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ORIGINAL ARTICLE

Preventative Effect of a Flavonoid, Enzymatically Modified Isoquercitrin on Ocular Symptoms of Japanese Cedar Pollinosis

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ABSTRACT

Background: Flavonoids are nutrients that exert anti-allergic effects. We investigated the preventative effect of enzymatically modified isoquercitrin (EMIQ), a flavonoid, to relieve the symptoms of Japanese cedar pollinosis.

Methods: In a parallel-group, double-blind placebo-controlled study design, 24 subjects with Japanese cedar pollinosis took 100 mg EMIQ or a placebo for 8 weeks, starting 4 weeks prior to the onset of pollen release. Subjective symptoms, ADL scores and the usage of drugs were recorded daily, and the QOL score was obtained every 4 weeks. Blood sampling was performed before and after the study to measure serum levels of IgE and flavonoids.

Results: During the entire study period, ocular symptom + medication score for the EMIQ group was significantly lower ($p < 0.05$) than that of the placebo group. When limited to the period, ocular symptom scores ($p < 0.05$, weeks 5–6), and ocular congestion scores ($p < 0.05$, weeks 5–6) for the EMIQ group was significantly lower than that for the placebo group while other scores for the EMIQ group, such as ocular itching scores ($p = 0.09$, weeks 4–5), lacrimation scores ($p = 0.07$, weeks 5–6), and ocular congestion scores ($p = 0.06$, weeks 4–5), all tended to be lower. However no significant differences were found in nasal symptoms between the two groups. Serum concentrations of IgE were not significantly downregulated but the serum concentrations of quercetin and its derivatives were elevated significantly by the intake of EMIQ.

Conclusions: Intake of the quercetin glycoside EMIQ proved to be effective for the relief of ocular symptoms caused by Japanese cedar pollinosis.

KEY WORDS

diet therapy, flavonoid, Japanese cedar pollen, pollinosis, quercetin

INTRODUCTION

The number of allergic patients suffering from allergic rhinitis, asthma and atopic dermatitis, has been increasing globally, with changes in environmental factors, in the past few decades.[1,2] Diet has also been proposed as one of the environmental factors.[3-7] According to the hypothesis, changes in intake of lipids or antioxidant substances during the last two decades are thought to have increased the prevalence of allergic diseases and/or to have worsened allergic symptoms. If this is the case, an appropriate daily intake of

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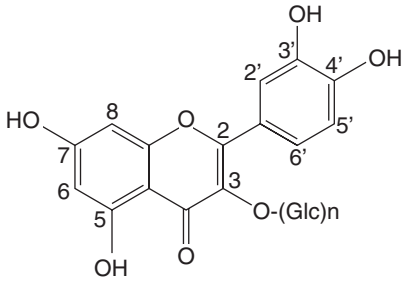
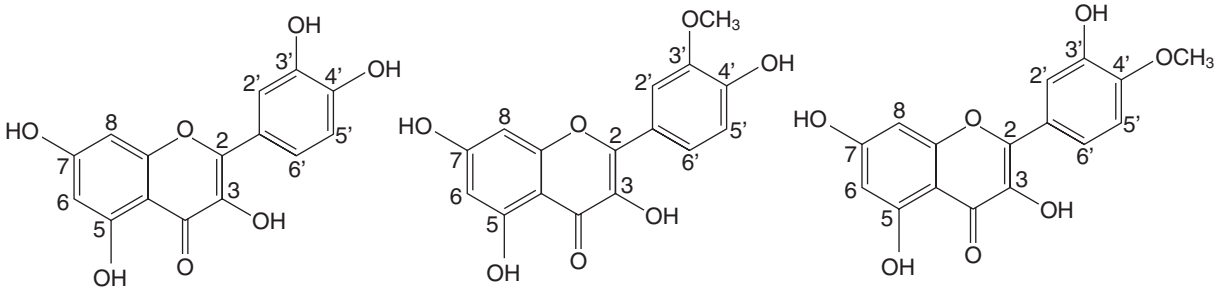


Enzymatically modified isoquercitrin
EMIQ (n = 1–8)



Quercetin Isorhamnetin Tamarixetin

Fig. 1 Chemical structure of enzymatically modified isoquercitrin (EMIQ), quercetin, isorhamnetin and tamarixetin.

lipids or antioxidants may reduce the onset of allergic diseases or ameliorate allergic symptoms.

