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Inhibition of T-cell-Dependent Antibody Production by Quercetin in Mice

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Abstract − The immunosuppressive properties of flavonoids were examined for the first time by testing their effects on T-cell-mediated antibody production, using a classical plague-forming cell (PFC) assay in mice. Among the tested flavonoids including naringenin, chrysin, flavonol, galangin, quercetin, morin, myricetin and biochanin A, only quercetin, orally administered at 25 mg/kg, significantly inhibited the number of IgM-producing PFCs induced by sheep red blood cells (SRBC). Interestingly, biochanin A (isoflavone) increased the number of PFCs, suggesting an immunostimulatory effect. The other flavonoids tested did not inhibit or enhance PFC response significantly. Quercetin was also found to show thymus atrophy dose-dependently at 5-500 mg/kg. All these results indicate that quercetin inhibits in vivo antibody production probably by inhibiting T-cell function.

Keywords: Flavonoid, Quercetin, Immunosuppression, Plague-forming cell assay

INTRODUCTION

Flavonoids, the anti-inflammatory plant constituents, are known to possess anti-inflammatory and immunomodulatory activity (For review: Middleton and Kandaswami, 1993; Kim *et al.,* 2004). It was repeatedly reported that certain flavones/flavonols having a C-ring 2,3-double bond inhibited in vitro mitogen-induced lymphoblastosis, particularly lymphoblastosis of T-cells, but not of B-cells (Mookerjee *et al.,* 1986; Hirano *et al.,* 1989; Namgoong *et al.,* 1994). These compounds also modulate other T-cell functions such as cytotoxicity (Schwartz *et al.,* 1982). Several in vivo studies demonstrated their inhibitory activity against allergic responses and delayed-type hypersensitivity (Kimata *et al.,* 2000; Kim and Chung, 1990). Although these results show the effects of flavonoids on T-cell-mediated immunity, the in vivo effects of flavonoids on antibody production have not yet been established. Therefore, the present study examined the effects of flavonoids on in vivo antibody production using a classical plague-forming cell (PFC) assay in mice. Thymus atrophy of quercetin was also examined to determine in vivo systemic effect on thymus, an organ that is crucial for T-cell maturation.

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MATERIALS AND METHODS

Chemicals

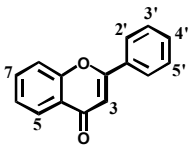
All flavonoids (Fig. 1) used in this study and dexamethasone were purchased from Sigma-Aldrich (St. Louis, MO). All cell culture reagents and guinea-pig complement was obtained from Gibco (Grand Island, NY).

Animals

Male ICR mice (20-25 g) were obtained from Experimental Animal Center, College of Pharmacy, Seoul National University (Seoul, Korea). The animals were maintained in an animal room under conditions of 22 ± 2°C, 40-60% relative humidity and 24 h/24 h L/D cycle. Mice were fed with laboratory chow (Purina Korea) and water ad libitum. The mice were acclimatized at least 7 days before experiment.

Plague-forming cell (PFC) assay

From male sheep (Animal Farm, Kangwon National University), blood was withdrawn in Alsever's solution and used within 21 days. On the day of immunization, sheep red blood cells (SRBC) were washed with Earle's balanced salt solution (EBSS) and 5×10^8 SRBC was intraperi-



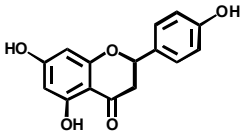
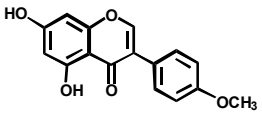
Flavonoids	C-3	C-5	C-7	C-2'	C-3'	C-4'	C-5'
Naringenin							
Chrysin	H	OH	OH	H	H	H	H
Flavonol	OH	H	H	H	H	H	H
Galangin	OH	OH	OH	H	H	H	H
Quercetin	OH	OH	OH	H	OH	OH	H
Morin	OH	OH	OH	OH	H	OH	H
Myricetin	OH	OH	OH	H	OH	OH	OH
Biochanin A							

Fig. 1. The chemical structures of the flavonoids tested in this study.

toneally administered to each mouse. Flavonoids including quercetin were administered orally 1 h prior to immunization. After 4 days of immunization, mice were sacrificed by cervical dislocation and the spleens were excised. The isolated splenocytes were obtained after treatment with a hemolytic solution (0.8% NH_4Cl, 0.1 M Hepes). The final erythrocyte cell pellet obtained by centrifugation at 200 g for 10 min was added to an appropriate volume of EBSS. Following the modified procedure of Bullock and Moller (1972), splenocytes (50 μl), 400 μl agar solution (250 mg agar/50 ml EBSS, pH 7.2), SRBC (25 μl) and diluted (1:3) guinea pig complement (25 μl) were mixed together, poured into a bacteriological culture dish and overlayed with cover glass (45×50 mm). The agar plates were allowed to solidify and were incubated at 37°C in 5% CO_2 incubator for 3 h. PFCs were counted using a dissecting microscope.

