

# Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells

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## Summary

**Background** Flavonoids have a variety of activities including anti-allergic activities, and are known to inhibit histamine release from human basophils and murine mast cells.

**Objective** The effects of luteolin, a flavone, on the immunoglobulin (Ig) E-mediated allergic mediator release from human cultured mast cells (HCMCs) were investigated and compared with those of baicalein and quercetin.

**Methods** HCMCs were sensitized with IgE, and then treated with flavonoids before challenge with antihuman IgE. The amount of released mediators was determined as was mobilization of intracellular  $\text{Ca}^{2+}$  concentration, protein kinase C (PKC) translocation and phosphorylation of intracellular proteins were detected after anti-IgE stimulation.

**Results** Luteolin, baicalein and quercetin inhibited the release of histamine, leukotrienes (LTs), prostaglandin  $\text{D}_2$  ( $\text{PGD}_2$ ), and granulocyte macrophage-colony stimulating factor (GM-CSF) from HMC in a concentration-dependent manner. Additionally, the three flavonoids inhibited A23187-induced histamine release. As concerns  $\text{Ca}^{2+}$  signalling, luteolin and quercetin inhibited  $\text{Ca}^{2+}$  influx strongly, although baicalein did slightly. With regard to PKC signalling, luteolin and quercetin inhibited PKC translocation and PKC activity strongly, although baicalein did slightly. The suppression of  $\text{Ca}^{2+}$  and PKC signalling might contribute to the inhibition of mediator release. The activation of extracellular signal-regulated kinases (ERKs) and c-Jun NH2-terminal kinase (JNK), that were activated just before the release of LTs and  $\text{PGD}_2$  and GM-CSF mRNA expression in IgE-mediated signal transduction events, were clearly suppressed by luteolin and quercetin. In contrast, the flavonoids did not affect the activation of p38 mitogen-activated protein kinase (p38 MAPK) pathway.

**Conclusion** These results indicate that luteolin is a potent inhibitor of human mast cell activation through the inhibition of  $\text{Ca}^{2+}$  influx and PKC activation.

**Keywords:**  $\text{Ca}^{2+}$  signalling, flavonoids, granulocyte macrophage-colony stimulating factor, histamine, human cultured mast cells, leukotrienes, luteolin, mitogen-activated protein kinases, PKC signalling, prostaglandin  $\text{D}_2$

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## Introduction

Flavonoids are a group of naturally occurring polyphenolic compounds distributed in many plants [1]. They exhibit a

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variety of activities including anti-inflammatory and anti-allergic properties, and some compounds are well known to inhibit histamine release from murine mast cells [2,3] and human basophils [4,5]. The flavonoids display a remarkable array of biochemical and pharmacological actions, some of which are suggested to affect the function of immune system. A number of flavonoids are indicated to affect the function of enzyme systems critically involved in the immune response and inflammation, such as both tyrosine and serine-threonine protein kinases, phospholipase A<sub>2</sub> (PLA<sub>2</sub>), phospholipase C (PLC), and lipoxygenases [6,7].

Recently, Saito *et al.* have established a method for culturing human mast cells *in vitro*, and human cultured mast cells (HCMCs) have become available for biochemical and pharmacological studies [8]. HCMCs are obtained by culturing CD34<sup>+</sup> cord blood mononuclear cells for more than 12 weeks in the presence of stem cell factor (SCF) and interleukin (IL) -6. The characteristics of HCMCs have been studied by us and several other investigators, indicating that HCMCs are useful for analyses of the function of lung mast cells and for the pharmacological studies on lung mast cells because of their morphological features, protease expression patterns and the lack of responsiveness to several agents such as substance P and compound 48/80 [9]. Suzuki *et al.* examined HCMCs and reported the early and late signalling events in HCMCs [10]. Their findings indicate that HCMCs may be useful for analyses of IgE-mediated signal transduction in human mast cells.

Although quercetin is well investigated as a kinase inhibitor *in vitro* and baicalein is sometimes evaluated as an anti-allergic agent *in vivo*, the pharmacological action of luteolin is rarely investigated. So far, we have investigated the effects of luteolin on IgE-mediated allergic reactions as well as baicalein and quercetin in rodent systems [11]. The main purpose of this study is to clarify allergic actions and inhibitory mechanisms of luteolin in comparison with baicalein and quercetin using HCMCs.