Flavonoids are abundant in plant foods and possess antioxidant as well as anti-allergic activities.[8] We have demonstrated that flavonoids such as apigenin, fisetin, luteolin, quercetin and kaemferol suppress the production of Th2 cytokines and CD40 ligand expression in activated human basophils.[9-11] In addition, oral administration of astragalin, a kaemferol glucoside, showed a preventative and ameliorative effect on dermatitis in an atopic-dermatitis mouse model, NC/Nga.[12,13] We therefore proposed that an appropriate intake of flavonoids might constitute a dietary preventative or therapeutic strategy for allergic diseases.[14,15] Indeed, we have recently reported on the therapeutic efficacy of enzymatically modified isoquercitrin (EMIQ), a quercetin glycoside, by means of a randomized double-blind placebo-controlled trial, on ocular symptoms in patients with Japanese cedar pollinosis.[16] During the pollen season in 2007, EMIQ intake started from March 4, significantly suppressed an exacerbation of ocular symptoms at the beginning of April that was due to an increase in scattered cedar pollen or cypress pollen. Here, we investigated the preventative effect of EMIQ on symptoms in patients with Japanese cedar pollinosis.

METHODS

PARTICIPANTS

Patients enrolled in the study were 24 adult patients in Osaka Prefecture, who had been suffering from Japanese cedar pollinosis and showed positive blood IgE specific for Japanese cedar pollen. All participants provided written informed consent. The study protocols were approved by the Ethics Committee of Osaka University.

STUDY DESIGN

A randomized, double-blind, placebo-controlled design was used for the trial. Enzymatically modified isoquercitrin (EMIQ, San-Ei-Gen F.F.I. Inc., Toyonaka, Osaka, Japan)[17] and placebo were supplied in capsule form. Figure 1 shows the chemical structure of EMIQ, which is a glycoside of quercetin. EMIQ has been recognized as a food additive,[17] found to be safe by many evaluation studies including an acute toxicity study, 4-week toxicity study, 13-week toxicity study, chronic/carcinogenic study[18] and mutagenicity study (Ames assay), and its no-observed-adverse-effect level (NOAEL) was estimated at approximately 500 mg/kg/day. Since the acceptable daily intake for humans is thought to be 5 mg/kg/day, we selected 100 mg EMIQ for the study. The EMIQ capsule contained 50 mg EMIQ plus corn

Table 1 Subject characteristics

	Placebo	EMIQ
Number of participants	12	12
Age (mean ± SD)	37.6 ± 9.0	36.1 ± 10.3
Gender		
Male	10	9
Female	2	3
Total IgE (IU/ml)		
at the beginning	167 ± 212	215 ± 254
at the end	145 ± 158	196 ± 238
Specific IgE for cedar pollen (RAST score)		
at the beginning	3.3 ± 0.6	3.3 ± 1.0
at the end	3.3 ± 0.6	3.3 ± 1.0

starch, and the placebo capsule contained corn starch only with identical appearance to the EMIQ capsule.

Patients were randomly assigned to the EMIQ group or the placebo group and took one capsule twice a day for 8 weeks from January 27 to March 22, 2008. In 2008, according to the regional records for Osaka Prefecture, the onset of cedar pollen release occurred on February 22 and the amount of pollen showed a peak in mid- March.

Since EMIQ is used as an antioxidant in some commercially available food products, participants were prohibited from taking such products during this period.

OUTCOME MEASUREMENTS

The severity of subjective symptoms was evaluated by a scoring system based on the method by Okuda *et al*.[19] During the testing period, participants were asked to record symptom scores and activities of daily living (ADL) everyday for 10 weeks. Symptoms were evaluated using the following scale: sneezing and rhinorrhea (0: none, 1: 1–5 episodes, 2: 6–10 episodes, 3: 11–20 episodes, 4: >20 episodes), nasal obstruction (0: none, 1: mild, 2: moderate, 3: severe, 4: violent), ocular itching (0: none, 1: mild and bearable, 2: severe but bearable, 3: unbearable), lacrimation (0: none, 1: seldom, 2: sometimes, 3: always), and congestion of conjunctiva (0: none, 1: little, 2: mild, 3: severe). The ADL score ranged from 0 to 4, with 0: no problem, 1: mild symptoms not causing difficulties with ADL, 2: moderate symptoms with some difficulties with ADL, 3: severe symptoms with major difficulties with ADL, 4: critical symptoms. The total symptom score was calculated as the sum of the total nasal and ocular symptom scores. The total nasal score was calculated as the sum of specific nasal scores such as sneezing, rhinorrhea and nasal obstruction. The total ocular score was shown as the sum of specific ocular scores including those of ocular itching, lacrimation and ocular congestion. Thus, the range of these scores was 0–147/week for the total symptom score, 0–84/week for the total nasal score, 0–63/week for the total ocular score and 0–28/week for the ADL score. Participants were allowed to take anti-allergic drugs and use ocular drops and nasal sprays, depending on the severity of symptoms, but the usage of these drugs was recorded in the symptom diary and was evaluated according to medication scores,[19] defined by the Japanese Society of Allergology, using the following scale: 0: none, 1: oral antihistamines, oral histamine release inhibitors, nasal application of vasoconstrictor or anticholinergic drugs, histamine release inhibitive ocular drops, 2: focal administration of steroids, 3: oral administration of steroids. The total symptom plus medication score was calculated as the sum of the total symptom score and the medication score. The nasal symptom plus medication was the sum of the nasal symptom score and the medication score excluding the score for ocular drops. The ocular symptom plus medication score was calculated as the sum of the ocular symptom score and the medication score including the score for oral drugs, ocular drops and nasal sprays of corticosteroids since it has been shown that intranasal corticosteroids provide partial relief of ocular symptoms.[20,21]

