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New inhibitors for expression of IgE receptor on human mast cell

Satoru Tamura, Kunichika Yoshihira, Katsuaki Fujiwara, Nobutoshi Murakami *

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan

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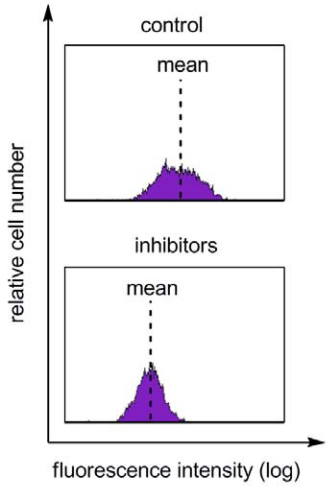
ABSTRACT

Exploration for inhibitors against expression of IgE receptor (FcεRI) on human mast cell, a significant trigger to acute and chronic allergic symptoms, disclosed epigallocatechin gallate (EGCG), epicatechin gallate, and gallocatechin gallate as active principles. Additionally, the anthocyanidin, delphinidin, and the flavone, tricetinidin, possessing a pyrogallol function were also revealed to suppress expression of FcεRI. Structure–activity relationship analysis among catechins, anthocyanidins, and flavones revealed the pyrogallol moiety to be crucial for biological potency. Furthermore, EGCG was clarified to reduce generation of γ-chain subunit to suppress expression of FcεRI on human mast cells.

The high-affinity IgE receptor, FcεRI, plays a key role in a series of acute and chronic human allergic reactions, such as atopic dermatitis, bronchial asthma, and allergic rhinitis. In humans, these receptors are found on surfaces of mast cells and basophils at high levels. Binding of allergen specific IgE to FcεRI and subsequent crosslinking of IgE with a multivalent antigen induces secretion of allergic mediators, histamine, proteases, chemotactic factors, and arachidonic acid metabolites, as well as sequential transcription of cytokine gene responsible for allergic symptoms.[1] In FcεRI knockout mice, binding of IgE to surface of mast cells was reported to be completely interrupted to induce neither degranulation of mast cells nor following allergic reactions. Furthermore, the knockout mice were confirmed to survive normally.[2] Thus, inhibition for FcεRI expression on mast cells should be recognized as an attractive target for preventing IgE-mediated allergic symptoms. In spite of significant expectation for scaffolds of anti-allergic agents with new mechanism of action, small-molecule inhibitors for FcεRI expression have been little found out.

In this circumstance, we undertook to search for anti-allergic seed principles with inhibitory activity for expression of FcεRI. To explore active principles, inhibitory potency was assessed by amount of IgE receptors on surfaces of human mast cells determined by indirect fluorescent antibody technique by flow cytometry.[3] In brief, HMC-1 cells,[4] human mast cell line established by Butterfield et al. were incubated with samples for 72 h, then the harvested cells were treated with anti-FcεRI α-chain antibody followed by FITC-labeled antibody. After this treatment, IgE receptors were detected as fluorescence of FITC with flow cytometer (Fig. 1).



Inhibition ratio for FcεRI expression (%)

$$= 100 \times \frac{\text{mean(cont)} - \text{mean(sample)}}{\text{mean(cont)} - \text{mean(back)}}$$

Figure 1. Evaluation for inhibitory activity for FcεRI expression.

* Corresponding author. Present address: Kyoto Pharmaceutical University. Tel.: +81 75 595 4634; fax: +81 75 595 4768.
E-mail addresses: murakami@poppy.kyoto-phu.ac.jp, murakami@phs.osaka-u.ac.jp (N. Murakami).

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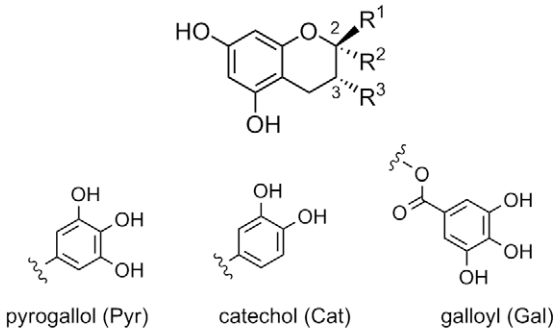


	R^1	R^2	R^3
EGCG (**1**)	H	Pyr	Gal
EGC (**2**)	H	Pyr	OH
ECG (**3**)	H	Cat	Gal
GCG (**4**)	Pyr	H	Gal
CG (**5**)	Cat	H	Gal
EC (**6**)	H	Cat	OH

