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Biochemical and Biophysical Research Communications 340 (2006) 1–7
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Luteolin, a flavonoid, inhibits AP-1 activation by basophils

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Received 21 November 2005
Available online 6 December 2005

Abstract

Flavonoids including luteolin, apigenin, and fisetin are inhibitors of IL-4 synthesis and CD40 ligand expression by basophils. This study was done to search for compounds with greater inhibitory activity of IL-4 expression and to clarify the molecular mechanisms through which flavonoids inhibit their expression. Of the 37 flavonoids and related compounds examined, ayanin, luteolin, and apigenin were the strongest inhibitors of IL-4 production by purified basophils in response to anti-IgE antibody plus IL-3. Luteolin did not suppress Syk or Lyn phosphorylation in basophils, nor did suppress p54/46 SAPK/JNK, p38 MAPK, and p44/42 MAPK activation by a basophilic cell line, KU812 cells, stimulated with A23187 and PMA. However, luteolin did inhibit phosphorylation of c-Jun and DNA binding activity of AP-1 in nuclear lysates from stimulated KU812 cells. These results provide a fundamental structure of flavonoids for IL-4 inhibition and demonstrate a novel action of flavonoids that suppresses the activation of AP-1.
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Keywords: Basophils; Cytokines; Flavonoid; Luteolin; AP-1

Mast cells and basophils play an important part in the pathogenesis of allergic diseases through the release of inflammatory mediators such as histamine and cysteinyl leukotrienes, cytokines, chemokines, and the expression of surface molecules including CD40 ligand [1–5]. Among the cytokines produced by these cells, IL-4 (interleukin-4) and IL-13 are the most important in relation to IgE production, Th2 differentiation, and allergic inflammation. We as well as others previously showed that flavonoids, natural constituents included in vegetables, fruits, and drinks, possess anti-allergic activities [6–9]. Luteolin, apigenin, and fisetin inhibit IL-4 and IL-13 production with an IC_{50} value of 2–6 μM, histamine release, and CD40 ligand expression (data submitted) but do not suppress leukotriene C4 production by activated basophils in response to cross-linkage of high affinity of IgE receptor (FcεRI) and IL-3.

This study was therefore undertaken to find compounds with a greater inhibitory effect on IL-4 synthesis by basophils in order to determine the fundamental structure for such inhibition and identify the molecular mechanisms through which luteolin suppresses IL-4 synthesis and CD40 ligand expression. Ayanin, apigenin, and compound 31 were the strongest inhibitors among flavonoids and related compounds, which show a similarity to luteolin in structure. Although luteolin did not suppress Syk and Lyn activation by anti-IgE antibody plus IL-3-stimulated purified basophils, nor inhibit p54/46 SAPK/JNK, p38 MAPK or p44/42 MAPK phosphorylation by A23187 plus PMA-stimulated KU812 cells, it did suppress phosphorylation of c-Jun and DNA-binding activity of AP-1 transcription factors.

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doi:10.1016/j.bbrc.2005.11.157

Materials and methods

Culture medium. RPMI 1640 (Nacalai Tesque, Kyoto, Japan) supplemented with heat-inactivated 10% FCS (Dainippon Pharmaceutical, Australia), L-glutamine (2 mM), 2-ME (0.05 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL) was used as the culture medium.

Chemicals. Flavonoids and related compounds were characterized as described elsewhere [10] and dissolved in DMSO at a concentration of 10 mM. A23187 and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Japan (Tokyo) and dissolved in DMSO at a dose of 10 mM. Anti-human IgE antibody was obtained from DAKO (Glostrup, Denmark) and IL-3 was purchased from Genzyme-Techne (Boston, MA).

