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REVIEW ARTICLE

Flavonoids and Related Compounds as Anti-Allergic Substances

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ABSTRACT

The prevalence of allergic diseases has increased all over the world during the last two decades. Dietary change is considered to be one of the environmental factors that cause this increase and worsen allergic symptoms. If this is the case, an appropriate intake of foods or beverages with anti-allergic activities is expected to prevent the onset of allergic diseases and ameliorate allergic symptoms. Flavonoids, ubiquitously present in vegetables, fruits or teas possess anti-allergic activities. Flavonoids inhibit histamine release, synthesis of IL-4 and IL-13 and CD40 ligand expression by basophils. Analyses of structure-activity relationships of 45 flavones, flavonols and their related compounds showed that luteolin, ayanin, apigenin and fisetin were the strongest inhibitors of IL-4 production with an IC_{50} value of 2–5 μM and determined a fundamental structure for the inhibitory activity. The inhibitory activity of flavonoids on IL-4 and CD40 ligand expression was possibly mediated through their inhibitory action on activation of nuclear factors of activated T cells and AP-1. Administration of flavonoids into atopic dermatitis-prone mice showed a preventative and ameliorative effect. Recent epidemiological studies reported that a low incidence of asthma was significantly observed in a population with a high intake of flavonoids. Thus, this evidence will be helpful for the development of low molecular compounds for allergic diseases and it is expected that a dietary menu including an appropriate intake of flavonoids may provide a form of complementary and alternative medicine and a preventative strategy for allergic diseases. Clinical studies to verify these points are now in progress.

KEY WORDS

allergy, basophil/mast cells, complementary and alternative medicine, flavonoid, prevention

INTRODUCTION

The worldwide prevalence of allergic diseases such as asthma, atopic dermatitis and allergic rhinitis has increased during the last two decades[1-3] and it is assumed that in Japan more than one-third of the population is now suffering from at least one of these diseases. For instance, the first case of Japanese cedar pollinosis was reported in the mid-1960s[4] but now half of the Japanese population has become sensitized with Japanese cedar pollens and 24–29% of the population is suffering from this disease.[5] This trend has led not only to a significant increase in patient morbidity but also in cost to the patients and their families and has placed a great burden on society.[6-8]

The interaction between genetic and environmental factors is generally accepted to cause individuals to be sensitized with environmental allergens and to suffer from allergic diseases.[9-12] However, it is believed that recent changes in the environment have contributed to the increase more significantly than genetic factors, since it seems unlikely that genes would change over one or two generations. Thus, it is a central issue to reveal what environmental factor(s) cause such high prevalence and to find strategies to prevent their development.[11,13,14] Recently the change of diet is considered to be one of the environmental factors responsible for such an increase.[15,16] Indeed, foods include both allergy-promoting and anti-allergic nutrients. In this article we will propose

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the possibility that an appropriate intake of flavonoids with anti-allergic activity may provide an effective form of complementary and alternative medicine and a preventative strategy for allergic diseases.

NUTRITION WITH ALLERGY PROMOTING AND INHIBITING ACTIVITY

Changes in environmental factors may contribute to the increase in the prevalence of allergic diseases. As environmental factors that influence the susceptibility to the development of asthma in predisposed individuals, the Global Strategy for Asthma Management and Prevention lists indoor and outdoor allergens, occupational sensitizers, air pollution, respiratory infection, parasitic infections, socioeconomic status, family size and obesity.[11] The Asthma Prevention and Management Guideline 2006, Japan indicates allergens, viral infection, in particular the RS virus, indoor and outdoor air pollution, smoking, food and its additives, parasite infection and drugs as triggering factors for the onset of asthma.[17] The hygiene hypothesis of allergic diseases indicates that environmental changes in the industrialized world have led to reduced microbial contact at an early age and thus resulted in the growing epidemic of allergic diseases.[18-20] The conditions of living, the development of anti-microbial agents and vaccination, and small numbers of siblings lead to decreased opportunities of infections including mycobacterium tuberculosis or type A hepatitis virus, which are hypothesized to change T helper (Th)1/Th2 balance. Secondly, increased exposure to allergens may contribute to the increase. Changes in living conditions such as maintaining the indoors at constant temperature and humidity levels also have resulted in conditions that are optimal for house dust mites to grow and there has been increased opportunity for keeping pets at home. Thirdly, air pollution including diesel particles, ozone and nitrogen oxide is thought to be associated with the increase.