## Materials and methods

### Agents

Luteolin (Sigma, St Louis, MO, USA), baicalein (Extrasynthese, Genay, France) and quercetin (Extrasynthese) were dissolved in dimethyl sulphoxide (DMSO) as 100 mmol/L dilutions and stored. In our preliminary experiments, DMSO at concentrations present in working dilutions used in the present experiments had no effect on mediator release from mast cells.

### Human mast cell culture

HCMCs were obtained from human umbilical cord blood

cells using a method described previously with some modifications. Briefly, mononuclear cells (MNCs) were separated from heparinized umbilical cord blood by Lymphocyte Separation Medium (ICN Biomedicals Inc., Aurora, OH, USA) gradient. CD34<sup>+</sup> cells were purified from MNC by using CD34<sup>+</sup> progenitor cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and Magnetic Cell Sorting System (MACS; Miltenyi Biotec). Purified CD34<sup>+</sup> cells were cultured in  $\alpha$ -minimum essential medium ( $\alpha$ -MEM; GIBCO-RBL Laboratories, Gland Island, NY) containing 15% fetal bovine serum (Filtron Pty Ltd, Brooklyn, Australia) in the presence of 80 ng/mL human recombinant SCF (Kirin Brewery, Maebashi, Japan) and 50 ng/mL human recombinant IL-6 (Kirin). The immunoperoxidase staining for tryptase and chymase was performed by the method previously established [12]. HCMCs employed in the present study were more than 15 weeks old, with >99% purity. Cells were almost 100% positive for tryptase and 14% positive for chymase in the immunophenotypic analyses.

### Chemical mediator release and cytokine production

HCMCs were sensitized with human myeloma IgE (Chemicon Int. Inc., Temecula, CA, USA) at 37 °C over night. In the experiment of IgE-mediated chemical mediator (histamine, leukotrienes [LTs] and prostaglandin D<sub>2</sub> [PGD<sub>2</sub>]) release, cells were resuspended at  $1 \times 10^5$  cells/mL in Tyrode's solution containing HEPES and bovine serum albumin (BSA; Seikagaku Co., Tokyo, Japan) (126 mmol/L NaCl, 4 mmol/L KCl, 0.64 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, 1 mmol/L CaCl<sub>2</sub>, 0.6 mmol/L MgCl<sub>2</sub>, 0.1% glucose, 10 mmol/L HEPES, 0.03% BSA, pH 7.4) and treated with flavonoids for 10 min. The cells were then challenged with antihuman IgE antibody (anti-IgE; DAKO, Glostrup, Denmark) at 4  $\mu$ g/mL for 30 min. To examine the effect of agents on the cytokine (granulocyte macrophage-colony stimulating factor; GM-CSF) production, cells were resuspended at  $1 \times 10^5$  cells/mL in fresh culture media and were treated with examined agents for 10 min, and then were challenged with anti-IgE at 2  $\mu$ g/mL for 6 h. Calcium ionophore A23187 (Sigma) was also used as a nonimmunological stimulant for histamine release. Histamine was quantified by a method of post-column derivatization as previously reported [13]. For the analysis of LTs, PGD<sub>2</sub> and GM-CSF, we used a commercial ELISA kit (LTs – Böhmann Laboratory, Allschwil, Switzerland, PGD<sub>2</sub> – Cyman Chemical Co., Ann Arbor, MI, USA, GM-CSF – R & D Systems, Inc., Minneapolis, MN, USA).

### Isolation of RNA and reverse transcriptase (RT)-PCR

Total cellular RNA was isolated from HCMCs using Isogen (Nippon gene Co, Tokyo, Japan). The first-strand cDNA

was synthesized from 1 µg of total RNA using the super-script pre-amplification system (GIBCO-RBL Laboratories), according to the manufacturer's instructions. First-strand cDNA was used in the PCR amplification of  $\beta$ -actin and GM-CSF. PCR was performed as follows: 94 °C, 90 s denaturing; 62 °C, 90 s annealing; and 72 °C, 90 s extension for 30 cycles ( $\beta$ -actin) or 32 cycles (GM-CSF). Primers used were as follows:  $\beta$ -actin (product size; 275 bp, sense; 5'-CAA GAGATGGCCACGGCTGCT-3', antisense; 5'-TCCTTCT GCATCCTGTCGGCA-3') and GM-CSF (product size; 279 bp, sense; 5'-CTGAACCTGAGTAGAGACACTG-3', antisense; 5'-GGGGATGACAAGCAGAAAGTC-3').