The degree of quality of life (QOL) was assessed by using the Japanese allergic rhinitis QOL questionnaire (JRQLQ)[22] prior to and at the 4th and 8th week of the study. The JRQLQ comprises questions about rhinorrhea, sneezing, nasal obstruction, nasal and ocular itching, lacrimation, general fatigue, irritability, depression and difficulties with daily activities such as working, housekeeping, studying, reading, doing sports, going outdoors, sleeping and having conversations.

BLOOD SAMPLING

Blood sampling was performed prior to and at the 4th and 8th week of the study. Serum concentrations of total IgE antibody and specific IgE for Japanese cedar pollen were analyzed by Shionogi Biomedical Laboratories (Osaka, Japan). Serum levels of quercetin, isorhamnetin and tamarixetin were measured by HPLC as described elsewhere,[23] after hydrolyzation of the samples.

STATISTICAL ANALYSIS

Symptom scores were analyzed with two-way ANOVA (analysis of variance) for repeated measurements with multiple comparisons. The data of serum flavonoids are expressed as the means +/− SE and compared using a two-tailed Student's *t*-test. In both tests, probability (p) values of <0.05 were considered to indicate a statistically significant difference.

RESULTS

CHARACTERIZATION OF PARTICIPANTS

Table 1 shows subject characteristics of the placebo and EMIQ group. The baseline characteristics were

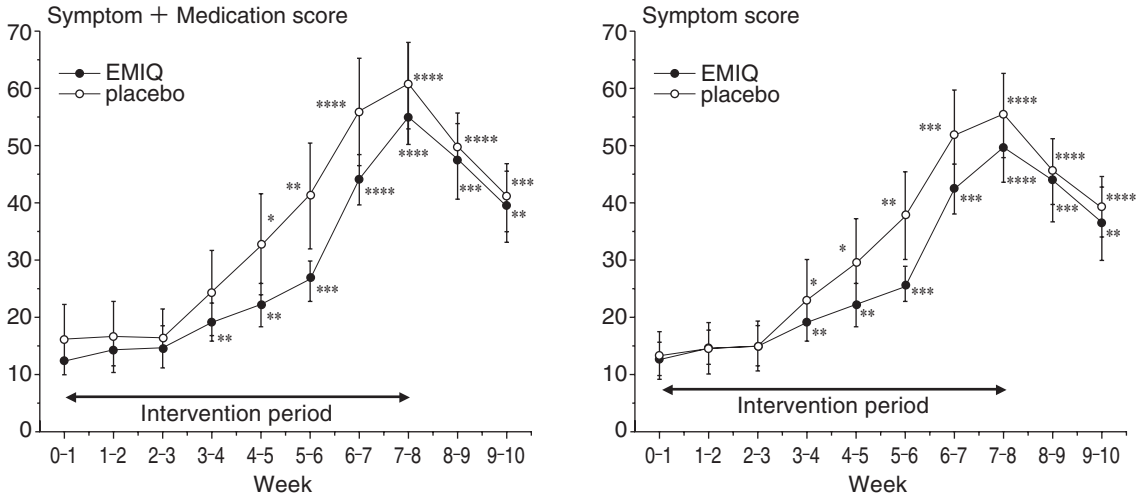


Fig. 2 Changes in total symptom + medication score and total symptom score in the EMIQ (solid dots) and the placebo (circles) group. Each value is the mean +/− SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared with the first week in each group.

similar for the two groups, and all participants showed positive serum IgE for Japanese cedar pollen. The average of total IgE levels for the EMIQ and the placebo groups were 215 IU/ml or 167 IU/ml, respectively. There were no significant differences in age, total IgE or sensitization to the cedar pollen between the two groups.

SYMPTOM ASSESSMENT

Figure 2 shows changes in the total symptom plus medication score and the total symptom score. Both scores for the placebo and EMIQ groups gradually increased until weeks 7–8 in accordance with an increase in the cedar pollen count. The scores of the EMIQ group were lower than those of the placebo group during weeks 3–8, but there was no statistically significant difference in the total symptom plus medication score and the total symptom score for the intervention period between the two groups. The changes in the total nasal symptom plus medication, total nasal symptom, sneezing, rhinorrhea and nasal obstruction scores are shown in Figure 3. Although no significant differences were found in these scores between the two groups, the ameliorative effect of EMIQ on ocular symptoms showed a statistically significant difference in the total ocular symptom plus medication score during the whole intervention period ($p = 0.04$) (Fig. 4). When limited to the period, total ocular symptom scores ($p < 0.05$, weeks 5–6), and ocular congestion scores ($p < 0.05$, weeks 5–6) for the EMIQ group was significantly lower than that of the placebo group while other scores for the EMIQ group, such as the ocular itching scores ($p = 0.09$, weeks 5–6), lacrimation scores ($p = 0.07$, weeks 5–6), and ocular congestion scores ($p = 0.06$, weeks 4–5), all tended to be lower. The changes in the QOL score, ADL score and medication score are shown in Figure 5. There were no differences in the QOL, ADL and medication scores between the two groups.

