Intestinal Bacterial Metabolism of Flavonoids and Its Relation to Some Biological Activities

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Flavonoid glycosides were metabolized to phenolic acids via aglycones by human intestinal microflora producing α-rhamnosidase, exo-β-glucosidase, endo-β-glucosidase and/or β-glucuronidase. Rutin, hesperidin, naringin and poncirin were transformed to their aglycones by the bacteria producing α-rhamnosidase and β-glucosidase or endo-β-glucosidase, and baicalin, puerarin and daidzin were transformed to their aglycones by the bacteria producing β-glucuronidase, C-glycosidase and β-glycosidase, respectively. Anti-platelet activity and cytotoxicity of the metabolites of flavonoid glycosides by human intestinal bacteria were more effective than those of the parental compounds. 3,4-Dihydroxyphenylacetic acid and 4-hydroxylphenylacetic acid were more effective than rutin and quercetin on anti-platelet aggregation activity. 2,4,6-Trihydroxybenzaldehyde, quercetin and ponciretin were more effective than rutin and ponciretin on the cytotoxicity for tumor cell lines. We insist that these flavonoid glycosides should be natural prodrugs.

Key words : Intestinal bacterial metabolism, Flavonoids, Flavonoid glycosides

INTRODUCTION

Flavonoid glycosides are polyphenolic compounds produced by most fruits, vegetables and herbal medicines. These compounds are resistant to boiling and fermentation and ingested daily more than 1 g by human. After ingestion of flavonoid glycosides, most of them are not easily absorbed in mammalian gut and meet intestinal microflora in the intestine. Therefore, these compounds are metabolized by intestinal bacteria. Aglycones related to mutagenesis are formed continuously in mammalian gut by bacterial hydrolysis of ingested flavonoid glycosides. Evidence for the involvement of the intestinal microflora in the metabolism of flavonoid compounds *in vivo* has been presented by Griffiths (1964) and Griffiths and Smith (1972). Particularly, it was reported that the formation of ring fission products from orally administered flavonoids was significantly decreased by the coadministration of oral antibiotics (Griffith & Barrow, 1972; Nakagawa *et al.*, 1965). The metabolites from the urine of rats orally treated with flavonols, such as quercetin and kaempherol, were phenolic acids. We also reported metabolism of some flavonoid glycosides, such as rutin, na-

ringin, poncirin and heperidin, to their aglycone by human intestinal bacteria (Kim *et al.*, 1994; Jang and Kim, 1996). However, the study on biotransformation of their aglycones to ring-fission metabolites by human intestinal bacteria was not complete. It is important to know how these components are metabolized by intestinal bacteria and how the biological activities of these components are changed before these components are delivered to target site such as liver and kidney.

In the present paper, we reported the isolation of the bacteria metabolizing flavonoid glycosides from human intestinal bacteria and the biological activity of their metabolites.

MATERIALS AND METHODS

Materials

Rutin, naringin, hesperidin, quercetin, naringenin, hesperetin and phenolic metabolites were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Isoquercetin, poncirin, hesperetin β-D-glucopyranoside, poncirenin, ponciretin and prunin were prepared according to our previous method. General anaerobic medium (GAM) was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). The other media were purchased from Difco Co. (Detroit, MI, U.S.A.)

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Isolation and identification of the intestinal bacteria, which degrade flavonoids

The intestinal bacteria were isolated from fresh Korean feces according to our previous method (Kim et al., 1994). The isolated bacteria were inoculated and incubated in GAM containing flavonoids. Flavonoids-transforming bacteria were judged by TLC analysis of the cultured media. The transformant-positive bacteria were identified according to Bergey's manual (Holt, 1984).

Determination of the metabolite of flavonoids by human intestinal bacteria

Fresh Korean feces were suspended 10-fold with anaerobic dilution media and centrifuged for 5min at 500rpm. The resulting supernatant was used as human intestinal bacteria. It was inoculated into 50ml GAM broth containing 5mg flavonoids. The inoculated GAM broth was incubated anaerobically for 2 days. The cultured broth was septically taken out, acidifed to pH 2 and extracted with ethylacetate for 1 min, and evaporated under nitrogen at 40°C to 45°C. This extract was assayed by TLC according to the method of Griffith et al. (1972). TLC was performed as follows; developing solvents A system, $CHCl_3$:AcOH:H_2O=2:1:1 (lower layer); B system, $CHCl_3$:MeOH=4:1; C system, $CHCl_3$:MeOH=6:1; D system, benzene: $CHCl_3$:MeOH= 5:3:2; E system, benzene:acetone:$CHCl_3$:MeOH=8:2:3:2.