PCR products were analysed by agarose gel electrophoresis.

#### *Measurement of intracellular $\text{Ca}^{2+}$ concentration ( $[\text{Ca}^{2+}]_i$ )*

Intracellular  $\text{Ca}^{2+}$  concentrations were determined with an automatic  $\text{Ca}^{2+}$  analyser (CAF-100; JEOL, Tokyo, Japan) by staining the cells with 2 µmol/L of fura-2 AM (Mikroprobe, Funakoshi, Tokyo, Japan). After being washed twice with Tyrode's solution, cell pellets were resuspended in the solution at  $2 \times 10^6$  cells/mL. Ratio of fura-2 signals between two waves of excitation (340 nm and 380 nm) was measured at 510 nm emission by a  $\text{Ca}^{2+}$  analyser.

#### *Detection of protein kinase C (PKC) translocation*

Sensitized cells were prepared at  $2 \times 10^6$  cells/tube. After pre-incubation, anti-IgE was added and the cells were incubated further for the time indicated. After the buffer was discarded, the cells were washed with ice-cold PBS. The cells were scraped with 1 mL of buffer A (20 mmol/L TRIS-HCl; pH 7.5, containing 5 mmol/L EDTA, 10 mmol/L EGTA, 50 µg/mL phenylmethylsulphonyl fluoride, 10 mmol/L benzimidazole, 0.3% v/v  $\beta$ -mercaptoethanol), sonicated for 30 s, and then centrifuged at 100 000 g for 60 min at 4 °C. The pellet was resuspended in buffer A containing 1% IGEPAL CA-630 (Sigma) for 20 s, and left for 60 min at 4 °C. Separate aliquots from the supernatant (cytosolic fraction) and the resuspended pellet (membrane fraction) were assayed for PKC translocation by Amersham's PKC enzyme assay system (Amersham, UK). This kit is based upon the PKC catalysed transfer of the  $\gamma$ -phosphate group of adenosine-5'-triphosphate to a peptide which is specific for PKC. Data are expressed as pmoles transferred phosphate per min per  $10^7$  cells.

#### *PKC assay*

For the analysis of the effect of flavonoids on PKC activity, we prepare the PKC fraction from HCMCs. The cytosol fraction of non-stimulated HCMCs was prepared as described above, and then used as the PKC fraction. At

the time when we employed Amersham's PKC enzyme assay system, each concentration of flavonoids was added, and the extent of PMA-induced reactions were detected. Staurosporin (Sigma), a non-specific PKC inhibitor, was used as positive control.

#### *Detection of mitogen-activated protein kinases (MAPKs) activation*

Extracellular signal-regulated kinases (ERKs), c-Jun NH<sub>2</sub>-terminal kinases (JNKs) and p38 MAPK are known as MAPKs. Drugs and anti-IgE-treated mast cells were lysed with ice-cold lysis buffer (50 mmol/L TRIS-HCl, pH 7.5; 0.15 mol/L NaCl; 1% IGEPAL CA-630; 10 µg/mL aprotinin; 10 µg/mL leupeptin; 1 mmol/L EDTA; and 1 mmol/L Na<sub>3</sub>VO<sub>4</sub>). Cell lysates were prepared separately,  $1 \times 10^5$  cells/lane for ERK and p38 MAPK, and  $3 \times 10^5$  cells/lane for JNK. Analyses of proteins by SDS-PAGE and transfer to PVDF membrane (PVDF-Plus, pore size = 0.45 micron; Micron Separations, Inc., Westboro, MA, USA) were performed as described elsewhere. Proteins were detected by the immunoblotting technique with antibodies against phosphorylated forms of ERK (Phospho-p44/42 MAP Kinase antibody; New England Biolabs Inc., Beverly, MA, USA), JNK (Phospho-SAPK/JNK antibody; New England Biolabs) and p38 MAPK (Phospho-p38 MAPK antibody; New England Biolabs). Horse-radish peroxidase (HRP)-conjugated antibody against rabbit IgG (Promega, Madison, WI, USA) was used as a secondary antibody. Finally, proteins were visualized by the ECL-plus system (Amersham). Because JNK2 activation was slightly after IgE-mediated stimulation, we evaluated JNK1 activation as JNK activation in HCMCs.

