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Intestinal and Hepatic Glucuronidation of Flavonoids

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Abstract: Flavonoids are polyphenolic phytochemicals present extensively in our daily diets, beverages, medicinal plants, and herbal remedies. The diverse biological effects of flavonoids have aroused great interest in scientists. In the past decade, various studies demonstrated extensive conjugate metabolisms, especially glucuronidation, of flavonoids in intestine and liver implying an important role of the glucuronidation in causing low oral bioavailability of flavonoids. The present article aims to review the up-to-date information on the studies of the first-pass metabolism, in particular glucuronidation, of flavonoids in the gastrointestinal tract and the liver, and also the isoformic enzymes involved in the metabolism and disposition of flavonoids. In addition, the role of efflux transporters, enterohepatic circulation, and enteric cycling in the disposition of flavonoid glucuronides has also been illustrated. Despite low oral bioavailabilities of the parent compounds, flavonoids and some of their bioactive phase II conjugates may accumulate adequate amount in the body to produce their pharmacological activities. Further investigation on the correlation between the accumulated concentrations of flavonoids and their pharmacological activities after their repeated oral administration is warranted.

Keywords: First pass metabolism; glucuronidation; flavonoids

Background

Flavonoids are polyphenolic phytochemicals present extensively in our daily diets, beverages, medicinal plants, and herbal remedies. As indicated in Figure 1, the basic chemical structures of flavonoids consist of three six-membered rings referred to as A, B, and C rings, respectively. The benzene ring A is condensed with heterocylipyrone ring C that is connected with ring B at the C2 or C3 position. According to the substitution and saturation of B ring, flavonoids are structurally further subdivided into flavones, flavonols, isoflavones, flavonols, and flavanones (Figure 2). Flavonoids occur in plants in forms of either aglycons or glycosides, which have one or more sugar molecules covalently bonded to the aglycon with the majority as *O*-glycosides.

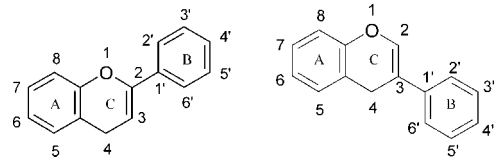

Figure 1. Basic structures of flavonoids.

The diverse biological effects of flavonoids have aroused great interests of scientists. The major reported pharmacological activities of flavonoids include antioxidative effects,[1–3]

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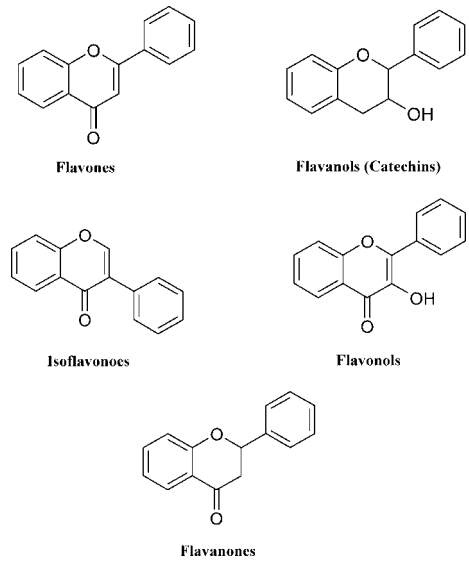

Figure 2. Subgroups of flavonoids.

protection against cardiovascular disease,[4,5] and anticancer effects, etc.[6–9] For a long time period, the beneficial effects of flavonoids have been ascribed to their antioxidant properties. However, more recent studies begin to emphasize the bioactivities of flavonoids through modulating several cellular signaling pathways such as phosphatidylinositol-3-kinase (PI 3-kinase), mitogen-activated protein kinase, protein kinase C (PKC), nuclear factor-κB (NF-κB) and activator protein-1 (AP-1).[10–16] Since these pathways regulate cell apoptosis, proliferation, survival, and inflammatory responses, the mechanisms of various biological effects elicited by flavonoids have been suggested to be associated with these signal transduction pathways. It is well known that biological effects require sufficient delivery of flavonoids from the site of administration, usually from gastrointestinal tract for dietary flavonoids, to the sites of target organs and receptors. However, whether flavonoids are orally bioavailable or not and in which form they exist the blood circulation after their oral intake have been the focus of various investigations in the past decade. Due to the complicity of absorption, metabolism and disposition nature of various flavonoids, a large number of data on the pharmacological, pharmacokinetic and biopharmaceutical studies of flavonoids have been generated, although some of the results are controversial, and that the overall effects in the body are contributed by which form, i.e., the parent flavonoid or its metabolite(s), is still largely unclear.