Assay of cytotoxic activities

The MTT method (Carmichael et al., 1987) was applied for evalutation of cytotoxic activity. IC_{50} value, the concentration of sample caused the 50% inhibition of cell growth, was determined by plotting the net growth rate versus the concentration of test sample. Cancer cell lines used for cytotoxicity test were as follows: SNU-1 (human stomach cancer cell line), SNU-C4 (human colon cancer cell line), P-388D1 (mouse lymphoid neoplasma cell line), L-1210 (mouse lymphocytic leukemia cell line), HepG2 (human hepatoblastoma cell line), A549 (human lung cancer cell line) and MA-104 (Macacus' rhesus monkey kidney cell line). Each cell except MA-104 was maintained in RPMI 1640 medium supplied with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere at 5% CO_2. MA-104 was maintained in DMEM meidum with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere at 5% CO_2.

Anti-platelet aggregation activity

The anti-platelet aggregation activity was determined according to the smearing method of Yun-Choi et al. (1985). Platelet rich plasma (PRP) was prepared by centrifugation of the citrated blood at 200×g for 10 min and platelet poor plasma (PPP) was obtained from the residue by centrifugation at 900×g for 30 min. Platelet number was adjusted to 3×10^{11}/L by mixing PRP and PPP. PRP (20 μl)and 10 μl of 4 mM inhibitor were incubated at 37°C for 8 min and then 10 μl of 1 mM ADP or collagen (1 mg/ml) was added to induce platelet aggregation. The reaction mixture was smeared into a slide glass, then stained with Wright-Giemsa and measured with a microscope.

Inhibitory activity of trypsin

The reaction mixture containing 0.36 ml of 1% casein, 0.1 ml inhibitor (test sample) was preincubated for 10 min at 37°C. 40 μl of tyrpsin was added to the reaction mixture for 20 min, then stopped by adding 1 ml of 5% TCA and centrifuged at 2000×g for 10min. Protein of the resulting supernatant was determined according to Lowry et al. (1956).

RESULTS

Metabolites of flavonoid glycosides by human intestinal bacteria

When flavonoid glycoside, rutin, was incubated with human intestinal microflora for 6-12h, main metabolite was quercetin. After 12 h incubation, 3,4-dihyroxy-

Table I. Metabolites of flavonoid glycosides by human intestinal bacteria

Flavonoid glycoside	Metabolite (aglycone)	Metabolite (phenolic acids)
Baicalin	Baicalein	4-hydroxybenzoic acid 3,4-dihydroxybenzoic acid pyrogallol phenylacetic acid
Puerarin Daidzin	Daidzein	4-hydroxybenzoic acid 2,4-dihydroxybenzoic acid resorcinol 2,4-dihydroxyacetophenone 4-hydroxyphenylacetic acid
Rutin	Quercetin	4-hydroxybenzoic acid 3,4-dihydroxybenzoic acid 3,4-dihydroxyphenylacetic acid
Naringin	Naringenin	4-hydroxybenzoic acid phloroglucinol 2,4,6-trihydroxybenzoic acid 4-hydroxyphenylacetic acid
Hesperidin	Hesperetin	resorcinol phloroglucinol 2,4-dihydroxyphenylacetic acid
Poncirin	Ponciretin	4-hydroxybenzoic acid 2,4-dihydroxyacetophenone phloroglucinol pyrogallol
Catechin	Catechin	4-hydroxybenzoic acid 2,4,6-trihydroxybenzoic acid phloroglucinol 4-methoxysalicylic acid