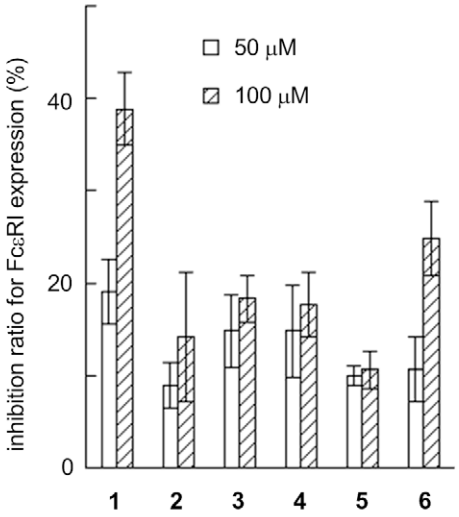
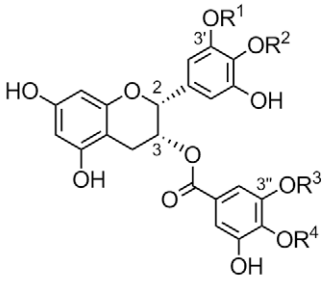


Figure 2. Inhibitory activity of catechins for FcεRI expression.

Average of fluorescence in the presence of each tested sample was compared with that of control, and inhibitory ratio was calculated according to the equation listed in Figure 1. In this assay, treatment of HMC-1 with only the secondary antibody provided background fluorescence and the score was indicated as mean(back).

By use of this assay for the extracts from about 400 kinds of medicinal plants, the 80% aq acetone extract of Theae Folium (Leaves of *Thea sinensis*) was revealed to show inhibitory activity for expression of FcεRI on human mast cells. Bioassay-guided separation of the extract through liquid–liquid partition and reversed phase HPLC furnished three active principles which showed 38.9%, 14.2%, and 18.3% of inhibition for FcεRI expression at the concentration of 100 μM, respectively. These compounds were identified to be epigallocatechin gallate (EGCG, **1**),[5,6] epigallocatechin (EGC, **2**),[5,7] and epicatechin gallate (ECG, **3**)[5,8] by comparison of their spectroscopic data, ^{1}H and ^{13}C NMR, FAB-MS, and optical rotations, with those reported. In addition to the isolated active catechins, gallocatechin gallate (GCG, **4**), catechin gallate (CG, **5**), and epicatechin (EC, **6**) were also evaluated for inhibitory activity for FcεRI expression. Of the six principles, EGCG (**1**) inhibited FcεRI expression most potently. Moreover, ECG (**3**) and GCG (**4**) exhibited similar potency as the second most potent principles. Curiously, EC (**6**) potently inhibited FcεRI expression at the concentration of 100 μM.

When inhibitory potency was respectively compared between **1** and **4**, **3** and **5**, the epi-type catechins (**1** and **3**) with 2,3-cis config-



	R^1	R^2	R^3	R^4
EGCG (**1**)	H	H	H	H
3'-OMe (**7**)	Me	H	H	H
4'-OMe (**8**)	H	Me	H	H
3''-OMe (**9**)	H	H	Me	H
4''-OMe (**10**)	H	H	H	Me

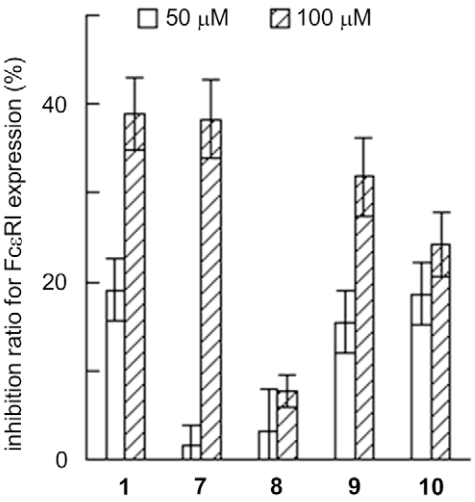


Figure 3. Inhibitory activity of *O*-methyl EGCGs for FcεRI expression.

uration were shown to exhibit more potent activity. By individual comparison of biological scores between **1** and **3**, **4** and **5**, a consecutive trihydroxyl structure in B-ring would significantly contribute to the inhibitory potency. On the basis of inhibitory efficacy of the four catechins (**1**, **2**, **3**, and **6**), a galloyl function was shown to potentiate the biological activity (Fig. 2).

Furthermore, to elucidate participation of both the pyrogallol function in the B-ring and the galloyl moiety in bioactivity of EGCG (**1**) in detail, biological scores were determined with regard to four kinds of mono-O-methylated epigallocatechin gallates (**7**–**10**). Consequently, introduction of a methyl function was shown to bring about reduction of bioactivity in general. Especially, introduction of the methyl group to 4′- or 4″-hydroxyl moiety resulted in extreme decrease in biological potency (Fig. 3).