## Results

#### *Effects on histamine release*

In the preliminary experiments, the concentration and the incubation time of anti-IgE to obtain an appropriate histamine release were examined. Consequently, an incubation with 4 µg/mL of anti-IgE for 30 min resulted in a release of  $45 \pm 3.9\%$  of total histamine. The following experiments were carried out under this condition. As for A23187-induced histamine release, the incubation with 0.3 µmol/L A23187 for 30 min resulted in an appropriate histamine release ( $66.3 \pm 5.8\%$ ). Under these conditions, the effects of luteolin, baicalein and quercetin on histamine release from HCMCs were examined. The three flavonoids (1–100 µmol/L) showed a concentration-dependent inhibition of IgE-mediated histamine release from HCMCs (Fig. 1a). Additionally, long exposure of flavonoids did not modulate the degree

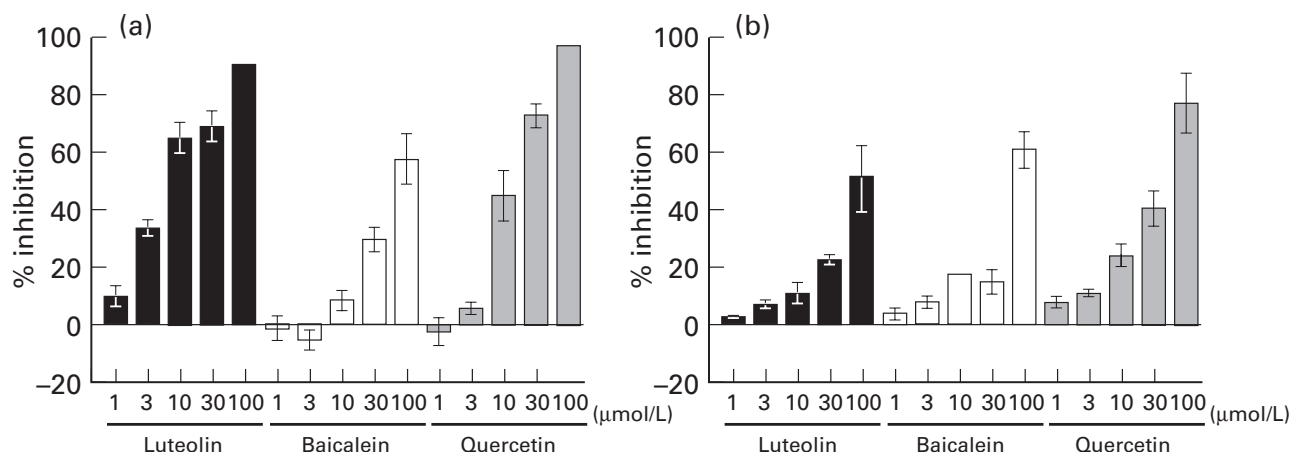


**Fig. 1.** Effects of luteolin and the other two flavonoids on IgE-mediated (a) or A23187-induced (b) histamine release from HCMCs. Sensitized HCMCs were challenged with anti-IgE for 30 min, and non-sensitized cells were challenged with A23187 for 30 min. Flavonoids were added 10 min before anti-IgE or A23187 challenge. Values are expressed as the percentage inhibition of the control histamine release. The mean net control antigen- and A23187-induced histamine releases were  $45.0 \pm 3.9$  and  $66.3 \pm 5.8\%$ , respectively. Each value represents the mean  $\pm$  SEM of three different experiments

of inhibition as far as 60 min pre-incubation (data not shown). Calcium ionophore A23187-induced histamine release was also inhibited by the three flavonoids in a concentration-dependent manner. The inhibition was less than 70% even in the highest concentration, 100  $\mu\text{mol/L}$  (Fig. 1b).

#### Effects on LTs, $\text{PGD}_2$ and GM-CSF release

An incubation of HCMCs with 4  $\mu\text{g/mL}$  anti-IgE for 30 min caused a release of LTs,  $69 \pm 19 \text{ ng}/10^6$  cells and  $\text{PGD}_2$   $92 \pm 29 \text{ ng}/10^6$  cells. In addition, an incubation with 2  $\mu\text{g/mL}$  anti-IgE for 6 h resulted in a production of  $1.2 \pm 0.4 \text{ ng}/10^6$  cells GM-CSF. Similar to histamine release, the release of LTs and  $\text{PGD}_2$  was inhibited concentration-dependently

(Fig. 2a,b). The inhibition of LT release was stronger than those of other mediators. The  $\text{IC}_{50}$  values of the flavonoids for LT release were almost 10 times lower than those for other mediators. Next, effects of flavonoids on GM-CSF production from HCMCs were examined. The flavonoids inhibited GM-CSF production in a concentration-dependent manner (Fig. 2c). They also inhibited GM-CSF mRNA expression when examined at 1.5 h after IgE-mediated stimulation (Fig. 3).