All participants completed the study, and no side effects, such as gastrointestinal symptoms, allergic reactions, cardiovascular symptoms, were observed during the entire period. The mean percentage of intake for the EMIQ and placebo groups was 96.9% and 97.8%, respectively.

BLOOD PARAMETERS

In a previous study in 2007 we measured trough values of serum quercetin concentrations before and after the study and found that there was no accumulation of serum quercetin level after continuous daily intake of 100 mg EMIQ for 8 weeks.[16] In this study we measured serum concentrations of quercetin and its methylated products, such as isorhamnetin and tamarixetin, at 2 hours after intake of EMIQ or placebo capsule at 4 and 8 weeks of the study (Fig. 6). Indeed, after taking EMIQ, the concentrations of these EMIQ-derived products were significantly elevated while the quercetin concentration remained unchanged and serum levels of isorhamnetin and tamarixetin were below 1 ng/ml in the placebo group. During this study, there was no significant change in serum concentration of total IgE and specific IgE for Japanese cedar pollen in either group (Table 1).

DISCUSSION

In this study we set out to determine whether the intake of quercetin glycoside EMIQ proved to be effective for the relief of ocular symptoms caused by Japanese cedar pollinosis. Previously we found that oral intake of EMIQ inhibited the exacerbation of ocular

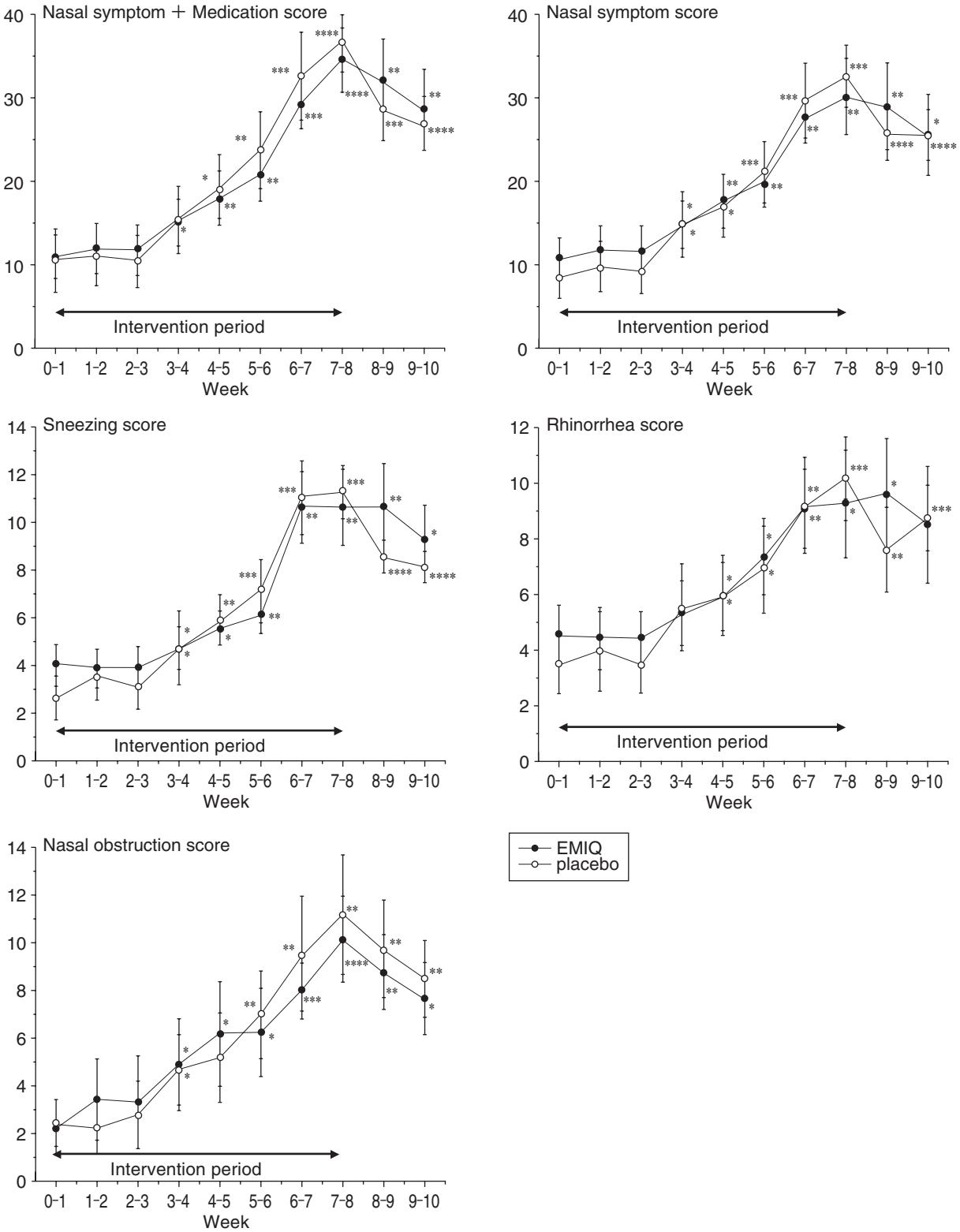


Fig. 3 Changes in nasal symptom + medication score, total nasal score, sneezing score, rhinorrhea score and nasal obstruction score in the EMIQ (solid dots) and the placebo (circles) group. Each value is the mean +/− SE. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ compared with the first week in each group.

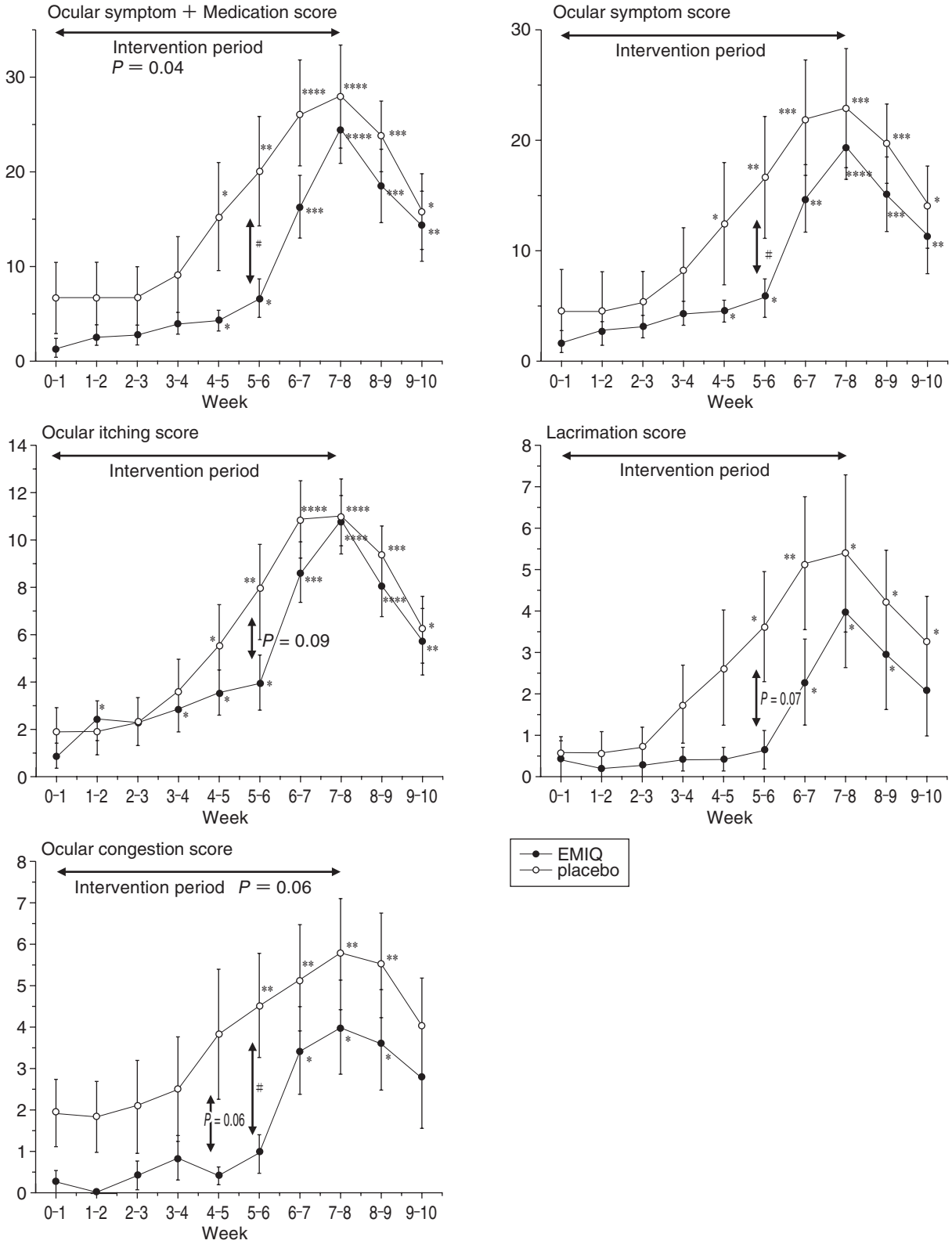


Fig. 4 Changes in ocular symptom + medication score, ocular total score, ocular itching score, lacrimation score and ocular congestion score in the EMIQ (solid dots) and the placebo (circles) group. Each value is the mean +/− SE. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ compared with the first week in each group. Arrow with $^{\#}p < 0.05$ shows comparison between both groups in the same week.