Recently it has been pointed out that dietary change might contribute to the onset of allergic diseases.[16] Indeed, nutrients with anti-allergy and allergy-promoting activities are included in foods and beverages.[15] Vitamins A, C, E, selenium and copper are antioxidants and vitamin C and E also have other anti-inflammatory and anti-allergic effects. Magnesium, when given intravenously, shows bronchodilation effect and inhibits degranulation by mast cells. Omega-3 polyunsaturated fatty acids (PUFA) stabilize the mast cell membrane and decrease leukotriene C4 synthesis, whereas omega-6 PUFA are precursors for leukotriene C4 and thus promote allergic inflammation. A high sodium intake may be associated with increased airway responsiveness. Based on the activity of the nutrients and the epidemiological studies, dietary manipulation of these nutrients may ameliorate allergic symptoms. However, intervention studies so far have reached no consistent conclusion. Devereux *et al.* recently reported that reduced consumption of foods containing antioxidants (fruits and vegetables), increased omega-6 PUFA intake and reduced omega-3 PUFA intake have been implicated for the increase in asthma and atopic diseases and they pointed out that dietary antioxidant and lipid intakes during pregnancy and early childhood might decrease the onset of allergic diseases.[16]

Guidelines for prevention of asthma and allergic diseases were recently proposed by the World Allergy Organization, based on the results of numerous clinical studies.[21] As primary prevention, four points were raised as follows. Firstly, maternal dietary restrictions during breastfeeding are not recommended. Secondly, breastfeeding for at least the first 4–6 months is exclusively recommended. Thirdly, maternal smoking and passive smoking of children should be avoided. Fourthly, avoidance of exposure to inhaled allergens is recommended for infants at high risk for development of allergic diseases. The reason why breastfeeding is beneficial to prevent the onset of allergic diseases remains to be determined, but factors including soluble IgA, soluble CD14, transforming growth factor (TGF)-β, omega-3 PUFA or others in breast milk are considered to play an inhibitory role.[22] Therefore, to reveal what foods or beverages are recommended during the pregnancy and lactation period for mothers whose children are at high risk for sensitization of allergens and development of allergic diseases is an important issue. Indeed, it was reported that administration of probiotics, Lactobacillus GG, prenatally to mothers and postnatally for 6 months to their infants, resulted in a frequency of atopic eczema in children aged 2 years of half that of the placebo group[23] and this preventative effect of probiotics on atopic eczema persisted at least 4 years.[24] Moreover fish oil (omega-3 PUFA) supplementation in pregnancy from the 20th week of gestation until delivery was shown to result in decreased severity of dermatitis in children at 1 year of age although there was no difference in the frequency of atopic dermatitis in comparison with the placebo group.[25]

FLAVONOIDS ARE CANDIDATE NUTRIENTS FOR PREVENTION AND COMPLEMENTARY AND ALTERNATIVE MEDICINE FOR ALLERGIC DISEASES

FLAVONOIDS INHIBIT INTERLEUKIN (IL)-4, IL-13 AND CD40 LIGAND EXPRESSION BY ACTIVATED BASOPHILS

Although there are many kinds of traditional remedies for allergic diseases in the world, whether most of such remedies are truly complementary or alternative medicine remains unknown and some of these are now being tested.[26] More than 10 years ago we evaluated the clinical effect of one kind of traditional vegetarian diet on adult patients with atopic dermati-

tis.[27] After a 2-month period of treatment, the severity of dermatitis decreased from 49.9 ± 18.6 to 27.4 ± 16.8 based on a score of atopic dermatitis severity, the SCORAD index and on serological parameters including lactate dehydrogenase-5 activity and a number of peripheral eosinophils. The amount of urinary secretion of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage, was also decreased.[28] Since this study was open and not placebo-controlled, what factor(s) led to decreased SCORAD scores remained unknown. Subsequent studies showed that one of the characteristics of this remedy was a high intake of flavonoids. By this vegetarian diet, it was calculated that 17 mg of apigenin, 1.6 mg of luteolin, 19.5 mg of quercetin, and 29 mg of kaempferol were consumed daily. Thus, we focused our attention on the biological activities of flavonoids.

Flavonoids are comprised of a large group of low molecular weight polyphenolic secondary plant metabolites and are found in fruits, vegetables, nuts, seeds, stems, flowers, roots, bark, tea, wine and coffee and are thus common substances in our daily diet.[29,30] Flavonoids have been recognized to exert antioxidant, anti-bacterial and anti-viral activity, and possess anti-inflammatory, anti-angionic, analgesic, hepatoprotective, cytostatic, apoptotic, estrogenic or anti-estrogenic properties as well as anti-allergic effects.[31,32] Based on their skeleton, flavonoids are categorized into eight groups: flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidins, chalcones and flavonolignans (Fig. 1). Their skeleton is a heterocyclic hydrocarbon, chromane, and substitution of its ring C in position 2 or 3 with a phenyl group (ring B) results in flavans or isoflavans. An oxo-group in position 4 leads to flavanones and isoflavanones. The presence of a double bond between C2 and C3 provides flavones and isoflavones. An additional double bond in between C1 and C2 makes these compounds colorful anthocyanidins. Natural flavonoids usually occur as glycosides (e.g. glucosides, rhamnoglucosides, and rutinosides) and more that 8,000 different flavonoids have been identified.[31,32]