A basophilic cell line, KU812 and purification of peripheral blood basophils. A basophilic cell line, KU812, was characterized previously [11]. Purified peripheral blood basophils were obtained by anticoagulating peripheral blood buffy coats from healthy transfusion donors with 10 mM ethylenediaminetetraacetic acid (EDTA) and mixing them with the same amount of phosphate-buffered saline (PBS). This mixture was then layered onto Ficoll–Paque PLUS (Amersham Pharmacia Biotech, Buckinghamshire, UK) and centrifuged at 400*g*, for 20 min. This was followed by negative selection with the aid of a MACS Basophil Isolation Kit (Miltenyi BioTech, Bergisch Gladbach, Germany) [12]. The number of total cells and their viability were determined by staining with Trypan blue solution and counting with a hemocytometer. The purity of the basophils in this fraction was assessed by examining 1000 cells on cytospins treated with May–Grünwald stain. The basophil purity was 80–96% in various experiments and the contaminating cells consisted mainly of small lymphocytes and a few monocytes. For convenience, these basophil-enriched fractions are henceforth simply referred to as purified basophils.

Measurement of IL-4 synthesis by purified basophils. Purified basophils ($0.2–1 \times 10^6$ cells/mL) were incubated without or with various concentrations of flavonoids for 15 min and then stimulated for 24 h with anti-IgE antibody (1 μg/mL) and IL-3 (20 ng/mL), which was determined to be the most appropriate stimulation for maximal induction of IL-4 synthesis by basophils. The supernatant was harvested and the level of IL-4 concentration was measured by means of ELISA according to the manufacturer's instructions. An ultrasensitive human IL-4 ELISA kit (Biosource International, Camarillo, CA) was used to detect IL-4 and the measurable range of IL-4 was 65–25,000 fg/mL.

Western blots. KU812 cells (1×10^6 cells/mL) or purified basophils (2×10^6 cells/mL) were pre-incubated without or with flavonoid (30 μM) for 15 min and then stimulated with A23187 (1 μM) plus PMA (10 ng/mL) or anti-IgE antibody (1 μg/mL) plus IL-3 (20 ng/mL), respectively, for the indicated time. Cytoplasmic and nuclear proteins were obtained with a Nuclear Extract Kit (Active Motif, Carlsbad, CA). Briefly, stimulated cells were washed with ice-cold PBS/phosphatase inhibitors twice and suspended in 500 μl of hypotonic buffer for 15 min on ice, followed by the addition of 25 μl of detergent to the suspension. After centrifugation, the supernatant was obtained in the form of a cytoplasmic fraction and stored at −80 °C until use. The nuclear extract was subsequently harvested by resuspending the nuclear pellet in 50 μl of complete lysis buffer. After 30 min on ice, the suspension was centrifuged for 10 min at 14,000*g*. The supernatant was obtained as a nuclear extract and stored at −80 °C until use. The amount of protein in the cytoplasmic and nuclear extract was measured by using a BCA protein assay (Pierce, Rockford, IL). The extracts were boiled in SDS and electrophoresed on 4–12% SDS–polyacrylamide gels (Nacalai Tesque), after which the proteins in the gel were transferred into nitrocellulose membranes. The blots were then probed with each of the following antibodies: anti-phospho (P)-p38 MAPK, anti-P-p42/44 MAPK, anti-P-c-Jun (Ser63 and Ser73), anti-P-p54/46 SAPK/JNK, anti-p38 MAPK, anti-p42/44 MAPK, anti-p54/46 SAPK/JNK (Cell Signaling Technology, Beverly, MA), anti-P-Syk, anti-P-Lyn or anti-P-tyrosine (Santa Cruz Biotechnology, Santa Cruz, CA). This was followed by treatment of the blots with HRP-linked donkey anti-rabbit immunoglobulin (Amersham Biosciences, Piscataway, NJ), developed in chemiluminescent reagent (Western Lightening; PerkinElmer Life Science, Boston, MA) and then exposed to X-ray film.

