-1.

Histamine assay

To assess degranulation, the released histamine was measured using a spectrofluorometric assay as previously described [21]. The kaempferol-pretreated KU812F cells (1×10^6) were added into Tyrode's buffer and induced with 1 μg/ml CRA-1. The 100 μl of supernatant were mixed with 40 μl of 1 N NaOH and 20 μl of 0.2% OPA and were incubated on ice for 40 min. The reaction was terminated by the addition of 10 μl of 3 N HCl and was measured an excitation wavelength of 360 nm and emission wavelength of 450 nm.

Western blot analysis

GATA-1 protein expression was examined by Western blot analysis. Briefly, CRA-1-induced cells were lysed in cell lysis buffer containing 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% Triton X100, 1 mM Na_3VO_4, 1 mM NaF, 2 mM EDTA, and a protease inhibitor cocktail. The proteins were separated by 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to PVDF membrane, and blocked with 10% skim in plain buffer (50 mM Tris-HCl, pH 7.5, 34 mM NaCl, and 0.001% Tween 20). The membrane was incubated with primary antibodies followed by anti-HRP conjugated secondary antibody. And then, the chemoreactive proteins were visualized using enhanced detection reagents in accordance with the manufacturer's instructions and the membrane was then exposed to x-ray film, after which it was quantified.

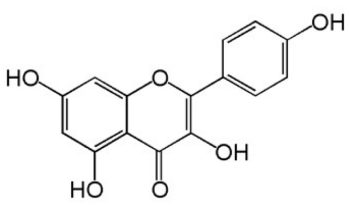

Fig. 1. Chemical structure of kaempferol isolated from *Nelumbo nucifera* stamens.

Table 1. Primer sequences used in this study.

Name		Sequences
GATA-1	Sense	5′-ATCAGCACT GGCCTACTACAGAG-3′
	Antisense	5′-GAGAGAAGAAAG GACTGGGAAAG-3′
G3PDH	Sense	5'-GCTCAGACACCATGGGGAAGGT-3'
	Antisense	5'-GTGGTGCAGGAGGCATTGCTGA-3'

Reverse-transcriptase polymerase chain reaction (RT-PCR)

The total RNA was isolated using TRIZOL reagent in accordance with the manufacturer's instructions and reverse-transcribed using a oligo $(dT)_{15}$ primer and MMLV reverse transcriptase. The cDNA was subjected to PCR amplification in the presence of specific primers (Table 1). The PCR reaction was accomplished as follows; 94℃, 30 sec for denaturing; 55℃, 30 sec for annealing; and 72℃, 1 min for extension.

Statistical analysis

All experiments were carried out independently in triplicate. Comparison between the control and test compounds group were determined by a Student's *t*-test and statistical significance was considered at $p < 0.05$.

Results and Discussion

We previously found that kaempferol exerted no cytotoxicity at ≤40 μM (data not shown) [22]. Therefore, the concentration range of 1–40 μM was selected for further experiments. Flow cytometric analysis revealed that kaempferol inhibited cell surface FcεRI expression in a dose dependent-manner as previously described [20, 21].

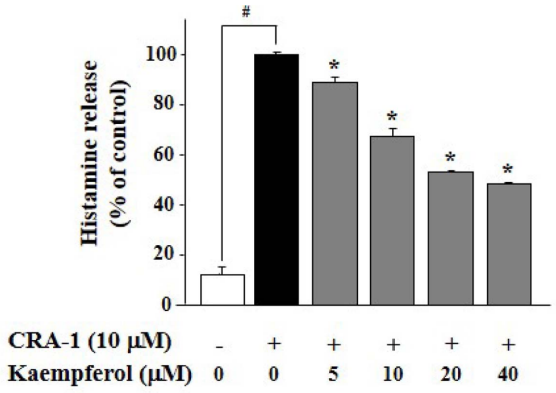

Fig. 2. Effects on FcεRI-mediated histamine release. Data are presented as means ± SD of three independent experiments. $^{\#}p < 0.05$ vs. control and $^{*}p < 0.05$ vs. CRA-1-treated group.