Mast cells and basophils expressing high affinity IgE receptor (FcεRI) play an important role in allergic inflammation through releasing chemical mediators such as histamine and cysteinyl leukotrienes, cytokines and chemokines.[33-36] Fewtress and Gomperts first demonstrated the anti-allergic effect of flavone inhibition of transport ATPase in histamine secretion from rat mast cells.[37] Quercetin inhibition of allergen-stimulated human basophils was reported subsequently.[38,39] Similarly, Cheong *et al.* showed the structure activity relationship of flavonoids for anti-allergic actions through analyses of their inhibitory activity on hexosaminidase release from rat mast cells. Apigenin, luteolin, 3.6-dihydroxy flavones, fisetin, kaempferol, quercetin, and myricetin were

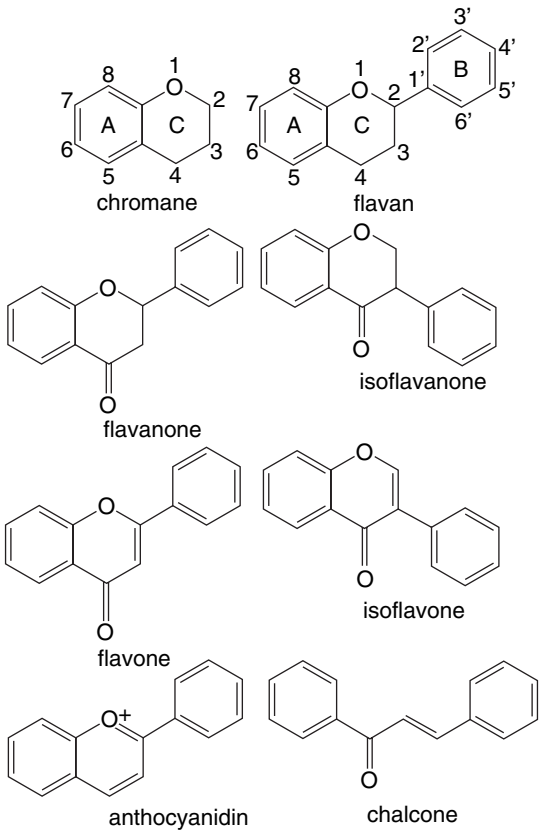


Fig. 1 Structures of basic flavonoid skeletons

found to inhibit such release with an IC_{50} value of less than 10 μM.[40] Flavonoids have also been shown to suppress cysteinyl leukotriene synthesis through an inhibition of phospholipase (PL) A_2 and/or 5-lipoxygenase (5LO). Quercetin was first demonstrated to be an inhibitor of PLA_2[41] and later it was reported that quercetagetin, kaempferol-3-O-galactoside, and scutellarein also possessed this activity with IC_{50} values ranging from 12.2 to 17.6 μM.[42] A number of flavonoids possess 5LO inhibitory activity. For instance, cirsiliol (3', 4', 5-trihydroxy-6,7-dimethoxy flavone) caused 97% inhibition of 5LO activity from rat basophilic cells and 99% suppression of release of cysteinyl leukotrienes from guinea pig lung.[43] Kimata *et al.* reported that luteolin, quercetin and baicalein inhibited not only the release of histamine, leukotrienes and prostaglandin D_2 but also the secretion of granulocyte macrophage-colony stimulating factor by human cultured mast cells in response to cross-linkage of FcεRI[44] and subsequently showed that these compounds also inhibited IgE-mediated tumor necrosis factor (TNF)-α and IL-6 production by bone marrow-derived cultured murine mast cells.[45] Thus these reports indicate that flavonoids are inhibitors of chemical mediator release and cytokine production by mast cells.

One of the characteristic features of allergic dis-

eases is overproduction of IgE to environmental allergens. For the differentiation of B cells into IgE producing cells, both the interaction of CD40 ligand with CD40 and the action of IL-4 or IL-13 on B cells are required.[46] The cells which are able to send these signals to B cells are reportedly Th2 cells, basophils and mast cells.[47] Thus, the effect of flavonoids on IL-4, IL-13 and CD40 ligand expression was examined by basophils. Purified peripheral blood basophils or a human basophilic cell line, KU812, were stimulated with anti-IgE antibody or PMA+A23187, respectively, in the presence or absence of various concentrations of flavonoids. In KU812 cells, fisetin suppressed IL-4, IL-5 and IL-13 production but the suppressive effect of fisetin on IL-6, IL-8 and IL-1β was relatively weak.[48] Luteolin, apigenin and fisetin strongly suppressed both IL-4 and IL-13 synthesis by allergen or anti-IgE antibody-stimulated peripheral blood basophils and the IC_{50} value of these flavonoids for inhibition of IL-4 synthesis was 2–6 μM.[49,50] These flavonoids also inhibit IL-4 production by anti-CD3 antibody-stimulated T cells but the required concentration was higher (IC_{50} = 10–19 μM). Similarly, Matsuda *et al* reported that apigenin, luteolin and fisetin inhibited IL-4 and TNF-α synthesis in rat mast cell line, RBL-2H3 cells.[51] In addition, luteolin, apigenin and fisetin in a dose-dependent manner suppressed CD40 ligand expression by PMA+A23187-stimulated basophils and KU812 cells, whereas myricetin even at 30 μM did not possess such activity.[52] These inhibitory activities of luteolin on the expression of IL-4, IL-13 and CD40 ligand were accompanied with its suppressive activity of mRNA expression of IL-4, IL-13 and CD40 ligand. Therefore, flavonoids such as luteolin, apigenin and fisetin are considered as potential natural IgE inhibitors.