Thymus atrophy in vivo

In order to evaluate the thymus atrophogenic activity, quercetin was administered orally to mice. Three days later, the mice were sacrificed. Thymus tissue and spleen were excised and weighed.

Table I. Effects of flavonoids on IgM-producing plaque-forming cell (PFC) response in mice

Compounds[a]	Total cell no./ spleen ($\times10^8$)	Total PFCs/ spleen ($\times10^3$)	PFCs/ 10^6 cells
Control	0.72 ± 0.07[b]	0.1 ± 0.1	0.9 ± 1.6
SRBC-treated	1.05 ± 0.19	41.8 ± 13.8	390.3 ± 74.7
Naringenin	1.05 ± 0.21	36.3 ± 9.3	357.0 ± 118.2
Chrysin	1.02 ± 0.11	39.2 ± 12.9	415.7 ± 102.6
Flavonol	1.01 ± 0.15	40.1 ± 4.8	402.5 ± 89.1
Galangin	0.99 ± 1.13	41.0 ± 10.0	416.9 ± 93.5
Quercetin	0.79 ± 0.13[c]	21.9 ± 3.8[d]	284.3 ± 72.6[c]
Morin	1.05 ± 0.20	35.3 ± 11.6	349.1 ± 126.7
Myricetin	1.12 ± 0.19	41.3 ± 11.8	412.5 ± 201.7
Biochanin A	0.93 ± 0.18	57.5 ± 20.7	621.7 ± 167.9[c]
Dexamethasone	0.40 ± 0.23[d]	20.2 ± 10.3[d]	505.7 ± 82.1[c]

[a]All flavonoids were orally administered at 25 mg/kg, while dexamethasone dissolved in acetone was subcutaneously injected at 5 mg/kg. [b]Arithmetic mean ± SD (n=8). [c]$p<0.02$, [d]$p<0.01$, significantly different from the SRBC-treated control group (n=8).

Statistics

All results were represented as arithmetic means ± SD. Unpaired Student *t*-test was used for comparing the statistical significance.

RESULTS AND DISCUSSION

In a preliminary experiment, the optimum number of SRBC for immunization was determined to be 5×10^8 cells/mouse. Under this condition, SRBC provoked high PFC response as demonstrated in Table I. When the flavonoids were orally administered at 25 mg/kg, it was found that quercetin significantly inhibited the IgM-producing PFC response and reduced the total cell number of spleen, while biochanin A increased the PFCs. The other flavonoids tested did not significantly alter the PFC response at a dosage of 25 mg/kg. As expected, dexamethasone (potent steroidal immunosuppressive anti-inflammatory agent), used as a reference drug, significantly inhibited the total PFCs, but increased PFCs/10^6 cells. To clearly establish its immunosuppressive property, the dose-dependent effect of quercetin was evaluated. Table II shows that quercetin reduced the total number of PFCs and PFCs/10^6 cells dose-dependently after oral administration of 5-500 mg/kg of quercetin. To investigate the in vivo effect of quercetin on T-cells, the extent of thymus atrophy was examined. In this experiment, quercetin clearly showed dose-dependent thymus atrophy (Table III). Dexamethasone also showed potent thymus atrophy.

All these results suggest that quercetin may possess in

Table II. Dose-dependent inhibition of quercetin on IgM-producing plague-forming cell (PFC) response in mice

Compounds[a]	Dose (mg/kg)	Total cell no./ spleen ($\times 10^8$)	Total PFCs/ spleen ($\times 10^3$)	PFCs/10^6 cells
Control		0.73 ± 0.10[b]	0.1 ± 0.2	1.8 ± 2.5
SRBC-treated		0.96 ± 0.10	35.1 ± 3.6	365.6 ± 36.5
Quercetin	5	0.95 ± 0.08	34.9 ± 9.8	367.4 ± 93.4
	50	0.77 ± 0.17	16.1 ± 4.1[d]	209.1 ± 48.6[d]
	500	0.72 ± 0.14[c]	14.8 ± 4.4[d]	205.6 ± 50.3[d]

[a]Quercetin was orally administered. [b]Arithmetic mean ± SD (n=8). [c]$p < 0.02$, [d]$p < 0.01$, significantly different from the SRBC-treated control group (n=8).