phenylacetic acid, 3,4-dihydroxybenzoic acid and 4-hydroxybenzoic acid, were produced (Table I). The other flavonoid glycosides, poncirin, hesperidin, naringin, puerarin, baicalin, daidzin and catechin, were metabolized to the corresponding aglycones at early time and then to the phenolic acids. Puerarin and daidzin were metabolized to daidzein and then to 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid 2,4-dihydroxyacetophenone and 4-hydroxyphenylacetic acid. Baicalin was metabolized to baicalein and then to 3,4-dihydroxybenzoic acid and pyrogallol and phenylacetic acid. Hesperidin was metabolized to hespertetin and then to 2,4-dihydroxyphenylacetic acid, resorcinol and phlorogpucinol. Naringin was metabolized to naringenin and then to 4-hydroxybenzoic acid, phloroglucinol, 2,4,6-trihydroxybenzaldehyde and 4-hydroxyphenylacetic acid. Poncirin was metabolized to ponciretin and then to 4-hydroxybenzoic acid, 2,4-dihydroxyacetophenone, pyrogallol and phloroglucinol. Catechin was metabolized to 4-hydroxybenzoic acid, 2,4,6-trihydroxybenzadehyde, phloroglucinol and 4-methoxysalicylic acid. Generally, flavonoid glycosides were metabolized to their aglycones at early time of incubation time and then to phenolic acids.

Glycosidation bacteria of flavonoid glycosides and flavonoid ring fission bacteria

We screened the bacteria hydrolyzing glycosides or fissuring the B-ring of rutin from human feces. Rutin glycosidating bacterium was *Bacteroides* JY-6 and its ring fission bacteria were *Pediococcus* Q-5, *Streptococcus* S-3, *Bacteroides* JY-6 and *Bifidobacterium* B-9. The isolated bacteria hydrolyzing flavonoid glycosides and/or fissuring B-ring of the flavonoid glycosides were shown Table II. The rhamnoglycosides, such as rutin, hesperidin, naringin and poncirin, were transformed to their aglycones by *Bacteroides* JY-6, which produced α-rhamnosidase and β-glucosidase. Baicalin was transformed to baicalein by *E. coli* HGU-3 and *Eubacterium* LG-22, which produced β-glucuronidase. We previously reported that the substrate specificity of the glycosidases produced by intestinal bacteria was different from those of mammalian tissue enzymes (Kim *et al.*, 1994). Furthermore, the flavonoid B-ring fissuring enzyme of mammalian tissues seems to be different from that of intestinal bacteria, because the homogenate of the rat liver could not fissure the B-ring of the flavonoids. Naringenin was transformed to phenolic acids by *Streptococcus* L-2

Table II. Intestinal bacteria transforming flavonoid glycosides to phenolic acids, which are isolated from human intestinal bacteria

Metabolic process	Transforming bacteria	Metabolic process	Transforming bacteria
Baicalin-baicalein	*E. coli* HGU-3 *Bacteroides* J-37	Baicalein-Phenolic acids	*Streptococcus* S-2 *Lactobacillus* L-2 *Eubacterium* A-44 *Bifidobacterium* B-9
Puerarin-daidzein	*Peptostreptococcus* YK-10	Daidzein-phenolic acids	*Bacteroides* JY-6
Daidzin-daidzein	*Bacteroides* J-37 *Eubacterium* A-44 *Fusobacterium* K-60		
Rutin-quercetin	*Bacteroides* JY-6 *Fusobacterium* K-60 *Eubacterium* YK-4	Quercetin-phenolic acid	*Streptococcus* S-2, *Lactobacillus* L-2, *Bifidobacterium* B-9, *Bacteroides* JY-6
Naringin-naringenin	*Bacteroides* JY-6 *Eubacterium* YK-4 *Feptostreptoccous* YK-10 *Fusobacterium* K-60	Naringenin-phenolic acid	*Streptococcus* S-2, *Lactobacillus* L-2 *Bacteroides* JY-6
Hesperidin-hesperetin	*Fusobacterium* K-60 *Eubacterium* YK-4 *Bacteroides* JY-6	Hesperetin-phenolic acid	*Streptococcus* S-2, *Lactobacillus* L-2, *Bifidobacterium* B-9, *Bacteroides* JY-6
Poncirin-ponciretin	*Fusobacterium* K-60 *Eubacterium* YK-4 *Bacteroides* JY-6	Ponciretin-phenolic acid	*Streptococcus* S-2, *Lactobacillus* L-2, *Bifidobacterium* B-9, *Bacteroides* JY-6
Catechin		Catechin-phenolic acids	*Lactobacillus* L-2, *Bifidobacterium* B-9 *Bacteroides* JY-6

and *Bacteroides* JY-6. Hesperetin and ponciretin were transformed by *Bifidobacterium* B-9 and *Bacteroides* JY-6. Catechin was transformed to phenolic acids by *Bifidobacterium* B-9 and *Lactobacillus* L-2 and *Bacteroides* JY-6. Baicalein was transformed to phenolic acids by *Streptococcus* S-2 and *Lactobacillus* L-2 and

Bifidobacterium B-9. Daidzein was transformed to phenolic acids by *Bacteroides* JY-6.