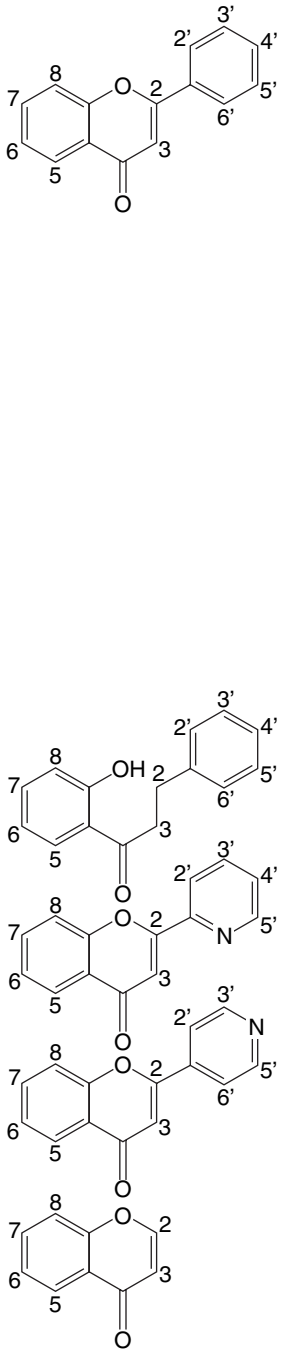
HIERARCHY OF FLAVONOIDS AND RELATED COMPOUNDS IN THE INHIBITORY ACTIVITY ON IL-4 SYNTHESIS BY BASOPHILS

In order to determine the basic structure of flavonoids for the inhibition of IL-4 production and search for more active compounds, 45 kinds of flavones, flavonols and their related compounds were then screened. Purified basophils were pre-incubated with various concentrations of flavonoids (1 to 30 μM), and then stimulated with anti-IgE antibody and IL-3 for 12 hours. IL-4 in the supernatant was measured by means of ELISA and the inhibitory activity of each compound was shown as an IC_{50} value (Fig. 2). Ayanin, luteolin, apigenin, and compound 31 are the strongest inhibitors with an IC_{50} value of 2.2–3.2 μM. Next, diosmetin, fisetin, ombuin and compound 5 showed an inhibitory activity with an IC_{50} value of 5.2–6.5 μM. Compound 11, in which carbon is exchanged for nitrogen in position 4' in the B ring, possesses an inhibitory activity with an IC_{50} value of 4.8 μM, while compound 10, in which carbon is replaced for nitrogen in the position 6', does not have such activity. Quercetin and kaempferol are representative flavonoids consumed substantially daily and have an intermediate activity of inhibition for IL-4 synthesis with an IC_{50} value of 15.7–18.8 μM. However, myricetin lacks such activity. These analyses of structure-activity relationship revealed the fundamental structure for the activity. For maximal inhibition, hydroxylation in positions 7 and 4' is essential and additionally the presence of OH in either position 3 or 5 is required. The glycosylation of position 3 decreases the activity.

MECHANISMS THROUGH WHICH FLAVONOIDS INHIBIT IL-4, IL-13 AND CD40 LIGAND EXPRESSION BY BASOPHILS

Signaling pathways through FcεRI in mast cells[33,53] and basophils[54] were reviewed. In basophils, crosslinking of FcεRI induces activation of various tyrosine kinases including Lyn and Syk, which lead to the stimulation of phosphatidylinositol 3 (PI_3)-kinases, mitogen-activated protein kinases (MAPK) 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 P44/42 MAPK. P44/42 MAPK activation is reported to lead to the synthesis of cysteinyl leukotrienes, while protein kinase C (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. 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.[55] The stimulation of T cells with PMA stimulates activating protein 1 (AP-1) through c-Jun N-terminal kinase (JNK) and P44/42 MAPK activation and synergistically enhances CD40 ligand transcription with the nuclear factor of activated T cells (NFAT).[56]