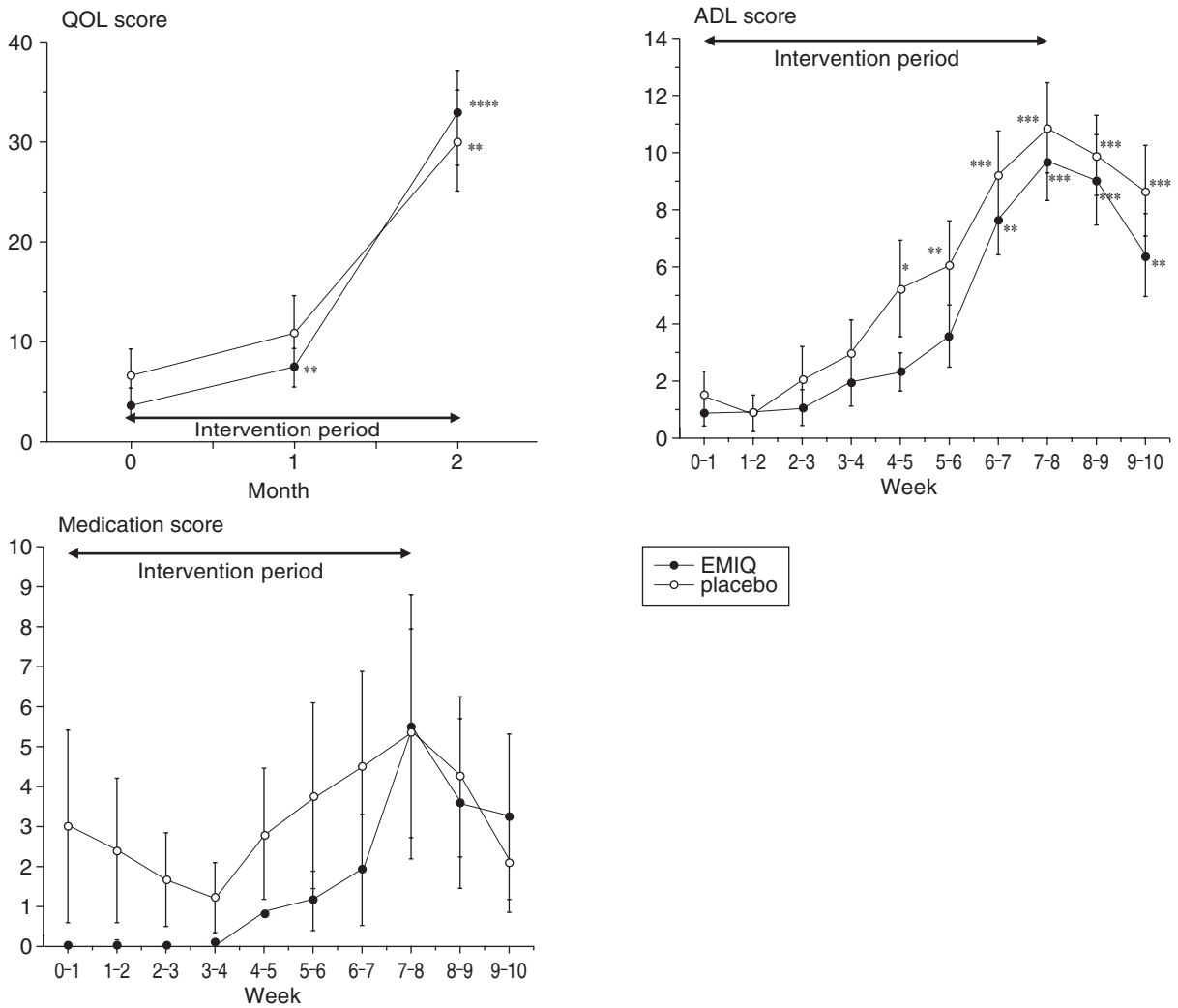


Fig. 5 Changes in QOL score, ADL score and medication score in the EMIQ (solid dots) and the placebo (circles) group. The data presented are the mean +/− SE. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared with the first week in each group.