Table III. Thymus atrophogenic activity of quercetin in mice

Compounds	Dose[a] (mg/kg)	Thymus wt. (mg)	Thymus wt. (mg/100 g body wt.)	Spleen wt. (mg)
Control	–	48.6 ± 7.4[b]	215.2 ± 21.7	112.6 ± 22.0
Quercetin	5	41.0 ± 6.4	199.4 ± 31.2 (7.3)[c]	97.1 ± 25.9
	50	32.4 ± 7.5[d]	158.7 ± 36.1[d] (26.3)	91.1 ± 11.3
	500	31.4 ± 5.7[e]	153.0 ± 29.7[e] (28.9)	111.2 ± 27.0
Dexamethasone	2	28.8 ± 5.5[e]	137.7 ± 27.3[e] (36.0)	76.0 ± 13.3[d]

[a]Quercetin was orally administered while dexamethasone dissolved in acetone was injected subcutaneously. [b]Arithmetic mean ± SD. [c]The values in the parenthesis represent % reduction of thymus weight compared to the control group. [d]$p < 0.01$, [e]$p < 0.001$, significantly different from the control group (n=8).

vivo immunosuppressive activity, particularly on T-cell-mediated antibody production since SRBC is a T-cell-dependent antigen and PFC used in this study is a marker for IgM-producing B-cells. It was interesting that biochanin A (isoflavone), in contrast to quercetin, enhanced the number of PFC, suggesting increased antibody production in vivo. Other isoflavones that are structurally similar to biochanin A remain to be further tested in order to establish their effects on antibody production.

Among the flavonoid derivatives studied, the best representative example is quercetin, a flavonol that is most abundant in nature. The immunosuppressive activity of this compound has been extensively investigated. For instance, quercetin was found to inhibit T-cell lymphoblastosis and mixed lymphocyte reaction (Mookerjee *et al.,* 1986; Hirano *et al.,* 1989; Namgoong *et al.,* 1994). The same compound also inhibits the cytotoxic effect of cytotoxic T-cells (Schuwartz *et al.,* 1982). Recently, quercetin was also demonstrated to enhance interferon (IFN)-γ production, but inhibited interleukin (IL)-4 production, suggesting a suppressive action of humoral immunity (Nair *et al.,* 2002). However, no report has been available to describe the in vivo effects of quercetin on antibody production, and to our knowledge this is the first report showing the inhibitory activity of quercetin on antibody production in vivo.

The results of the present study are well correlated with previous findings of in vitro experiments showing the immunosuppressive properties of quercetin. The suppressive mechanism of antibody production in vivo by quercetin may be mediated mainly by its inhibitory action on T-cell function, but not directly on B-cells as previously described (Schuwartz *et al.,* 1982; Hirano *et al.,* 1989; Namgoong *et al.,* 1994). Actually quercetin also showed thymus atrophy after oral administration, suggesting the preferential effect on thymus, an organ involved in T-cell maturation.

It is significant to note that other flavonoid derivatives such as chrysin, flavonol, galangin, morin and myricetin, structurally similar flavones and flavonols to quercetin, did not inhibit PFC response in vivo at doses of 25 mg/kg. Whereas some of these flavonoids including chrysin, flavonol and myricetin inhibited in vitro T-cell lymphoblastosis in similar concentration ranges as those of quercetin (Namgoong *et al.,* 1994), quercetin was the only flavonoid that inhibited the in vivo PFC response. The precise reason for the discrepancy of in vitro and in vivo effects of these flavonoids is not understood at present. But it is speculated that the bioavailability/ metabolism of these flavonoids may be the problem rather than their intrinsic activities (Ross and Kasum, 2002; Murota and Terao, 2003). It is possible that these flavonoids, except for quercetin and biochanin A, might be rapidly converted to inactive derivatives by metabolism, after oral uptake in mice.

In conclusion, quercetin inhibited the PFC response in

mice after oral administration. The same compound also showed thymus atrophy. But, other structurally related flavonoids that were tested did not alter the PFC response. Quercetin may reduce antibody production, at least in part, by inhibiting T-cell-mediated function in vivo. It is still not clear, however, whether quercetin suppresses antibody production in humans and this needs to be addressed by future clinical studies.

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