Then, in order to reveal acting points of flavonoids in basophils, whether or not flavonoids might inhibit activation of several signaling molecules was asked. The effect of luteolin or fisetin as an active reagent or the effect of myricetin as an inactive reagent on the activation of Syk or Lyn by anti-IgE antibody+IL-3-stimulated basophils or on the activation of MAPK family including P38 MAPK, P44/42 MAPK, P54/46 SAPK/JNK and on the activation of transcriptional factor including NFAT and AP-1 by A23187+PMA-stimulated KU812 cells was examined. Luteolin failed to inhibit Syk, Lyn and MAPK family activation whereas it inhibited both AP-1 and NFAT activation.[49,50] However, myricetin did not inhibit AP-1 and NFAT activation by KU812 cells. Thus, in our analy-



	3	5	6	7	8	2'	3'	4'	5'	6'	IC50 (μM)
Ayanin	OCH_3	OH	H	OCH_3	H	H	OH	OCH_3	H	H	2.2
Luteolin	H	OH	H	OH	H	H	OH	OH	H	H	2.7
Apigenin	H	OH	H	OH	H	H	H	OH	H	H	3.1
Compound 31	OH	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	H	3.2
Diosmetin	H	OH	H	OH	H	H	OH	OCH_3	H	H	5.2
Fisetin	OH	H	H	OH	H	H	OH	OH	H	H	5.8
Ombuin	OH	OH	H	OCH_3	H	H	OH	OCH_3	H	H	6.3
Compound 5	H	OAc	H	H	H	H	OAc	OAc	OAc	H	6.5
Compound 6	H	OH	H	H	H	H	OH	OH	OH	H	11.4
Scutellarein	H	OH	OH	OH	H	H	H	OH	H	H	14.0
3-Hydroxyflavone	OH	H	H	H	H	H	H	H	H	H	15.0
Kaempferol	OH	OH	H	OH	H	H	H	OH	H	H	15.7
Quercetin	OH	OH	H	OH	H	H	OH	OH	H	H	18.8
Compound 32	OCH_3	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	H	18.9
4',7-Dihydoxyflavone	H	H	H	OH	H	H	H	OH	H	H	19.4
Eriodictyol (2-3)	H	OH	H	OH	H	H	H	OH	OH	H	20.8
Compound 3	H	OAc	H	H	H	H	OCH_3	OCH_3	OCH_3	H	21.0
Fustin (2-3)	OH	H	H	OH	H	H	OH	OH	H	H	23.0
Compound 4	H	OH	H	H	H	H	OCH_3	OCH_3	OCH_3	H	23.0
7-Hydroxyflavone	H	H	H	OH	H	H	H	H	H	H	26.5
3',4' -Dihydroxyflavone	H	H	H	H	H	H	OH	OH	H	H	>30
Myricetin	OH	OH	H	OH	H	H	OH	OH	OH	H	>30
Galangin	OH	OH	H	OH	H	H	H	H	H	H	>30
Morin (dihydrate)	OH	OH	H	OH	H	OH	H	OH	H	H	>30
Chrysin	H	OH	H	OH	H	H	H	H	H	H	>30
Baicalein	H	OH	OH	OH	H	H	H	H	H	H	>30
Rhamnetin	OH	OH	H	OCH_3	H	H	OH	OH	H	H	>30
Compound 1	H	OH	H	H	H	H	H	OCH_3	H	OCH_3	>30
Compound 2	H	OH	NH_2	H	H	H	H	H	H	H	>30
Compound 7	CO-Phe-$(OCH_3)_2$	OAc	H	H	H	OCH_3	H	OCH_3	H	H	>30
Compound 8	CO-Phe	OH	H	H	H	H	H	H	H	H	>30
Compound 9	H	OCH_3	H	H	H	H	H	H	H	H	>30
Astragalin	O-Glc	OH	H	OH	H	H	H	OH	H	H	>30
Rutin	O-Rutinose	OH	H	OH	H	H	OH	OH	H	H	>30
Gossypin	OH	OH	H	H	O-Glc	H	OH	OH	H	H	>30
Isoquercitrin	O-Glc	OH	H	OH	H	H	OH	OH	H	H	>30
Myrictrin	O-Rhamnoside	OH	H	OH	H	H	OH	OH	OH	H	>30
Phloretin	H	OH	H	OH	H	H	H	OH	H	H	>30
Phloridzin	H	O-Glc	H	OH	H	H	H	OH	H	H	>30
Compound 10	H	OCH_3	H	H	H	H	H	H	H		>30
Compound 11	H	OCH_3	H	H	H	H	H		H	H	4.8

	2	3	5	6	7	8	IC50 (μM)
Compound 12	COOH	H	OCH_3	H	H	H	>30
Compound 13	$COOC_2H_5$	H	OCH_3	H	H	H	>30
Compound 14	$COOC_3H_7$	H	OCH_3	H	H	H	>30
Compound 15	$COOC_4H_9$	H	OCH_3	H	H	H	>30