DNA binding activity of nuclear transcription factor proteins. KU812 cells (1×10^6 cells/mL) were first incubated with luteolin, myricetin (30 μM) or an equivalent dose of DMSO for 15 min, and then stimulated with A23187 (1 μM) and PMA (10 ng/mL) for 1 h at 37 °C. After washing, nuclear protein was extracted from the cell pellets as described earlier. The nuclear extracts (1 μg of protein/aliquot) were assayed by means of ELISA (TransAM kits; Active Motif) for DNA-binding activity of phospho-c-Jun, c-Fos, FosB, Fra-1, Fra-2, JunB, and JunD. The transcription factor proteins, bound to immobilized oligonucleotides corresponding to appropriate consensus gene responsive elements, were detected with specific antibodies, followed by HRP-conjugated anti-immunoglobulin, according to the supplier's instructions. DNA-binding activity was expressed as OD.

Results

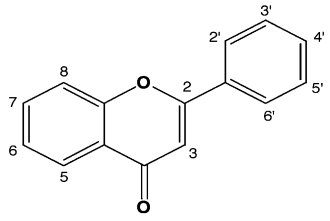
A hierarchy of the inhibitory activity of flavonoids and related compounds on IL-4 synthesis by basophils

In order to develop and find compounds which inhibit IL-4 synthesis by basophils more strongly, the inhibitory effects of 37 different flavonoids and related compounds on IL-4 synthesis were examined. Purified basophils were pre-incubated with various concentrations of compounds for 15 min and then stimulated with anti-IgE antibody plus IL-3 for 24 h. The IL-4 concentration in the culture supernatant was measured by means of ELISA. More than three independent experiments for each compound were carried out and the results are summarized in Fig. 1. Ayanin, luteolin, apigenin, and compound 31 were the strongest inhibitors of IL-4 synthesis with an IC_{50} value of around 2.2–3.2 μM. Followingly diosmetin, fisetin, ombuin, and compound 5 proved to have an effect with an IC_{50} value of 5.2–6.5 μM. Quercetin and kaempferol showed an intermediate effect. These results of analyses of the structure–activity relationships of flavonoids and related compounds thus indicated a fundamental structure for IL-4 inhibition. The presence of OH or OCH_3 in position 7 and 4′, either position 3 or 5 is required for the inhibitory activity.

Effect of luteolin on signaling cascades by activated basophils

Luteolin was selected to clarify the mechanism(s) through which flavonoids inhibit IL-4 secretion. Luteolin but not myricetin at 30 μM almost completely suppressed IL-4 synthesis and CD40 ligand expression by anti-IgE plus IL-3-stimulated basophils and by A23187 plus PMA-stimulated KU812 cells, so that we used 30 μM of luteolin to study its effect. To examine the effect of luteolin on Syk and Lyn activation, purified basophils were pre-incubated without or with luteolin at 30 μM for 15 min, and further stimulated with anti-IgE antibody and IL-3 for another 30 min. The phosphorylation of Syk or Lyn was examined by using Western blots. Fig. 2 shows that the presence of luteolin did not suppress phosphorylation of these proteins, which became more phosphorylated in response to anti-IgE and IL-3.

Subsequent experiments to examine the effect of luteolin on other signaling cascades were at first performed by using purified basophils but because it was difficult to repeat the