Due to the availability and utilization of more advanced and specific analytical techniques, such as application of LC-MS/MS, some ambiguous issues on ADME of flavonoids are being clarified. More recent studies demonstrated that phase II conjugates of aglycon such as glucuronides, sulfates, and methylated metabolites rather than flavonoid aglycons nor flavonoid glycosides mainly existed in blood circulation after ingestion of flavonoids.[8] It appears that oral bioavailabilities of both flavonoid aglycons and their glycoside may be too low (plasma levels at $\leq$nM level) to reach the effective levels ($\geq\mu$M level) at which various *in vitro* biological activities are reported. Since intestinal and hepatic first-pass effects are widely acknowledged to be responsible for low oral bioavailabilities of numeral therapeutic drugs and xenobiotics, it is expected that the intestine and the liver may also play an important role in contributing to low oral bioavailabilities of flavonoids. In the recent decade, extensive conjugate metabolisms, especially glucuronidation, of flavonoids in the intestine and the liver have been demonstrated in various studies. The present article aims to review the

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up-to-date information on the studies of the first-pass metabolism, in particular glucuronidation, of flavonoids in the gastrointestinal (GI) tract and the liver, and also the isoformic enzymes and transporters involved in the metabolism and disposition of flavonoids.

Intestinal Absorption of Flavonoids

In the studies conducted in early years, flavonoid glycosides were used to be considered absorbable. For example, quercetin glycosides were found to be absorbed by the small intestine in ileostomy patients and the glucoside of quercetin was more efficiently absorbed than its aglycon.[18] Further study from the same group found that quercetin glucoside was more rapidly and readily absorbed than quercetin rutinoside in human. Therefore, it was proposed that sugar moiety was a major determinant for the absorption of dietary flavonoid glycosides and the involvement of sodium-dependent glucose transporter (SGLT-1) was suggested to be responsible for the efficient absorption of quercetin glucoside.[19] In addition, studies from other research groups also claimed that quercetin glucosides were directly absorbed orally and present in human blood circulation.[11,12] Furthermore, the role of SGLT-1 in the transport of quercetin 4′-β-glucoside was confirmed in the later studies using both Caco-2 cell and SGLT-1-transfected CHO cell models.[22]

However, that flavonoids were absorbed in their aglycon forms was suggested subsequently. With the availability of *in vitro* absorption models such as Caco-2 cell monolayer model, flavonoid aglycons were found to be much more permeable across the human intestinal membrane than their glycosides in several studies. For example, in Caco-2 cell model, the apical to basalateral apparent permeability (P_{app}) of quercetin (5.8×10^{-6} cm/s) was significantly higher than those of quercetin 4′-*O*-glucoside (P_{app}: $<0.02 \times 10^{-6}$ cm/s) and quercetin 3,4′-O-diglucoside (P_{app}: 0.09×10^{-6} cm/s).[23] Similarly, studies from our group also demonstrated that both quercetin 3-*O*-glucoside and quercetin 3-*O*-galatoside were not readily absorbed in Caco-2 cell model and had a low P_{app} of 0.06×10^{-6} and 0.14×10^{-6} cm/s, respectively.[24] Moreover, the reported permeabilities of isoflavone aglycons including genistein, daidzein, glycitein, biochanin A, formononetin, and prunetin ranged from 1×10^{-6} cm/s to 5×10^{-5} cm/s, which were also much greater than those of their corresponding glycosides.[25]