Fig. 2 Hierarchy of inhibitory activity of 45 flavonoids and related compounds on IL-4 production by basophils Purified basophils were pre-incubated with various concentrations of flavonoid or related compound for 15 minutes, and stimulated with anti-IgE antibody plus IL-3 for 12 hours. The concentration of IL-4 in the supernatant was measured. The inhibitory activity of each compound is shown as an average of IC_{50} values.

ses the inhibitory activity of flavonoids on IL-4, IL-13 and CD40 ligand expression is considered to be mediated by their suppressive action on these transcriptional factors. Park *et al.* recently reported that apigenin inhibits IL-4 production by mouse T cells via down-regulation of NFAT DNA binding activity.[57] How flavonoids inhibit AP-1 and NFAT activation awaits clarification, but it has been demonstrated that nitric oxide also inhibits IgE-dependent cytokine production and Fos/Jun activation and NFAT without inhibition of MAPK family in RBL-2H3 mast cells,[58] so that the actions of flavonoids resemble those of nitric oxide.

In previous studies regarding the action of fla-

vonoids on basophils or mast cells, several findings have been reported. Kimata *et al*. showed that luteolin and quercetin inhibited Ca^{2+} influx, PKC activity, p44/42 MAPK and JNK activation but not p38 MAPK by human cultured mast cells in response to cross-linkage of FcεRI.[44] Shichijo *et al*. demonstrated that flavonoids inhibited Syk activation, also in human cultured mast cells in response to cross-linkage of FcεRI.[59] 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 week suppressor of degranulation. 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.[60] 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, stimulation, doses of flavonoids or it may be due to the presence of several acting points of flavonoids. Further studies are required to clarify these points.

CLINICAL EFFECTS OF FLAVONOIDS

Based on the anti-allergic activity, whether intake of flavonoids might be effective for the amelioration of allergic symptoms or for prevention of the onset of allergic diseases was tested. NC/Nga mouse spontaneously develops severe eczema, scratching behaviour and serum IgE elevation with aging under nonspecific pathogen free circumstances.[61] To see the preventative effect, the mice were orally given astragalin, kaempferol 3'glucoside (1.5 mg/kg) or a control diet.[62] Development of dermatitis was observed in the control group with aging and the severity of dermatitis was evaluated by scoring. Oral intake of astragalin remarkably inhibited the appearance of the skin symptoms, scratching behaviour and serum IgE elevation. Moreover, astragalin significantly diminished the severity of dermatitis, scratching behaviour and transepidermal water loss even after onset.[63] Similarly, by using this model mouse, it has been subsequently shown that administration of extract from petals of *Impatiens balsamina* L. including flavonoids such as kaempferol 3-rutinoside and 2-hydroxy-1,4-naphthoquinone as active gradients suppressed scratching behaviour and dermatitis.[64] Moreover, in an asthmatic model mouse sensitized with ovalbumin, it was demonstrated that oral administration of luteolin, even at a dose of 0.1 mg/kg, led to a significant suppression of bronchial hyperreactivity and bronchoconstriction.[65] Nobiletin, a polymethoxyflavonoid, when given at a dose of 1.5 or 5 mg/kg intraperitoneally into OVA-sensitized rats, was recently reported to reduce OVA-induced increases in eosinophils and eotaxin expression.[66] Substances, used in traditional remedies for allergic diseases are found to include flavonoids. For instance, *Perilla frutescens* BRITTON, a medicinal herb prescribed in Saiboku-to contains luteolin glucoside, apigenin glucoside, scutellarin and rosmarinic acid and oral administration of *Perilla frutescens* inhibits PCA reaction in mice.[67] Isoquercitrin from *Argemone platyceras*, traditionally used for cough, bronchitis and pneumonia in Mexico, inhibits carbachol and leukotriene D_4-induced contraction in guinea-pig airways.[68] Also, extract of flowers of *Impatiens textori* MIQ containing apigenin, apigenin 7-glucoside and luteolin is shown to inhibit antigen-induced anaphylaxis in ddY mice.[69] Thus these reports raise the possibility that the active gradients used in some traditional remedies for allergic diseases are flavonoids.

We attempted to examine the clinical effect of persimmon leaf extract on adult patients with atopic dermatitis in randomized controlled trials (Takigawa *et al.* unpublished data), since persimmon leaf extract tea included 0.45–0.72% astragalin, 0.38–0.55% isoquercitrin (quercetin 3-glycoside) and 0.32% toriphorin (kaempferol 3-galactoside). Patients were treated with placebo, or 1 *g* or 2 *g* of persimmon leaf extract for 4 weeks without any change in medication, and the severity of their skin lesions was measured according to the SCORAD index. The intake of persimmon leaf extract at a dose of 1 *g* and 2 *g*/day significantly decreased the SCORAD index, which was accompanied by a decrease in the number of peripheral blood eosinophils. In order to verify that flavonoid is effective in patients with atopic dermatitis, further studies involving a large number of participants are required, but these preliminary data, we believe, seem to be promising as complementary and alternative medicine for atopic dermatitis.