Biological activities of flavonoids and their metabolites

The biological activities, such as trypsin inhibition,

Table III. Inhibitory effect of trypsin activity

Flavonoid glycoside	Inhibition (%)	Metabolite (phenolic acids)	Inhibition (%)
Diosmin	– [2]	1) 4-hydroxyphenylacetic acid	25
Rutin	–	2) 3,4-dihydroxyphenylacetic acid	–
		3) 4-hydroxybenzoic acid	38
Puerarin	–	4) 2,4-dihydroxybenzoic acid	–
Hesperidin	–	5) 3,4-dihydroxybenzaldehyde	–
Neohesperidin	–	6) 2,4,6-tryihydroxybenzaldehyde	–
Naringin	–	7) phloroglucinol	13
Poncirin	–	8) resorcinol	–
		9) 4-methoxysalicylic acid	–
Baicalin	–	10) 2',4'-dihydroxyacetophenone	21
Metabolite	**Inhibition**	11) 2'-hydroxy-4'-methoxy acetophenone	–
(aglycone)	**(%)**	**12) pyrogallol(pyrogallic acid)**	33
Daidzein	6.7	13) phenylacetic acid	–
		14) 3-hydroxyphenylacetic acid	–
Quercetin	–	15) 3-hydroxybenzoic acid	20
Hesperetin	–	16) protocatechuic acid	19
Naringenin	–	17) 2,4,6-trihydroxybenzoic acid monohydrate	20
Ponciretin	–	18) 4-hydroxy-3-methoxybenzoic acid	25
Baicalein	–	**19) salicylic acid**	52
		20) p-coumaric acid	–
Catechin	–	21) caffeic acid	–

[1]final concentration, 1 mM
[2]not inhibited.

Table IV. Anti-platelet aggregation effect of flavonoids and metabolites

Favonoid glycoside[1]	Platelet aggregation activity	Metabolite	Platelet aggregation activity
Diosmin	+	**1) 4-hydroxyphenylacetic acid**	–
Rutin	+	**2) 3,4-dihydroxyphenylacetic acid**	–
Puerarin	+ +	**3) 4-hydroxybenzoic acid**	–
		4) 2,4-dihydroxybenzoic acid	–
Hesperidin	±	5) 3,4-dihydroxybenzaldehyde	±
Neohesperidin	±	6) 2,4,6-trihydroxybenzaldehyde	+
Naringin	+	7) phloroglucinol	+
Poncirin	±	**8) resorcinol**	–
Baicalin	±	9) 4-methoxysalicylic acid	–
		10) 2',4'-dihydroxyacetophenone	+
Metabolite	**Platelet aggregation**	11) 2'-hydroxy 4'-methoxy acetophenone	±
(aglycone)	**activity**	12) pyrogallol(pyrogallic acid)	±
Diosmetin	+	13)'phenyl acetic acid	±
Quercetin	+	14) 3-hydroxyphenylacetic acid	±
Daidzein	+	**15) 3-hydroxybenzoic acid**	–
Hesperetin	±	16) Protocatechuic acid	±
Naringin	±	17) 2,4,6-trihydroxybenzoic acid monohydrate	+
		18) 4-hydroxy 3-methoxybenzoic acid	+
Ponciretin	–	19) salicylic acid	+
Baicalein	+ +	20) caffeic acid	–
Catechin	–	**21) p-coumaric acid**	±
Positive control	**+ +**	**Negative control**	–
		Aspirin	±