Interestingly, more recent evidence further demonstrated that flavonoid aglycons could be directly absorbed whereas their glycosides need to be hydrolyzed to aglycons prior to the intestinal absorption. An *in vivo* study discovered that neither flavonoid glycosides nor aglycon appeared in systemic circulation after oral intake of quercetin glucoside enriched diet. Instead, there was extensive accumulation of phase II metabolites of quercetin in the plasma.[26] Since then, several similar observations were reported.[27,28] Therefore, flavonoid glycoside was proposed to be hydrolyzed to their corresponding aglycons before their intestinal absorption.[29] In 2000, solid evidence on the hydrolysis of flavonoid glycosides in human intestine was demonstrated,[30] and quercetin glucosides were found to be completely converted to their corresponding aglycon prior to their intestinal absorption in ileostomy patients. In addition, several studies also discovered the existence of β-glucosidase in the small intestinal epithelium in rats and human and their hydrolysis activities toward flavonoid glycosides.[31,32] In addition, lactase phlorizin hydrolase (LPH), which is usually bound to intestinal lumen for hydrolysis of lactose demonstrated, was also demonstrated to be able to hydrolyzed flavonoid

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glucosides.[29] The role of LPH was further confirmed in an absorption study using rat intestinal perfusion model by the same group.[33] The results demonstrated that *N*-(*n*-butyl)deoxygalactonojirimycin, an LPH inhibitor, significantly inhibited the hydrolysis of quercetin 3-*O*-glucoside in a dose-dependent manner. Recently, our research group also compared the stability of quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside in the *in vitro* intestinal villi preparations. Our results demonstrated that quercetin 3-*O*-glucoside was readily hydrolyzed to quercetin, whereas no significant hydrolysis of quercetin 3-*O*-galactoside was observed. Furthermore, such preference of hydrolysis resulted in a rapid and complete absorption of quercetin glucoside but no absorption of quercetin galactoside in the rats.[34] In addition to the intestine, hydrolysis of flavonoid glucosides such as quercetin 4′-glucoside and genistein 7-glucoside were also found in the oral cavity by both bacteria and shedded oral epithelial cells.[35,36] Since intestinal permeability of flavonoid glycosides are found to be poor and subject to enzymatic hydrolysis, the previous report on the existence of flavonoid glycoside in blood circulation might be due to inadequate analytical methods that could not distinguish differences in sugar moieties of the parent flavonoid glycosides from flavonoid glucuronides, the phase II conjugates of flavonoid.

From the studies published after year 2000, a number of studies demonstrated that the intestinal hydrolysis of flavonoid glycosides to their corresponding aglycons was the prerequisite for their intestinal absorption. Under the most circumstance, flavonoid glycosides only served as the precursor of their aglycon during intestinal absorption and after the absorption flavonoid conjugates such as glucuronides rather than flavonoid aglycons were predominant in the systemic circulation. Therefore, it is necessary to further investigate the metabolism and disposition of flavonoid aglycons and also re-evaluate the pharmacological effects of the parent flavonoid aglycons and glycosides *in vivo*, especially in different tissue targets in the body including the gastrointestinal tract and colons. On the other hand, because of its physiological location, the intestine is the first organ that renders first-pass metabolism of flavonoid. Although it is generally accepted that the expressions of metabolizing enzymes in the intestine are much lower than those in the liver, the role of intestine in contributing to low oral bioavailabilities of flavonoids should not be underestimated.

First-Pass Glucuronidation of Flavonoids

Glucuronidation in the Intestine. Although flavonoids aglycons were supposed to be rapidly absorbed after oral ingestion, their plasma concentrations are found to be very low whereas the phase II metabolites such as glucuronides, sulfates, and methylated conjugates seem to be predominant in blood circulation. Therefore, liver and intestine are thought to be responsible for the extensive first-pass metabolism of flavonoids, and glucuronidation mediated by various UDP-glucuronosyltransferases (UGTs) is suggested to be one of the most important metabolic pathways of flavonoids in both liver and intestine. Quite a number of studies in human have demonstrated the contribution of UGTs to the first-pass glucuronidation of flavonoids. For instance, after intake of kaempferol in human, 3-*O*-glucuronide conjugate of kaempferol was found to be the predominant form in plasma.[25] Epicatechin glucuronide was detected as the main metabolite in human plasma after ingestion of flavonoid procyanidins and flavan-3-ols enriched cocoa milk drinks.[38] The conjugate metabolites, namely epicatechin-3′-*O*-glucuronide, 4′-*O*-methyl-epicatechin-3′-*O*-glucuronide, and 4′-*O*-methyl-epicatechin-5 or 7-*O*-glucuronide, were identified in human after intake of epicatechin.[39] It was also found that quercetin-3-*O*-glucuronide together with quercetin-3′-*O*-sulfate and isorhamnetin-3-*O*-glucuronide were dominant in human plasma after oral administration of quercetin.[40] The intestinal absorption of quercetin was suggested to be rapid, and the human plasma C_{max} of quercetin-3′-*O*-sulfate, quercetin-3-*O*-glucuronide,