As the indicator of degranulation of CRA-1 stimulated basophils, histamine in the medium was measured. Kaempferol inhibited the release of histamine in CRA-1-stimulated KU812F cells in a dose-dependent manner (Fig. 2), and these results were similar to those reported in previous studies [20].

To evaluate the down-regulation of GATA-1 expression in FcεRI-mediated allergic reaction, KU812F was treated with or without kaempferol for 24 h, then stimulated with mouse anti-human FcεRI antibody (CRA-1) for 30 min. We employed western blot analysis to determine if GATA-1 suppression mediated by kaempferol could be attributed to the reduction in the levels of nucleic GATA-1 protein using anti-GATA-1 and anti-rabbit immunoglobulin HRP antibodies.

Kaempferol inhibited GATA-1 protein expression in FcεRI-mediated allergic reaction in a dose-dependent manner (Fig. 3A). The inhibitory effect of kaempferol on GATA-1 mRNA levels was investigated by RT-PCR. The total RNA was isolated using TRIZOL reagent in accordance with the manufacturer's instructions and reverse-transcribed using a oligo (dT)15 primer and MMLV reverse transcriptase. The cDNA were subjected to PCR amplification in the presence of specific primers as shown in Table 1.

The PCR condition was 94℃, 30 sec for denaturing; 55℃, 30 sec for annealing; and 72℃, 1 min for extension. We found that kaempferol inhibited GATA-1 mRNA levels in a dose-dependent manner (Fig. 3B).

We previously reported that kaempferol showed anti-allergic activity that suppressed FcεRI α chain expression by inhibiting phosphorylation of Syk, Lyn, and ERK -1 [20, 21]. In the present study, we determined that kaempferol inhibited GATA-1 protein and gene expression in FcεRI-mediated human basophilic KU812F cells. Taken together, our results suggest that kaempferol negatively regulated FcεRI α chain expression and basophil activation via down-regulation of GATA-1 expression. It is reported that omega-3 fatty acids inhibited Th2-type cytokines expression such as IL-4, IL-5 and IL-13, which might be associated with down-regulated nuclear GATA-1 expression [23]. Therefore, further research is certainly needed to study the effects of kaempferol on association

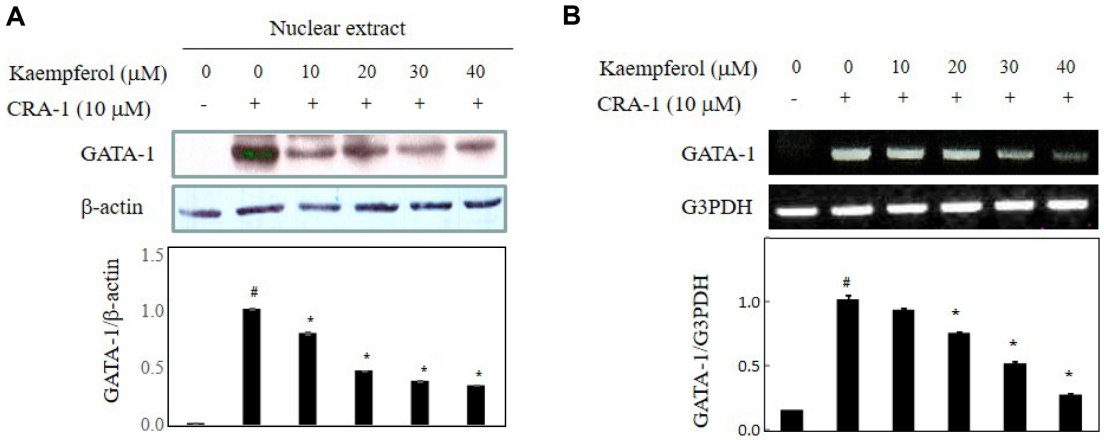

Fig. 3. Kaempferol inhibits protein (A) and mRNA (B) expression of GATA-1 in FcεRI-mediated allergic reaction. Data are presented as means ± SD of three independent experiments. $^{\#}p < 0.05$ and $^{*}p < 0.05$ indicates significant differences from the control group and CRA-1-treated group, respectively.

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between cytokine productions and GATA expression in FcεRI-mediated allergic reactions.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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