[1]final concentration 2 mM

Table V. Cytotoxic effect of flavonoid glycosides and their metabolites

Flavonoid glycoside	IC$_{50}$ (mM)						
	SNU-C4	SNU-1	P-388	L-1210	HepG2	A549	MA-104
Diosmin	0.8	>1	0.3	0.5	>1	1	>1
Rutin	>1	>1	0.3	0.2	>1	>1	>1
Puerarin	>1	>1	>1	>1	>1	>1	>1
Hesperidin	>1	0.5	0.5	>1	>1	1	>1
Neohesperidin	>1	>1	>1	0.8	>1	>1	>1
Naringin	>1	>1	>1	0.8	>1	>1	>1
Poncirin	>1	>1	>1	>1	>1	>1	>1
Baicalin	0.5	0.05	0.05	0.04	0.8	0.9	>1
Metabolites (aglycone)							
Diosmetin	0.5	0.1	0.08	0.5	0.4	0.5	>1
Quercetin	0.05	0.04	0.05	0.08	>1	>1	>1
Hesperetin	>1	0.6	0.3	0.1	>1	>1	>1
Naringenin	0.3	0.2	0.3	0.2	>1	>1	>1
Ponciretin	0.17	0.07	0.17	0.17	>1	>1	>1
Baicalein	0.1	0.04	0.05	0.01	0.5	0.7	>1
Catechin	>1	>1	>1	>1	>1	>1	>1
adriamycin	0.05	0.001	0.003	0.02	>1	0.1	1

Table VI. Cytotoxic effect of phenolic acid metabolites

Metabolite	IC$_{50}$ (mM)						
	SNU-C4	SNU-1	P-388	L-1210	HepG2	A549	MA-104
1) 4-hydroxyphenylacetic acid	>1	>1	>1	>1	>1	>1	>1
2) 3,4-dihydroxyphenylacetic acid	0.5	0.03	0.1	0.2	0.8	>1	>1
3) 4-hydroxybenzoic acid	>1	>1	0.55	>1	0.1	>1	>1
4) 2,4-dihydroxybenzoic acid	>1	>1	1	>1	1	>1	>1
5) 3,4-dihydroxybenzaldehyde	0.25	0.045	0.1	0.08	0.05	>1	>1
6) 2,4,6-trihydroxybenzaldehyde	0.03	0.3	0.5	0.005	1	0.1	>1
7) phloroglucinol	0.3	0.2	0.5	0.5	1	>1	>1
8) resorcinol	1	0.5	0.9	0.05	1	>1	>1
9) 4-methoxy salicylic acid	>1·	0.5	1	0.5	0.1	>1	>1
10) 2',4'-dihydroxyacetophenone	>1	>1	>1	>1	>1	>1	>1
11) 2'-hydroxy-4'-methoxy acetophenone	>1	>1	>1	0.1	>1	>1	>1
12) pyrogallol(pyrogallic acid)·	0.2	0.02	0.01	0.1	0.5	0.8	>1
13) phenylacetic acid	0.5	0.05	0.5	0.1	0.6	0.7	>1
141) 3-hydroxyphenylacetic acid	>1	>1	>1	1	>1	>1	>1
15) 3-hydroxybenzoic acid	>1	0.2	0.1	>1	>1	>1	>1
16) protocatechuic acid	>1	>1	0.6	>1	>1	>1	>1
17) 2,4,6-trihydroxybenzoic acid monohydrate	>1	>1	1	0.05	0.1	>1	>1
18) 4-hydroxy-3-methoxybenzoic acid	0.6	0.55	>1	0.5	>1	>1	>1
19) salicylic acid	>1	>1	>1	>1	>1	>1	>1
20) p-coumaric acid	0.5	0.03	>1	0.1	0.5	0.8	>1
21) caffeic acid	1	>1	>1	>1	>1	>1	>1
adriamycin	0.05	0.001	0.003	0.02	>1	0.1	1

anti-pletelet aggregation activity and cytotoxic actvity for tumor cell lines, of the metabolites of flavonoid glycosides by intestinal bacteria were investigated. First, the trypsin inhibitory effect of flavonoid glycosides and their metabolites were investigated (Table III). Most of tested compounds had low trypsin inhibitory activity. Among them, salicylic acid was best, followed by 4-hydroxybenzoic acid. Anti-pletelet aggregation activity of flavonoid glycosides and their metabolites was shown in Table IV. Anti-platelet agg-