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isorhamnetin-3-*O*-glucuronide and quercetin diglucuronide reached at 0.6–0.8 h after ingestion of fried onions.[41] After oral intake of soy meal containing genistein, 7-*O*-glucuronide (accounted for about 90% of all metabolites) and the 4′-*O*-sulfate (less than 10% of all metabolites) conjugate of genistein were demonstrated to be the major metabolites found in the blood circulation in human.[42] In a study characterizing the metabolism of isoflavones from soy milk in women, daidzein and genistein glucuronides determined in plasma were accounted for 62% and 53% of total daidzein and genistein ingested, respectively, and about 73% and 71% of total daidzein and genistein were excreted as their corresponding glucuronides in urine.[43]

In addition to human studies, extensive studies on glucuronidation of flavonoids in laboratory animal were reported. Quercetin-3-*O*-glucuronide and quercetin-4′-*O*-glucuronide readily appeared in blood circulation in rats after oral administration of quercetin.[44] Most of baicalein was transformed to its glucuronide in rat after oral administration.[45] After oral ingestion, hesperidin was primarily converted to hesperetin-3′-*O*-glucuronide and hesperetin-7-*O*-glucuronide with negligible level of sulfate in rat plasma.[46] Wogonin 7-*O*-glucuronide was also found as the major metabolite of wogonin in rat plasma after oral intake of wogonin.[47]

Based on the observations from both human and animal studies, several groups initiated the mechanistic studies on the intestinal absorption and disposition of various flavonoids. *In situ* rat intestinal perfusion approach was one of the most popular methods used to provide more information on the intestinal glucuronidation of flavonoids. The absorption of a series of flavonoid aglycons, including quercetin, kaempferol, luteolin, naringenin, and their respective glycosides, was conducted using isolated rat small intestine perfusion model, and the absorbed flavonoids at the serosal side of the intestine were found to be converted to their corresponding glucuronides.[48] In the perfusion study of quercetin and quercetin 3-*O*-glucose in the rat intestine, only conjugated forms (glucuronide and/or sulfate) of quercetin were detected in the mesentericvein blood implying the importance of first-pass metabolism of intestine.[49] In a perfused rat intestinal model, genistein was subject to extensive sulfation and glucuronidation.[50] Our recent studies discovered that over 90% of baicalein was rapidly metabolized to baicalein-7-*O*-glucuronide and then transported to the mesenteric blood when perfusing baicalein through rat jejunum, indicating that extensive glucuronidation may occur in the small intestine.[51]

Glucuronidation in the Liver. Comparing with the investigation of intestinal first-pass metabolism of flavonoids, studies on the hepatic first-pass glucuronidation of flavonoids were limited. By comparing the concentrations of quercetin after its intravenous and intra portal administration to rats, the hepatic extraction ratio was determined to be about 52.6%, and extensive hepatic glucuronidation of quercetin was suggested due to the finding of glucuronides as the major metabolites of quercetin.[52] Although limited studies were designed to specifically evaluate the contribution of glucuronidation of flavonoid in the liver, its importance should be aware of due to high content of UGTs present in the liver.