symptoms caused by exposure to either the cedar pollen or cypress pollen but did not suppress nasal symptoms.[16] Since the number of participants was small (10 persons in each group), the result was limited. In addition, the study started on March 4th in 2007 when participants already had nasal and ocular symptoms and therefore it was conducted to examine the therapeutic effect of EMIQ. In 2008, we investigated whether EMIQ might exert a preventative effect to reduce symptoms. Indeed, the oral intake of EMIQ inhibited ocular symptoms but not nasal symptoms, so the efficacy was reproducible. The reason(s) why EMIQ suppressed ocular symptoms but not nasal symptoms is yet to be determined. It is possible that the nasal allergic inflammation is more severe than in the eye. If that is the case, higher doses of EMIQ intake would reduce nasal symptoms. Alternatively, although ocular and nasal symptoms are mediated by immediate type allergic reaction, the inflammation of rhinitis is considered to be more complicated than that of conjunctivitis. In allergic rhinitis, it was demonstrated that the blood vessels, nerves and mucous glands as well as mast cells, eosinophils and T cells play a role in the generation of nasal symptoms.[24]

Quercetin glycosides are the most abundant flavonoids present in plant foods.[8] The sugar moiety has been shown to affect its solubility and absorption.[25] For instance, it was reported that the peak concentration of quercetin, after intake of the quercetin monoglucoside known as isoquercitrin, was 20 times higher, and that this flavonoid reached its target 10 times faster than the corresponding values for the quercetin rutinoside known as rutin, and that absorption of isoquercitrin was far better than that of aglycone quercetin by itself.[26,27] The presence of sugars