Since catechins belonging to polyphenols inhibited expression of FcεRI on human mast cells, we also examined inhibitory activity of other commercially available polyphenols, anthocyanidins and flavones. In the case of the anthocyanidins, only delphinidin (**11**) displayed nearly similar activity to EGCG (**1**) as depicted in Figure 4. Notably, delphinidin (**11**) inhibited FcεRI expression more potent than ECG (**3**) and GCG (**4**). On the contrary, the other three anthocyanidins resulted in morphological change in HMC-1 cells. With respect to the flavones tested, only tricetinidin (**15**) exhibited similar inhibitory activity to ECG (**3**) and GCG (**4**). The flavones **16**–**20** induced aberration form to HMC-1, while treatment with **21** or **22** caused cell death. Because not only EGCG (**1**) but also both active polyphenols **11** and **15** include pyrogallol moiety, this partial structure should be significantly concerned with inhibition of FcεRI expression (Fig. 4).

FcεRI molecule, consisting of three subunits, α, β, and γ-chains, was shown to adopt a tetrameric structure comprising one α-chain, one β-chain, and two disulfide-linked γ-chains. In particular, α-chain was recognized as a specific component of FcεRI in terms of predominant extension to extracellular region and direct binding to IgE.[2] Accordingly, we elucidated the subunit responsible for down regulation of FcεRI expression by EGCG (**1**) by monitoring generation of mRNA.[9] HMC-1 cells were cultured in the presence of **1** for 36 h, and total mRNA was collected by use of oligo (dT)-cellulose column in the usual way. Each mRNA level of α, β and γ-chain was determined by RT-PCR followed by stain of the resulting PCR product with ethidium bromide. As shown in Figure 5, EGCG (**1**) definitely suppressed mRNA expression of γ-chain, while no obvious decrease appeared with regard to those of α and β-chains. Thus, **1** was clarified to reduce generation of mRNA of γ-chain subunit to suppress expression of FcεRI on human mast cells.

In conclusion, we disclosed epigallocatechin gallate (EGCG, **1**), epicatechin gallate (ECG, **3**), and gallocatechin gallate (GCG, **4**) as the inhibitors for FcεRI expression on human mast cells, which would be recognized as promising seeds toward anti-allergic agents with new mechanism of action. Among them, EGCG (**1**) inhibited expression of FcεRI most potently. Based on structure–activity relationship of the several relatives, cooperation of the pyrogallol moiety in B-ring, the galloyl function, and the 2,3-cis configuration was shown to enhance the inhibitory activity of **1**. Additionally, the anthocyanidin, delphinidin (**11**), and the flavone, tricetinidin (**15**), possessing the pyrogallol function were also revealed to suppress expression of FcεRI. As a result of preliminary examination of mechanism of action of EGCG (**1**), **1** was clarified to reduce generation of γ-chain subunit to suppress expression of FcεRI principally at mRNA level.

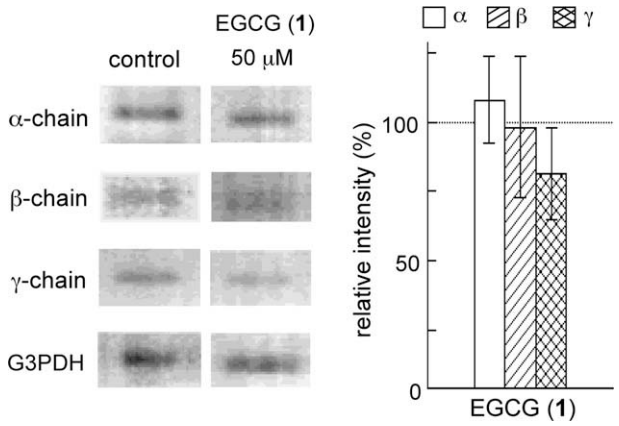
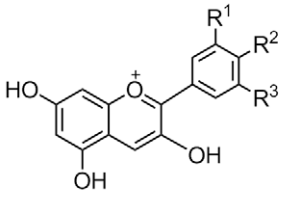
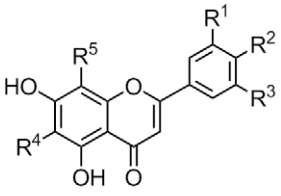


Figure 5. Inhibition for expression of mRNA of FcεRI subunits by EGCG.