	3	5	6	7	8	2'	3'	4'	5'	IC_{50}(μM)
Ayanin	OCH_3	OH	H	OCH_3	H	H	OH	OCH_3	H	2.2
Luteolin	H	OH	H	OH	H	H	OH	OH	H	2.7
Apigenin	H	OH	H	OH	H	H	H	OH	H	3.1
Compound 31	OH	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	3.2
Diosmetin	H	OH	H	OH	H	H	OH	OCH_3	H	5.2
Fisetin	OH	H	H	OH	H	H	OH	OH	H	5.8
Ombuin	OH	OH	H	OCH_3	H	H	OH	OCH_3	H	6.3
Compound 5	H	OAc	H	H	H	H	OAc	OAc	OAc	6.5
Compound 6	H	OH	H	H	H	H	OH	OH	OH	11.4
Scutellarein	H	OH	OH	OH	H	H	H	OH	H	14.0
3-Hydroxyflavone	OH	H	H	H	H	H	H	H	H	15.0
Kaempferol	OH	OH	H	OH	H	H	H	OH	H	15.7
Quercetin	OH	OH	H	OH	H	H	OH	OH	H	18.8
Compound 32	OCH_3	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	18.9
4',7-Dihydroxyflavone	H	H	H	OH	H	H	H	OH	H	19.4
Eriodictyol(2-3)	H	OH	H	OH	H	H	H	OH	OH	20.8
Compound 3	H	OAc	H	H	H	H	OCH_3	OCH_3	OCH_3	21.0
Fustin(2-3)	OH	H	H	OH	H	H	OH	OH	H	23.0
Compound 4	H	OH	H	H	H	H	OCH_3	OCH_3	OCH_3	23.0
7-Hydroxyflavone	H	H	H	OH	H	H	H	H	H	26.5
3',4'-Dihydroxyflavone	H	H	H	H	H	H	OH	OH	H	>30
Myricetin	OH	OH	H	OH	H	H	OH	OH	OH	>30
Galangin	OH	OH	H	OH	H	H	H	H	H	>30
Morin	OH	OH	H	OH	H	OH	H	OH	H	>30
Chrysin	H	OH	H	OH	H	H	H	H	H	>30
Baicalein	H	OH	OH	OH	H	H	H	H	H	>30
Rhamnetin	OH	OH	H	OCH_3	H	H	OH	OH	H	>30
Compound 1	H	OH	H	H	H	OCH_3	H	OCH_3	H	>30
Compound 2	H	OH	NH_2	H	H	H	H	H	H	>30
Compound 7	CO-Phe-$(OH_3)_2$	OAc	H	H	H	OCH_3	H	OCH_3	H	>30
Compound 8	CO-Phe	OH	H	H	H	H	H	H	H	>30
Compound 9	H	OCH_3	H	H	H	H	H	H	H	>30
Astragalin	O-Glc	OH	H	OH	H	H	H	OH	H	>30
Rutin	O-Rutinoside	OH	H	OH	H	H	OH	OH	H	>30
Gossypin	OH	OH	H	H	O-Glc	H	OH	OH	H	>30
Isoquercitrin	O-Glc	OH	H	OH	H	H	OH	OH	H	>30
Myricitrin	O-Rhamnoside	OH	H	OH	H	H	OH	OH	OH	>30

Fig. 1. A hierarchy of inhibitory activity of flavonoids on IL-4 synthesis by basophils. Purified basophils were pre-incubated without or with various doses of flavonoid for 15 min and then stimulated with anti-IgE antibody (1 μg/mL) and IL-3 (20 ng/mL) for 24 h. The IL-4 concentration was measured by means of ELISA. The suppressive effect of flavonoid was shown as IC_{50} value from more than three independent experiments.

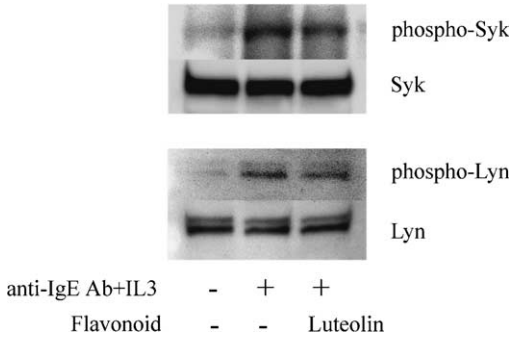


Fig. 2. Luteolin does not inhibit Syk and Lyn phosphorylation by anti-IgE plus IL-3-stimulated basophils. Purified basophils were pre-incubated without or with luteolin (30 μM) for 15 min, and then stimulated with anti-IgE antibody (1 μg/mL) and IL-3 (20 ng/mL) for 30 min. The cellular lysate was obtained and the activation of Syk and Lyn was examined by Western blots.