regation activity of 3,4-dihydroxyphenylacetic acid, 4-hydroxylphenylacetic acid and 2-hydroxy 4-methoxy acetophenone were best. Anti-platelet aggregation activity of the main metabolites, quercetin, poncirin and baicalein, were lower than those of the phenolic acid. Generally, anti-platelet aggregation activity of the phenolic acids were best, followed by aglycone and glycosides. We investigated cytotoxic activity on a stomach cancer cell line (SNU-1) and a colon cancer cell line (SNU-C4). (Table V and VI). As shown here, cytotoxic

activity of 3,4-dihydroxyphenylacetic acid was best, followed by quercetin, baicalein and baicalin on stomach cancer cell line. In colon cell line, cytotoxicity of 2,4,6-trihydroxybenzaldehyde was best, followed by quercetin, baicalin and ponciretin. On the other tumor cell line, cytotoxic activities of flavonoid glycosides and their metabolites were similar to those of the above cell lines. Generally, cytotoxic activities of the metabolites, phenolic acids and algycones, were higher than those of glycosides.

DISCUSSION

MacDonald et al. (1983) and Tamura et al. (1980) reported that rutin could be metabolized to quercetin by intestinal bacteria, which produce β-glucosidase and/or β-rhamnosidase, and the formed quercetin was strong mutagenic. Bokkenheuser et al. (1987) reported that Bacteroides distasonis transformed rutin to quercetin. In addition, Booth et al. (1956) reported that phenolic acids were detected from the urine of the rat orally treated with rutin and quercetin. We also reported that quercetin was not detected from the urine after orally administered less than 100 mg/kg rutin on rats.

We isolated the bacteria, which transformed flavonoid glycosides to the phenolic acids via their aglycones, from human intestinal bacteria. These intestinal bacteria produce several kinds of glycosidases and the enzyme(s) fissuring B-ring of the flavonoid: α-rhamnosidase, exo β-glucosidase and β-glucosidase catalyzing rutin, hesperidin, poncirin and naringin to their aglycones, β-glucuronidase catalyzing baicalin to baicalein, C-glycosidase catalyzing puerarin to daidzein, and the enzyme fissuring B-ring of the aglycones to the phenolic acids. β-Glucosidase(s) were produced by Bacteroides spp., Streptococcus spp., Pepetotreptococcus spp, Eubactrium spp. and Fusobacterium spp. α-Rhamnosidase(s) were produced by Bacteroides spp., Pepetotreptococcus spp, Eubactrium spp. and Fusobacterium spp. β-Glucuronidase(s) were produced by Eubacterium spp., E. coli and Bacteroides spp. C-glucosidase was produced by Bacteroides spp. The enzyme(s) fissuring B-ring of the flavonoids were produced by Streptococcus spp., Bifidobacterium spp. and Bacteroides, spp. so on. The substrate specificity of these glycosidases produced by intestinal bacteria was different from those of mammalian tissue enzymes. Furthermore, the flavonoid B-ring fissuring enzyme was not detected in the liver of the rat. These results suggested that flavonoid glycosides could be metabolized to phenolic acids by intestinal bacteria in the intestine of human. The other evidence for the involvement of the intestinal microflora in the metabolism of flavonoid compounds in vivo has been presented by Griffiths (1964) and Das and Griffiths

(1968).

On the preventive effect of chronic illness of flavonoid glycosides, the reaction transforming them to their aglycones and phenolic acids by intestinal bacteria should be important. These aglycones and phenolic acids were effective on anti-platelet aggregation activities and cytotoxic activities. Among the metabolites of flavonoid glycosides by human intestinal bacteria, 3,4-dihydroxyphenylacetic acid and 4-hydroxylphenylacetic acid were effective on anti-platelet aggregation activity. The cytotoxicity of 2,4,6-trihydroxybenzaldehyde, quercetin, baicalein and ponciretin were effective for tumor cell lines. These metabolites could be transformed from rutin, catechin or naringin by human intestinal bacteria. These results suggested that oriental medicines and vegetables 'containing rutin, poncirin, catechin and quercitrin may have the preventive effect of chronic illness.

Finally, by human intestinal bacteria, flavonoid glycosides were metabolized to phenolic acids via their aglycones and these metabolisms play an important role on the bilological activity of flavonoid glycosides, which should be natural prodrugs.

ACKNOWLEDGEMENTS

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