#### Effects on $\text{Ca}^{2+}$ signalling

Cross-linking of  $\text{Fc}\epsilon\text{RI}$  induced the elevation of  $[\text{Ca}^{2+}]_i$  and PKC translocation in a few minutes. As previously described,  $\text{Ca}^{2+}$  and PKC signalling are essential for

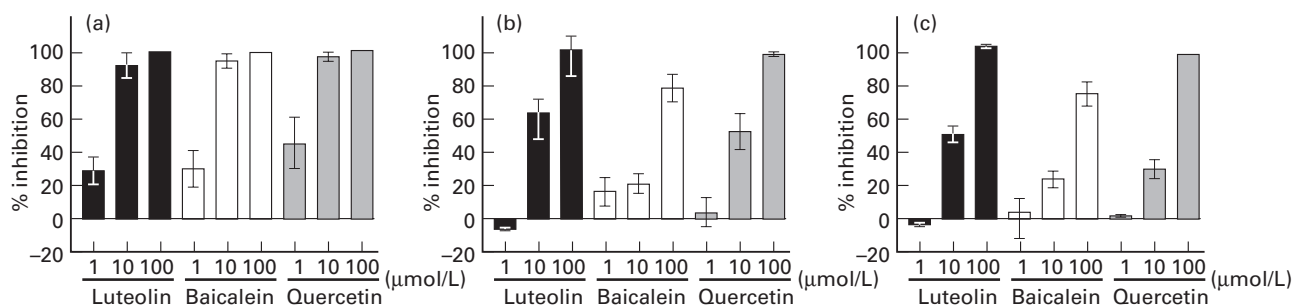


**Fig. 2.** Effects of luteolin and the other two flavonoids on IgE-mediated LTs (a),  $\text{PGD}_2$  (b), and GM-CSF (c) release from HCMCs. Sensitized cells were challenged with anti-IgE for 30 min (LTs and  $\text{PGD}_2$ ) or 6 h (GM-CSF). Flavonoids were added 10 min before anti-IgE challenge. Values are expressed as the percentage inhibition of the control mediators' release. The mean net control anti-IgE-induced LTs,  $\text{PGD}_2$  and GM-CSF release were  $69 \pm 19$ ,  $92 \pm 29$  cells and  $1.2 \pm 0.4 \text{ ng}/10^6$  cells, respectively. Each value represents the mean  $\pm$  SEM of three different experiments

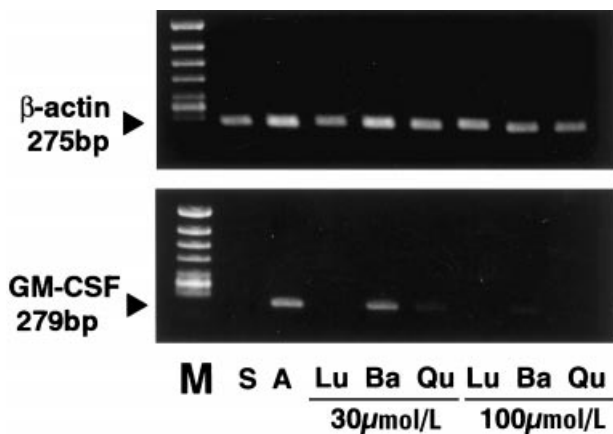


**Fig. 3.** Effects of luteolin and the other two flavonoids on IgE-mediated GM-CSF mRNA expression in HCMCs. Sensitized HCMCs were challenged with anti-IgE for 1.5 h, and analysed GM-CSF mRNA expression by RT-PCR. The results presented are representative of three different experiments. (M: molecular-weight marker, S: spontaneous, A: anti-IgE stimulation, Lu: luteolin, Ba: baicalein, Qu: quercetin)

mediator release, such as histamine, arachidonate metabolites and cytokines from rodent mast cells. As concerning  $\text{Ca}^{2+}$  signalling, flavonoids inhibited  $\text{Ca}^{2+}$  influx induced by IgE-mediated stimulation. Luteolin and quercetin inhibited about 90% of  $\text{Ca}^{2+}$  influx at  $30 \mu\text{mol/L}$ , although baicalein showed only about 50% inhibition at the same concentration (Fig. 4).