	R^1	R^2	R^3
delphinidin (**11**)	OH	OH	OH
cyanidin (**12**)	OH	OH	H
malvidin (**13**)	OMe	OH	H
peonidin (**14**)	OMe	OH	OMe



	R^1	R^2	R^3	R^4	R^5
tricetinidin (**15**)	OH	OH	OH	H	H
apigenin (**16**)	H	OH	H	H	H
luteolin (**17**)	OH	OH	H	H	H
baicalein (**18**)	H	H	H	OH	H
orientin (**19**)	OH	OH	H	H	Glc
wogonin (**20**)	H	OH	H	H	OH
norwogonin (**21**)	H	H	H	H	OH
scutellarein (**22**)	H	OH	H	OH	H

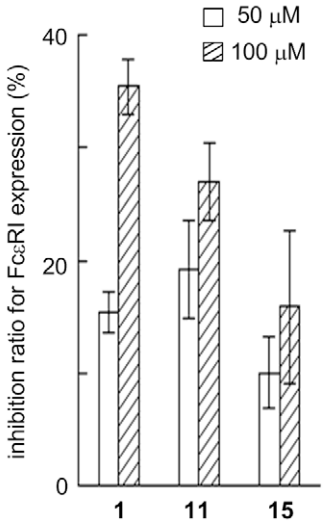


Figure 4. Inhibitory activity of anthocyanidins and flavones for FcεRI expression. The polyphenols except for **11** and **15** induced morphological change (**12**–**14**, **16**–**20**) or cell death (**21**, **22**) to HMC-1 cells at the concentration of 50 μM.

To date, no anti-allergic agents based on suppression of FcεRI expression have developed. Although the present active constituents may indeed require a little high concentration for this biological response, the three catechin congeners (**1**–**3**) are recognized as considerably potent suppressors owing to isolation from the fairly potent extract thorough the screening of about 400 medicinal plants. However, the potential of the active constituent described here for the anti-allergic agents with the novel mechanism of action is unconcluded at present. To open up a new avenue to this issue, further investigations including human clinical trial should be necessary.

Acknowledgments

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3. In 24-well microculture plates, HMC-1 cells (5.0×10^5 cells/mL) were cultured in 0.98 mL of IMDM medium (Sigma) containing 10% fetal bovine serum (Sigma), 2% L-Glutamine Stock Solution (Nacalai), 1.2% Penicillin-Streptomycin-Solution (Sigma), and 1.2 mM monothioglycerol (Sigma) in the presence of the test samples at 37 °C in 5% CO_2 for 72 h. The test samples were dissolved in DMF and diluted to the appropriate concentration using complete medium, then 20 μL of each sample solution was inoculated. The final concentration of DMF in the culture is 0.2%. After the whole was washed with PBS containing 0.5% BSA and 0.05% NaN_3 twice, the cells were treated with anti-human FcεRI α-chain antibody (0.001 μg, Kyokuto) on ice for 60 min. Then the cells were rinsed with the PBS twice and incubated with FITC labeled anti-mouse IgG antibody (0.001 μg, Cosmo Bio) on ice for 45 min in the dark. After duplicate washing with the PBS, the harvested cells were analyzed by flow cytometry (FACScalibur, Becton Dickinson). All samples were assessed for inhibitory activity for FcεRI expression in triplicate. In the cases of co-incubation with only DMF and treatment with only FITC labeled anti-mouse IgG antibody, the fluorescent scores were indicated as mean(control) and mean(back), respectively. Inhibition ratio of each sample was determined by the following equation:
Inhibition ratio (%) = 100 × [mean(control) − mean(sample)/mean(control) − mean(back)].
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9. In 6-well microculture plates, EGCG was co-incubated with HMC-1 cells (5.0×10^5 cells/mL) in 3 mL of the IMDM medium used for the bioassay at the concentration of 50 μM at 37 °C in 5% CO_2 for 36 h. Total cellular RNA was isolated using QuickPrep micro mRNA Purification Kit (Amersham) according to the manufacturer's instructions. First strand cDNA was synthesized using anchored oligo(dT) primers and ReverTra Ace reverse transcriptase (TOYOBO). The resultant cDNAs were respectively amplified by using the following primers: FcεRIα forward, 5′ataaaagctccgcgtgagaa3′, reverse, 5′tccttgagcacagacgtttc3′; FcεRIβ forward, 5′ttaccaggacctctaggagtgg3′, reverse 5′aggctggatgaaaaggtgtt3′; FcεRIγ forward 5′ccagcagtggtcttgctcttact3′, reverse 5′gcatgcaggcatatgtgatgcca3′; G3PDH forward 5′gatgacatcaagaaggtggtg3′, reverse 5′gctgtagccaaattcgttgtc3′. The amplified PCR products were subjected to electrophoresis on a 1.5% TAE (Tris–acetate EDTA buffer) agarose gel, then stained with ethidium bromide. Image analysis for each blot was conducted by Scion image (Scion) to determine amount of the expressed mRNA and quantification of each mRNA was carried out in triplicate. G3PDH was used as a control to correct expression of FcεRIα, β, and γ. The relative expression rates of the three subunits were described as compared with that of unstimulated HMC-1 cells.