Enzymatically modified isoquercitrin is a quercetin glycoside that consists of isoquercitrin and its maltooligosaccharides, and it is manufactured from rutin through enzymatic modification.[70] By this modification, the absorption rate through the intestine was remarkably increased. This flavonoid is recognized as a food additive in the List of Existing Food Additives (Notification No. 120, Japanese Ministry of Health and Welfare, April 16, 1996)[71] and is commonly used for various foods such as beverages etc. as an antioxidant which has high solubility in water. Therefore, whether or not intake of enzymatically modified isoquercitrin is effective for allergic diseases is now being tested.

EPIDEMIOLOGICAL REPORTS REGARDING THE ASSOCIATION OF INTAKE OF FLAVONOIDS WITH ALLERGIC DISEASES

Is it evident that an excess (or appropriate) intake of flavonoids contributes to decrease the prevalence of allergic diseases or ameliorate allergic symptoms?[72,73] So far, epidemiological evidence does not appear to lead to a definitive conclusion that fla-

vonoids have *in vivo* beneficial effects in allergic diseases. Two studies have suggested that a high intake of fresh fruit[74] and vegetables[75] may protect against asthma, but neither could determine which specific foods or nutrients were responsible. Subsequently Shaheen *et al.* reported that in a population-based case-control study in South London, apple consumption or red wine intake were negatively associated with asthma prevalence or severity, respectively, perhaps due to a protective effect of flavonoids.[76] Moreover, a 30-year longitudinal epidemiological study reported that the incidence of asthma is lower in populations with higher intake of flavonoids and that the relative risk was 0.65[77] whereas in a population-based, case-control study of 1471 adults in London, dietary intake of three flavonoid subclasses (catechins, flavonols and flavones) is reported not to be associated with asthma.[78] Since in the past studies the amount of daily intake of flavonoids was estimated by questionnaire and was the total amount of three major flavonoids including quercetin, kaempferol and myricetin, more precise analysis of flavonoid intake in quantity and quality will be required to lead to a conclusion.

IS AN APPROPRIATE INTAKE OF FLAVONOIDS EFFECTIVE AS A FORM OF COMPLEMENTARY OR ALTERNATIVE MEDICINE FOR THE TREATMENT OR PREVENTION OF ALLERGIC DISEASES?

The findings regarding *in vitro* or *in vivo* anti-allergic properties of flavonoids are summarized (Table 1). Flavonoids, particular luteolin, apigenin and fisetin have strong anti-allergic activities such as inhibition of histamine release, of IL-4 and IL-13 synthesis and of CD40 ligand expression by basophils and perhaps mast cells. The inhibitory activity of flavonoids on IL-4, IL-13 and CD40 ligand expression may be mediated through their inhibition of transcriptional factors such as AP-1 and NFAT. In some allergic model mice, oral administration of flavonoids has been shown effective as treatment or prevention. One longitudinal epidemiological study supports this idea.

On the basis of this evidence, as future studies, we consider the following points (Fig. 3). Firstly, novel anti-allergic drugs, which suppress IgE production and allergic inflammation through their inhibitory activity of IL-4 and IL-13 synthesis and of CD40 ligand expression by basophils and mast cells, will be developed. Further modifications of structure may lead to higher active compounds. The glycosylation may make flavonoids more soluble and absorptive through the small intestine if oral administration is considered. Secondly, it is essential to test whether an appropriate intake of flavonoids is truly effective for the prevention or dietary treatment of allergic diseases through well-controlled study. Thirdly, more epidemiological analyses regarding the association of flavonoid intake in quantity and in quality with prevalence or severity of allergic diseases will be required.

Flavonoids are included in plant foods and beverages, and people consume substantial amounts of flavonoids.[29,30] The daily intake of flavonoids, calculated by the amounts of quercetin, kaempferol and myricetin, in some reports, plus luteolin, apigenin and fisetin, has been reported in several countries.[73] The total amount of flavonoids varied from 2.6 to 68.2 mg/day in European countries, USA and Japan. In each country the major flavonoid was quercetin, ranging from 14 to 100% of the total amount of flavonoids, followed by kaempferol and myricetin with an average intake of 0.1 to 5.9 mg/day. Luteolin, apigenin and fisetin, strong inhibitors of IL-4 production, were shown to be ingested but in average amounts of less than 1 mg/day. However, our recent analyses revealed that in Japan middle-aged women consume daily 5.6 mg of luteolin and 1.4 mg of apigenin and vegetarian women take 1.6 mg of luteolin and 17 mg of apigenin.