#### Effects on PKC translocation (PKC activation) and PKC activity

In HCMCs, although,  $10.4 \pm 3.4\%$  of total PKC was present in membrane fraction in unstimulated condition,  $34.8 \pm 6.8\%$  of total PKC was translocated to membrane fraction after IgE-mediated stimulation. The effects of flavonoids on IgE-mediated increase in membrane-associated PKC were examined. Pretreatment of luteolin for 10 min inhibited the translocation of PKC in a concentration-dependent manner (Fig. 5). Luteolin and quercetin inhibited PKC translocation almost 75% compared with control translocation at  $30 \mu\text{mol/L}$ , although baicalein inhibited only 20%. Next, experiments were undertaken to measure the effects of flavonoids on PKC activity. The control PKC activity was  $5.16 \pm 0.63 \text{ nmol/min}/10^7$  mast cells. Luteolin and quercetin showed the inhibition of PKC activity by 48 and 38%, respectively, at a concentration of  $100 \mu\text{mol/L}$  (Fig. 6). Luteolin and quercetin inhibited PKC activity in a concentration-dependent manner between 1 and  $100 \mu\text{mol/L}$ . Baicalein inhibited PKC activity less than 20% of the control activity at  $100 \mu\text{mol/L}$ . Staurosporine, a PKC

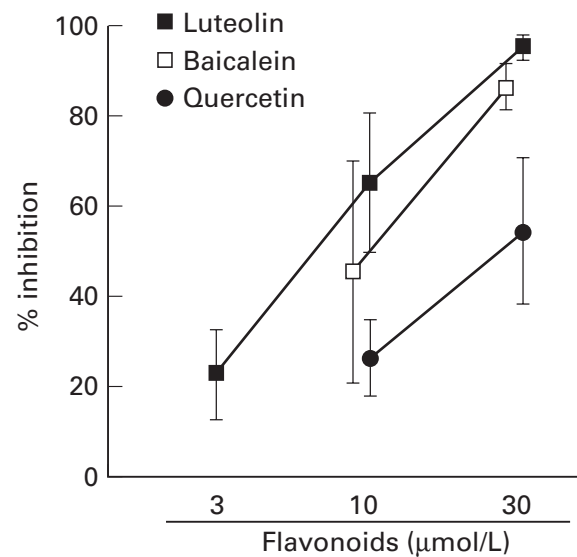


**Fig. 4.** Effects of luteolin and the other two flavonoids on IgE-mediated the elevation of  $[\text{Ca}^{2+}]_i$  in HCMCs. Sensitized cells were loaded fura-2 AM, and then challenged with anti-IgE. Luteolin, baicalein and quercetin were added 10 min before anti-IgE challenge. Ratio of fura-2 signals was measured by a  $\text{Ca}^{2+}$  analyser. Values are expressed as the percentage inhibition of the control response. Each value represents the mean  $\pm$  SEM of four different experiments.

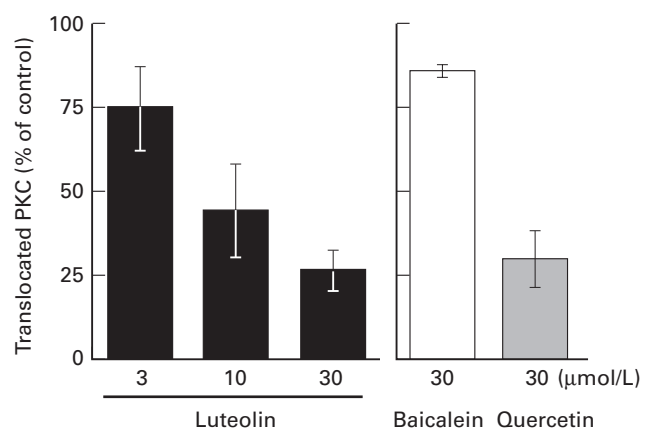


**Fig. 5.** Effect of luteolin and other two flavonoids on IgE-mediated PKC translocation to the membrane fraction in HCMCs. Sensitized HCMCs were challenged with anti-IgE, and then separated into the membrane fraction and the cytosol fraction. Flavonoids were added 10 min before anti-IgE challenge. PKC content in these fractions were determined by Amersham's PKC enzyme assay system, and values were expressed as the translocated PKC (% of control). Each value represents the mean  $\pm$  SEM of three different experiments