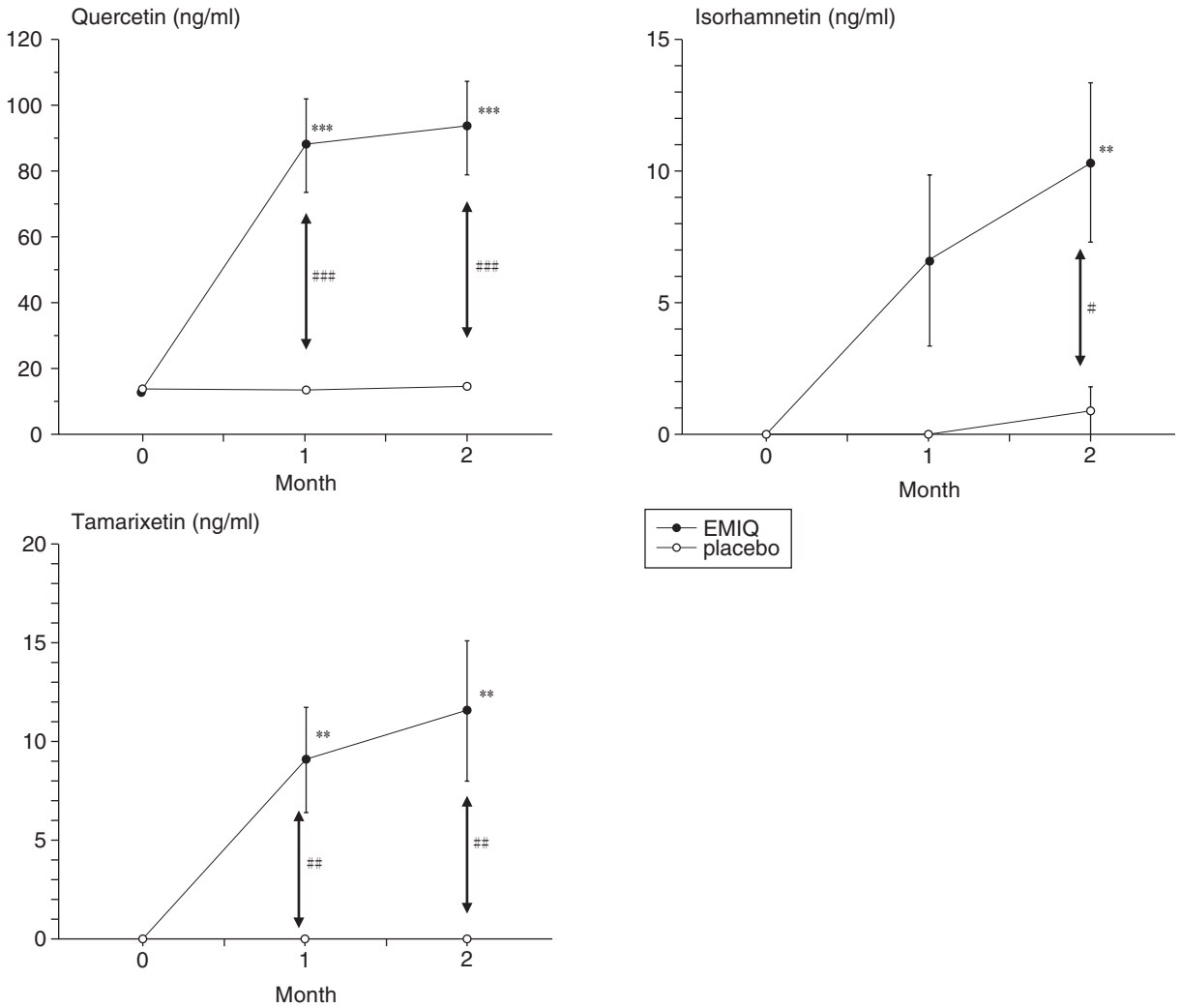


Fig. 6 Changes in serum concentrations of quercetin, and its methylated metabolites isorhamnetin and tamarixetin in the EMIQ (solid circle) and the placebo (circle) group. Each value is the mean +/− SE. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with the values at the beginning of the study in each group. Arrow with $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, shows comparison between both groups in the same week.

in the flavonoids is therefore an important determinant for their absorption. EMIQ is more soluble than quercetin and isoquercitrin, and is expected to be the highest absorptive flavonoid among these quercetin derivatives. In a previous study, we measured serum concentrations of quercetin and the trough value did not increase in the EMIQ group, so that successive intake of EMIQ for 8 weeks did not lead to an accumulation of quercetin in the blood.[16] Here, we showed that the serum levels of quercetin and its major metabolites, isorhamnetin and tamarixetin[28] were elevated at 2 hours after EMIQ intake and quercetin concentration reached around 90 ng/ml, indicating that EMIQ was indeed absorbed.

The ameliorative effect of EMIQ was found in ocular symptoms and was to a large extent mediated through its anti-allergic, antioxidant[29] or other activities.[8] We have found that some kinds of flavonoids possess novel anti-allergic activities: including apigenin, fisetin, luteolin, quercetin and kaemferol which suppress the expression of Th2 type (IL-4, IL-5 and IL-13),[9,10] and CD40 ligand[11] in basophils. In addition to these *in vitro* anti-allergic activities, numerous studies with allergic animal models have recently demonstrated that flavonoids exert preventative and therapeutic effects by modifying Th2 type cytokine expression and suppressing IgE elevation.[12,13,30-35] In a study performed in 2007, the serum levels of cytokines and chemokines before and after the study were measured but no significant reduction in Th2 cytokines (IL-4, IL-5 and IL-13) or IgE concentration as a result of EMIQ intake was found.[16] Similarly, in 2008, the serum level of IgE was not specifically reduced for the EMIQ group, indicating that systemic im-

mune responses were not modulated by EMIQ intake. On the other hand, the ferric-reducing antioxidant power tended to increase, and one oxidized product, oxidized LDL, decreased in the EMIQ group, suggesting that the ameliorative effect of EMIQ was due to its antioxidant activity. With regard to the involvement of oxidant stress in the development of allergic conjunctivitis, few sources have been available. In a murine model of allergic conjunctivitis oxidative stress generated by NAD(P)H oxidase was reported to augment immediate-type hypersensitivity reactions and pollen antigen-driven allergic conjunctivitis.[36] The serum concentration of quercetin at 2 hours after EMIQ intake reached around 90 ng/ml, which was 0.3 μM. The IC_{50} value of quercetin for the inhibition of IL-4 production by activated basophils obtained from *in vitro* analysis was, however, 18.8 μM,[10] and the IC_{50} value of quercetin for the inhibition of histamine or other chemical mediators release in basophils or mast cells is reportedly 3–15 μM.[37-40] Since other flavonoids are also obtained orally from foods and teas in significant amounts,[41] the possibility remains that the effect of flavonoids may be mediated through their anti-allergic activity. Recently the aryl hydrocarbon receptor has been demonstrated to be a regulator of Th17 and regulatory T cell development and these T cells are known to modulate autoimmune and allergic reactions.[42] The exogenous ligands for aryl hydrocarbon receptor are environmental toxins including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and flavonoids have shown to inhibit dioxin-induced activation of aryl hydrocarbon receptor even below 1 μM.[43] Therefore, the effect of EMIQ on ocular symptoms is possibly mediated by affecting T cell differentiation through inhibition of the activation of aryl hydrocarbon receptor. Alternatively, it is possible that EMIQ directly affects conjunctival residential cells including epithelial cells, resulting in the suppression of allergic conjunctivitis.

In the dietary hypothesis, it has been proposed that diet is one of the environmental factors that contribute to an increase in the prevalence of allergic diseases and/or worsening of allergic symptoms.[4-7] Furthermore, an epidemiologic study found that a higher intake of flavonoids was associated with a lower incidence of asthma.[44] In experimental models, oral administration of flavonoids has been demonstrated to prevent allergic diseases including asthma, anaphylaxis, food allergies and atopic dermatitis.[12,13,30-35] All these findings, in addition to ones reported here for EMIQ, strongly suggest that an appropriate intake of flavonoids can be a dietary means for the prevention and treatment of allergic diseases.

ACKNOWLEDGEMENTS

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