experiments due to the limited number of cells available, a human basophilic cell line, KU812, was employed instead. KU812 cells were pre-incubated with luteolin, myricetin at 30 μM or the equivalent dose of DMSO, and further stimulated with A23817 plus PMA for 30 min. The activation of p38 MAPK, p44/42 MAPK, and p54/46 SAPK/JNK in cytoplasmic fractions and of phosphorylated c-Jun in nuclear fractions was examined by Western blotting. The experiments were carried out more than three times and a representative result is shown in Fig. 3. The stimulation of KU812 cells with A23187 and PMA resulted in an increase in the phosphorylation of p38 MAPK, p44/42 MAPK, p54/46 SAPK/JNK, and c-Jun proteins. Luteolin as well as myricetin at 30 μM did not affect the phosphorylation of p38 MAPK, p44/42 MAPK, and p54/46 SAPK/JNK proteins. However, luteolin significantly suppressed phosphorylation of c-Jun at Ser63 and Ser73 residues in nuclear extracts whereas myricetin did not. The luteolin inhibition of c-Jun phosphorylation was significant over repeated experiments.

Effect of luteolin on DNA binding activity of AP-1

AP-1 and NFAT are important transcription factors in the regulation of various cytokines including IL-4 as well as of CD40 ligand expression [13–15]. We previously demonstrated that fisetin inhibited NFATc2 activation by A23187-stimulated KU812 cells through gel-shift mobility assay [8]. We next examined the effect of luteolin on the DNA-binding activity of AP-1 transcription factors, since luteolin inhibited c-Jun protein phosphorylation in the nuclear extract. The stimulation of KU812 cells with A23187 and PMA led to the enhancement of DNA-binding activity of phosphorylated c-Jun, JunB, JunD, c-Fos, and FosB (Fig. 4), but not of Fra-1 and Fra-2 in nuclear extracts (data not shown). We conducted four independent experiments and a representative result is shown in Fig. 4. The pre-incubation of luteolin but not myricetin diminished

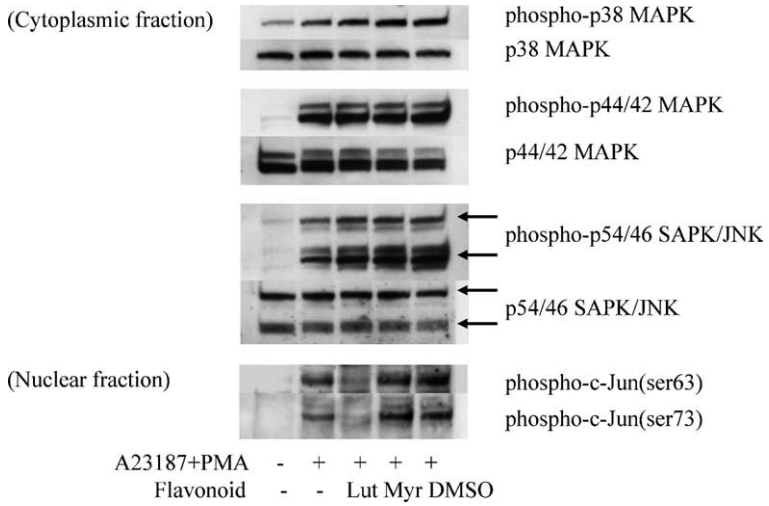


Fig. 3. Luteolin inhibits phosphorylation of c-Jun but not p38 MAPK, p44/42 MAPK, and p54/46 SAPK/JNK by PMA plus A23187-stimulated KU812 cells. KU812 cells were pre-incubated with luteolin or myricetin at 30 μM, or equivalent dose of DMSO, and then stimulated with PMA (10 ng/mL) and A23187 (1 μM) for 30 min. The cellular fraction and nuclear fraction were obtained and the activation of p38 MAPK, p44/42 MAPK, and p54/46 SAPK/JNK and of phosphorylation of c-Jun was examined by Western blots.