Regarding flavonoid content in foods and beverages, a database of the content of the major flavonoids in foods and beverages, including luteolin, apigenin, quercetin and kaempferol, has recently been established in the USA.[79] Quercetin levels in the edible parts of most vegetables are less than 10 mg/kg, except for onion (185–634 mg/kg), kale (110–120 mg/kg), broccoli (10–68 mg/kg), and lettuce (5–30 mg/kg). Quercetin is also included in apple (5–72 mg/kg), orange (18 mg/kg) and Japanese tea (12–90 mg/l). Kaempferol could only be detected in kale (211–470 mg/kg), endive (15–91 mg/kg), leek (11–56 mg/kg), broccoli (16–100 mg/kg), parsley (11–45 mg/kg), strawberry (8–19 mg/kg) and orange (32 mg/kg). Celery includes luteolin (22–200 mg/kg) and apigenin (17–750 mg/kg), both of which showed strong inhibitory activity on Th2 cytokine and CD40 ligand expression by basophils. Parsley also possesses apigenin (1850 mg/kg) and luteolin (<1–11 mg/kg). Fisetin is reported to be included in onion (5 mg/kg), apple (27 mg/kg) and strawberry (160 mg/kg). Thus, the data of flavonoid content in foods and beverages and the hierarchy of flavonoid activity lead to the concept that it is preferable for persons to take flavonoids from what they like on the basis of their quality and quantity. 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.[80] Therefore progress in research regarding the bioavailability of flavonoids will be essential for establishment of dietary management for allergic or other diseases.[80,81]

Table 1 Summary of anti-allergic properties of flavonoids

1. *Biological activities*
   Inhibition of histamine, Th2 cytokines (IL-4 and IL-13) and CD40 ligand expression in basophils and mast cells
2. *Presumable mechanisms*
   Inhibition of activation of transcriptional factors such as AP-1 and NFAT
3. *Hierarchy of inhibitory activity of representative flavonoids*
   Luteolin, apigenin, fisetin > kaempferol, quercetin > myricetin and others
4. *Clinical effect*
   Preventative and ameliorative effect on dermatitis in NC/Nga mice
   Ameliorative effect on dermatitis in adult patients with atopic dermatitis
5. *Epidemiological report*
   The higher the intake of flavonoids, the lower the incidence of asthma (relative risk = 0.65)

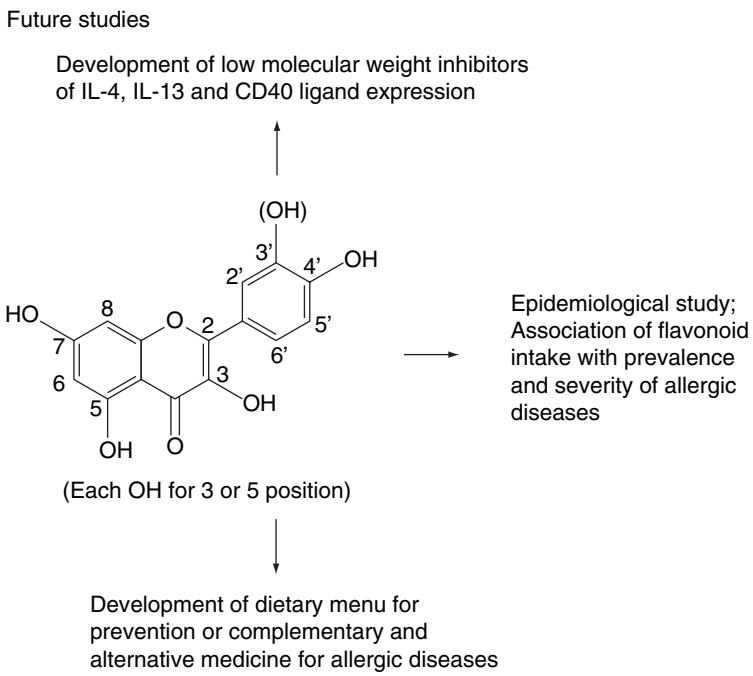


Fig. 3 Future studies are required for flavonoids or related compounds to be novel anti-allergic drugs or to be used in the dietary menu for treatment or prevention of allergic diseases.

CONCLUSIONS

Allergy, one of the common diseases throughout the world, is of growing concern because of its increasing rate of prevalence. Dietary change may contribute to this increase. Flavonoids, in particular luteolin, apigenin and fisetin, possess anti-allergic activities that inhibit histamine, IL-4, IL-13 and CD40 ligand expression by basophils and mast cells. The structure activity relationship of flavonoids and related compounds determines the fundamental structure for inhibitory activity of IL-4 synthesis and will be helpful for development of new anti-allergy drugs through modification. Since a database of the flavonoid content in major vegetables, fruits and beverages has been developed, an appropriate intake of flavonoids from foods and beverages is anticipated to ameliorate allergic symptoms and prevent the onset of allergic diseases. To achieve this goal, information regarding the bioavailability of flavonoids and clinical trails to verify the effectiveness will be required.

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