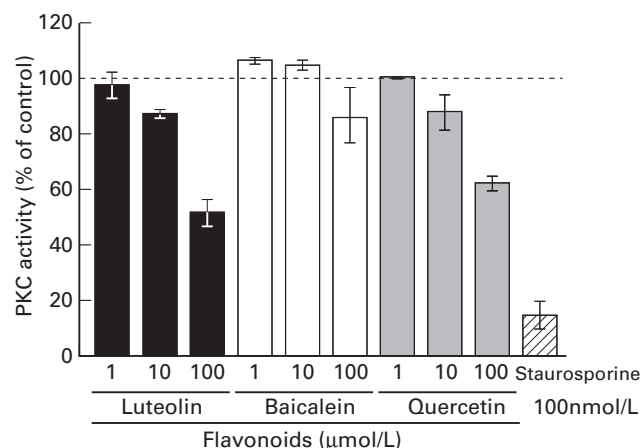


**Fig. 6.** Effects of luteolin and the other two flavonoids on PKC activity. The cytosol fraction of non-stimulated HCMCs was prepared and then used as the PKC fraction. The time when we employed Amersham's PKC enzyme assay system, each concentration of flavonoids was added, and the degree of PMA-induced reactions was detected. The inhibition of flavonoid-treated samples against flavonoid-non-treated sample was evaluated as PKC inhibition by flavonoids. The mean net control PKC activity was  $5.16 \pm 0.63$  nmol/min/ $10^7$  mast cells. Values are expressed as PKC activity (% of control). Each value represents the mean  $\pm$  SEM of three different experiments

inhibitor, inhibited PKC activity almost 90% of control activity.

#### Effects on MAPKs activation

In the preliminary experiments, the peaks of ERKs, JNK, and p38 MAPK activation were detected near 10 min after IgE-mediated stimulation. Therefore, this experiment was

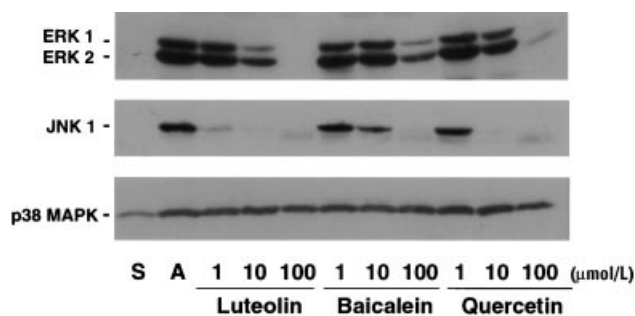


**Fig. 7.** Effects of luteolin and the other two flavonoids on IgE-mediated ERK, JNK and p38 MAPK activation in HCMCs. Sensitized HCMCs were challenged with anti-IgE, and total cellular proteins were analysed by SDS-PAGE and Western blotting. Phosphorylation of ERK, JNK and p38 MAPK were detected with phosphospecific antibodies. The results presented are representative of three different experiments. (S: spontaneous, A: anti-IgE stimulation)

conducted at 10 min after IgE-mediated stimulation. Luteolin and quercetin inhibited both ERKs and JNK activation concentration-dependently (1–100 µmol/L), although baicalein inhibited these kinase activations only weakly at 100 µmol/L. In contrast, p38 MAPK activation was not influenced by the treatment of flavonoids (Fig. 7).

#### Discussion

Previously, we have reported that luteolin and the other two flavonoids, baicalein and quercetin, inhibited rodent mast cell activation *in vitro* and *in vivo* [11]. In the present study, the effects and the inhibitory mechanisms of luteolin on IgE-mediated histamine, LTs, PGD<sub>2</sub> and GM-CSF release from HCMCs were investigated.