the DNA-binding activity of phosphorylated c-Jun, JunB, JunD, c-Fos, and FosB in nuclear extracts by KU812 cells. In each experiment, the presence of luteolin consistently suppressed the DNA-binding activity of phosphorylated c-Jun, JunB, JunD, c-Fos, and FosB at by factors of 2–4, >4, 2–4, >4, and >4, respectively. Finally, we incubated nuclear factors with DNA consensus in the presence of luteolin in order to determine whether luteolin might affect the direct binding of nuclear factors to the DNA consensus. However, the presence of luteolin did not interfere with the DNA binding capacity of AP-1.

Discussion

In this study, we demonstrated that flavonoids including ayanin, luteolin, apigenin, diosmetin, fisetin, and ombuin, and chemically produced compounds 31 and 5 inhibit IL-4 synthesis by anti-IgE antibody plus IL-3-activated basophils. This inhibition was found to have an IC_{50} value ranging from 2.2 to 6.5 μM. Analyses of the activity–structure relationships of flavonoids and related compounds indicate that the presence of OH or OCH_3 in position 7 and 4prime, either position 3 or 5, is required for the

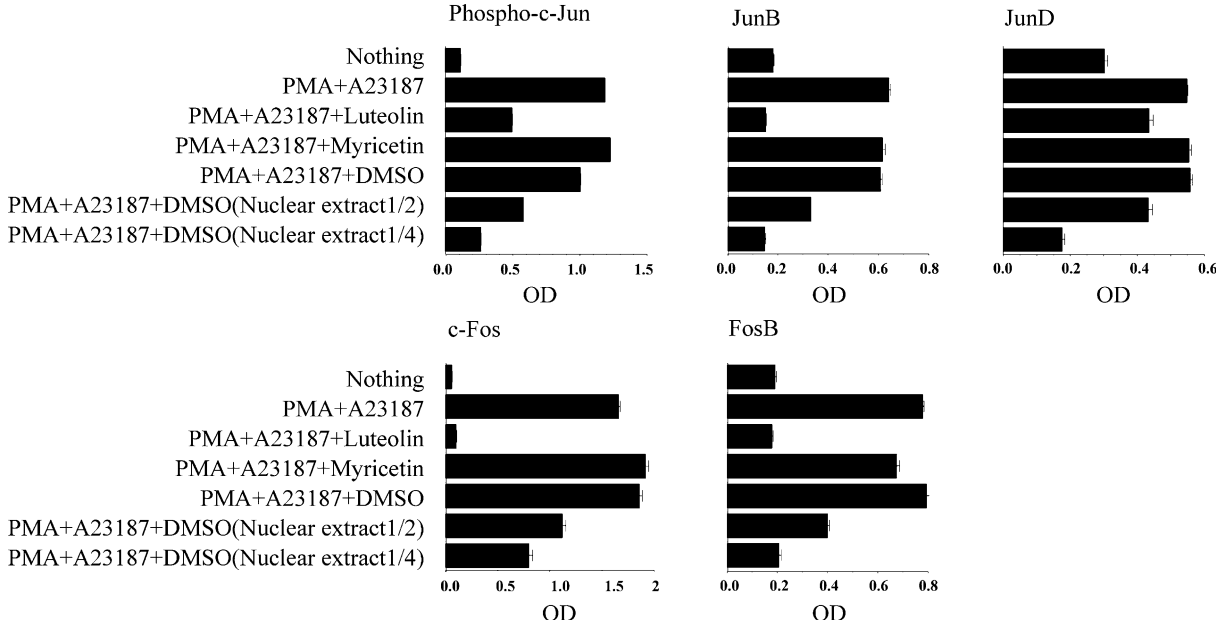


Fig. 4. Luteolin inhibits AP-1 DNA binding activity of nuclear extracts by PMA plus A23187-stimulated KU812 cells. KU812 cells were pre-incubated with luteolin or myricetin at 30 μM or equivalent dose of DMSO for 15 min, and then stimulated with PMA (10 ng/mL) plus A23187 (1 μM) for 1 h. AP-1 DNA binding activity of nuclear extracts (1 μg protein) was examined. Nuclear extract 1/2 and 1/4 mean that nuclear extract is 1/2 (0.5 μg) and 1/4 (0.25 μg).

activity. In the case of rat mast cell line, RBL-2H3 cells, similarly apigenin, luteolin, diosmetin, and fisetin showed an inhibitory activity on IL-4 synthesis with an IC_{50} value of 3.0–3.8 μM [10]. These findings can be expected to aid the development of new compounds of IL-4 inhibitors. Alternatively, since flavonoids are included in vegetables, fruits, and drinks [16], and thus are ingested daily in significant amounts, an appropriate intake of flavonoids is expected to alleviate allergic symptoms as complementary or alternative medicine, or to prevent allergic diseases [17]. Indeed, we as well as others have shown that administration of flavonoids to mice or rats suppresses the onset of allergic diseases and inhibits allergic symptoms [18–20]. The database of the content of the major flavonoids in foods and drinks including luteolin, apigenin, quercetin, and kaempferol has been recently established in the USA [21], which indicates the possibility that the greater the intake of selective flavonoids with higher activity, the stronger their effect is likely to be. In support of this concept, a cohort study in Finland found a lower asthma incidence for higher total flavonoid intakes [22]. It should be pointed out, however, that the establishment in in vitro experiments of a hierarchy of the inhibitory action of flavonoids does not simply mean that an increase in the intake of flavonoids with higher activity should be recommended, since glycosylation of flavonoids usually increases the absorption from the small intestine but decreases their inhibitory activity on IL-4 synthesis according to the in vitro experiments [23].