As shown in the results, luteolin and the other two flavonoids inhibited IgE-mediated histamine release from HCMCs as seen in rodent mast cells [10]. Although there are some discrepancies such as tyrosine phosphorylation of PLC-γ1 and Btk upon cross-linking of FcεRI between human and rodent mast cells, as concerns the effects of luteolin and the other two flavonoids, the inhibitory effects on histamine release were comparable. Because flavonoids inhibited A23187-induced histamine release from HCMCs, the targets of flavonoids are not only tyrosine protein kinases such as FcεRI-mediated activation of Syk and Lyn but also other intracellular events. So far now, although the efforts to investigate the effects of flavonoids on histamine release from rodent mast cells and human basophils were done vigorously [2–5,14], effects on arachidonate metabolites (LTs and PGD<sub>2</sub>) release and cytokine production were poorly examined. Interestingly, luteolin and the other two flavonoids inhibited LT release more potently than PGD<sub>2</sub> release. The production of arachidonic acid is regulated by Ca<sup>2+</sup> signalling and PLA<sub>2</sub> followed by ERK activation, and then arachidonic acid is metabolized via cyclo-oxygenase and lipoxygenase pathways [15]. ERK activation may be regulated by PKC signalling, because phorbol ester could directly activate ERKs in HCMCs (data not shown). In this study, we hypothesized that the discrepant effects on LT release and PGD<sub>2</sub> release might due to the powerful inhibition of lipoxygenase in relation to antioxidant activities, which were shared with all three flavonoids. Furthermore, we found that luteolin and the other two flavonoids inhibited not only the release of low-molecular weight mediators but also high-molecular weight mediators such as GM-CSF. Additionally, the inhibition of the expression of GM-CSF mRNA indicated the pretranslocation events were suppressed by these flavonoids. Although, so far now, flavonoids such as quercetin and baicalein have been thought to be as the inhibitory agents for chemical mediator release from mast cells *in vitro* [16,17] and allergic immediate phase reactions in skin and airway *in vivo* [18–20], the

inhibition of cytokine production may enlarge the possibility of luteolin and the other two flavonoids as inhibitors of IgE-mediated late phase reactions *in vivo*.

In this report, we showed that luteolin and other two flavonoids inhibited  $\text{Ca}^{2+}$  signalling and PKC signalling. As concerns PKC signalling, the flavonoids inhibited both PKC translocation, an index for PKC activation, and PKC activity. Although we used a crude fraction of HCMCs as a source of PKC, the results obtained are reliable because staurosporin reasonably inhibited the activity. Furthermore, structure–activity analysis on flavonoids revealed a model of the minimal essential features required for PKC and other enzyme inhibition [21]. As for PKC inhibition, luteolin and quercetin possessed the essential features but baicalein did not. This report supports our present results, and it might explain, in part, why luteolin and quercetin inhibited histamine release followed by PKC signalling stronger than baicalein. We have already investigated that both PKC signalling and  $\text{Ca}^{2+}$  signalling pathways are required for histamine and LT release from HCMCs as well as rodent systems [22]. With regard to  $\text{TNF}\alpha$ , the production is mainly regulated by  $\text{Ca}^{2+}$  influx, but the release process is regulated by additional mechanisms possibly involving activation of PKC in RBL-2H3 [23]. Therefore, the inhibition of  $\text{Ca}^{2+}$  and PKC signalling explains the suppression of histamine, arachidonate metabolites and cytokine release.

In signal transduction events via FcεRI, ERKs are activated through Raf/MEK/ERKs pathway for cytosol  $\text{PLA}_2$  activation followed by arachidonic acid release and activation of transcription factors such as NF-κB and AP-1 [24,25]. JNKs are activated through MEKK1/JNKK/JNK sequential protein kinase pathway for the activation of transcription factors such as AP-1 and ATF-2 [26–28]. p38 MAPK negatively regulates the activation of ERKs and the responses mediated by these kinases [29]. We visualized the activation of these pathways and evaluated the effects of flavonoids on these events in FcεRI signal transduction. Although luteolin and quercetin potently inhibited the activation of ERKs and JNK, baicalein did only slightly. The inhibition made us aware that immediately preceding events for LTs and  $\text{PGD}_2$  release and GM-CSF mRNA expression in FcεRI-mediated signal transduction events were suppressed in parallel with the mediator release. In contrast, flavonoids did not affect the p38 MAPK pathway. This result indicated the existence of a flavonoid-insensitive pathway after IgE-mediated stimulation.

In conclusion, luteolin inhibited arachidonate metabolites and cytokine release as well as histamine release. The action of luteolin is mediated through inhibiting PKC and  $\text{Ca}^{2+}$  signalling similar to quercetin. Therefore, luteolin could be used as one of the attractive and characteristic probes for studying allergic reactions.

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