Upon activation through cross-linkage of FcεR1, mast cells and basophils secrete chemical mediators, cytokines and chemokines, and express surface molecules. Through the actions of these soluble mediators and cell-to-cell interaction mast cells and basophils play an important part in the formation of allergic inflammation [1–5]. These cells produce IL-4 and IL-13 as well as express CD40 ligand (CD154), so that they can induce B cells to differentiate into IgE secreting cells. We previously showed that flavonoids suppress histamine release, IL-4 and IL-13, synthesis and CD40 ligand expression (submitted for publication) but not leukotriene C4 production by activated human basophils [8,9]. To explain this effect, we attempted in the study reported here to identify and characterize the molecular mechanisms of flavonoids.

The question we tried to answer was: What signaling cascade leads to the secretion of histamine, the production of leukotriene C4, the synthesis of IL-4 and IL-13, and the induction of CD40 ligand expression [3–5,24]? In basophils, cross-linking of FcεR1 induces activation of various tyrosine kinases including Lyn and Syk, which lead to the stimulation of PI_3-kinases, MAP kinases, and PLC. The PI_3-kinases then activate Rac/Rho GTPases and p38 MAPK, which are involved in the production of cytokines such as IL-4 and IL-13. Rac/Rho GTPases also affect the cytoskeletal processes during degranulation as well as extracellular signal-related kinase-activating kinase (MEK). P44/42 MAPK activation is reported to lead to the synthesis of cysteinyl leukotrienes, while PKC activated by diacylglycerol is involved in degranulation and may also affect cytokine transcription. The release of calcium by IP_3 affects degranulation, PLA_2 translocation, and calcineurin activity. Thus, several signaling molecules are associated with induction of IL-4 transcription, degranulation, and leukotriene C4 synthesis. While the precise mechanism for CD40 ligand expression in basophils remains unknown, it was found that in T cells, an increase in intracellular calcium is an essential signal for its expression [25], because induction of the CD40 ligand is inhibited by cyclosporin A [26], which blocks the phosphatase activity of calcineurin. Finally, the stimulation of T cells with PMA stimulates AP-1 through JNK and P44/42 MAPK activation and synergistically enhances CD40 ligand transcription with NFAT [13].

Previous studies regarding the points where flavonoids exert their action in cells have demonstrated that flavonoids and their metabolites act at PI_3-kinase, Akt/protein kinase B (Akt/PKB), tyrosine kinases, PKC, and MAP kinase signaling cascades [27]. As for the action of flavonoids on basophils or mast cells, several findings have been reported. Kimata et al. [28] showed that luteolin and quercetin inhibited Ca^{2+} influx, PKC activity, and p44/42 MAPK and JNK activation but not p38 MAPK by human cultured mast cells in response to cross-linkage of FcεRI. Shichijo et al. [29] demonstrated that flavonoids inhibited Syk activation, also in human cultured mast cells in response to cross-linkage of FcεRI. However, the relationship between the inhibition of Syk activation and degranulation was unclear, since myricetin proved to be a strong inhibitor of Syk activation but a weak suppressor of degranulation. In our study, we did not observe luteolin inhibition of Syk activation by basophils stimulated with anti-IgE and IL-3. In another study, flavones including apigenin were found to reduce the FcεRI α and γ chains, perhaps through down-regulating P44/42 MAPK phosphorylation in KU812 cells [30]. In this regard, we showed that luteolin did not suppress p54/46 SAPK/JNK, p38 MAPK, and p44/42 MAPK by A23187 + PMA-stimulated KU812 cells. Luteolin almost completely suppressed IL-4 synthesis and CD40 ligand expression under the same condition, suggesting that the inhibitory action of luteolin on IL-4 synthesis and CD40 ligand expression is not mediated through its inhibition of these signal transducers. The reasons for this discrepancy between our results and previously reported findings that flavonoids inhibited MAPK family members are not known at the present time but it may be due to differences in cells and/or stimulation.

The most striking finding of our study is that luteolin inhibited phosphorylation of c-Jun protein and AP-1 activation but not phosphorylation of p54/46 SAPK/JNK. Since luteolin did not directly suppress the DNA binding activity of AP-1 transcription factors to the consensus, it appears that luteolin blocked activation of nuclear factors including Fos and Jun after the stimulation of MAPK/JNK. In a previous study of ours, we found that fisetin

suppressed the NFAT-DNA binding activity in KU812 cells [8] and the pre-incubation of KU812 cells with luteolin also depressed the DNA binding activity of nuclear extracts to the NFAT consensus. This means that luteolin is a natural compound with an inhibitory effect on AP-1 and NFAT transcription factors, which play an important role in the transcriptional activation of cytokines including IL-4 and IL-13, and of CD40 ligand expression [13,15]. Consequently, the suppressive effect of luteolin on IL-4 and IL-13 synthesis, and CD40 ligand expression is thought to be mediated through its inhibitory action on AP-1 and NFAT activation. How flavonoids inhibit AP-1 and NFAT activation awaits clarification, but recently it has been demonstrated that nitric oxide also inhibits IgE-dependent cytokine production and Fos/Jun activation in RBL-2H3 mast cells [31], so that the actions of flavonoids resemble those of nitric oxide.

We hope that the evidence presented here may contribute to the clinical application for allergic patients of these flavonoids as alternative and complementary therapeutic agents, to the preventative strategy for allergic diseases through diet management [32] and to the development of compounds, which suppress cytokine synthesis and CD40 ligand expression.

Acknowledgments

This work was supported by funds of the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Health, Labour and Welfare of Japan, and The Osaka Foundation for Promotion of Clinical Immunology.

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