Enzymes Mediating Glucuronidation. In general, glucuronidation of flavonoids is considered as a rapid metabolic pathway. The rate of glucuronidation of galangin was reported to be 30-fold higher than that of the P450-mediated hydroxylation of galangin.[53] Comparing with typical glucuronidation substrates such as acetaminophen, morphine and androstanediol, formation rate of baicalein 7-*O*-glucuronide by intestine and liver microsomes was reported to be one and two times faster in both human and rats.[54] The intestinal

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Table 1. Summary of the Literature Reports on the in Vitro Glucuronidation of Flavonoids[a]

		metabolites	V_{max} (nmol/min/mg)	K_m (μM)	Cl_{int} (μL/min/mg)
baicalein[54]	HJM		41.89 ± 1.92	93.93 ± 7.71	446
	HLM		14.38 ± 0.57	23.25 ± 2.58	618
	RJM		12.50 ± 0.74	41.92 ± 5.80	298
	RLM		33.65 ± 3.03	77.12 ± 13.23	436
galangin[53]	HLM	M 1	1.52 ± 0.25	3.6 ± 0.7	422
		M2	34.3 ± 2.1	221 ± 31	155
quercetin	human liver extract [55]	quercetin glucuronides (Total)	$(12 \pm 0.6) \times 10^{-3}$	1.7 ± 0.3	7.1
		quercetin 7-*O*-glucuronide	$(7.6 \pm 0.4) \times 10^{-3}$	6.5 ± 0.9	1.2
		quercetin 3′-*O*-glucuronide	$(1.2 \pm 0.1) \times 10^{-3}$	0.6 ± 0.2	2
		quercetin 4′-*O*-glucuronide	$(5.2 \pm 0.3) \times 10^{-3}$	0.8 ± 0.2	6.5
	RLM [56]		60 ± 0.21	24 ± 0.05	2500
epicatechin[57]	RLM		0.418	15.6	27
isorhamnetin[56]	RLM		48 ± 0.02	148 ± 0.09	324
keampferol[56]	RLM		34 ± 0.02	110 ± 0.03	309
genistein[58, 59]	RDM		1.4	4.0	35
	RJM		3.2	6.6	485
	RIM		1.4	8.3	169
	RCM		0.4	3.2	125
	RLM		3.713	23.6	157
daidzein[58,59]	RDM		1.1	2.0	550
	RJM		0.6	2.4	250
	RIM		0.6	5.5	109
	RCM		0.8	4.3	186
	RLM		1.190	1.119	1063
formononetin[58,59]	RDM		0.8	2.8	286
	RJM		0.5	7.3	68
	RIM		0.6	9.0	67
	RCM		0.5	8.4	60
	RLM		1.384	2.225	622
glycitein[58,59]	RDM		2.9	0.7	4143
	RJM		1.9	2.7	703
	RIM		1.4	2.1	667
	RCM		2.5	0.6	4167
	RLM		3.720	1.034	3598
biochanin A[58,59]	RDM		1.4	5.3	264
	RJM		1.8	6.7	269
	RIM		0.4	7.1	56
	RCM		0.3	13	23
	RLM		1.250	0.196	6378
prunetin[58,59]	RDM		0.8	1.3	615
	RJM		1.6	2.1	762
	RIM		0.8	1.8	444
	RCM		0.1	0.7	143
	RLM		0.343	0.834	0.411

[a] Key: RDM, rat duodenum microsome; RJM, rat jejunum microsome; RIM, rat ileum microsome; RCM, rat colon microsome; RLM, rat liver microsome; HJM, human jejunum microsome; HLM, human liver microsome.

and hepatic glucuronidation activities toward various flavonoids have been investigated using *in vitro* microsomal studies. As summarized in Table 1, from the *in vitro* studies by incubating various flavonoids with variety of intestinal and liver microsomal preparations, several important meta-

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bolic parameters of glucuronidation have been reported. Although significant differences in metabolic rates of individual flavonoids were observed, the most of the flavonoids tested exhibited relatively high rates as indicated by a large intrinsic clearance (Cl_{int}) value, suggesting their extensive glucuronidation in both the liver and the intestine.

Glucuronidation is a process of metabolism catalyzed by UDP-glucuronosyltransferases (UGTs). To date, more than 20 UGT isoforms have been identified from the endoplasmic reticulum of different tissues responding for catalyzing the biotransformation of hydrophobic substrates to hydrophilic glucuronides.[60] Liver was found to contain most of UGT isoforms and UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 are though to be the most important for the drug glucuronidation in the liver.[61] Studies on the extrahepatic distribution discovered that intestine also contains a large number of UGTs. For example, UGT 1A1, 1A3, 1A4, 1A6, 2B15, and 2B4 were also revealed in the intestine, whereas UGT 1A7, 1A8, and 1A10 were found only expressed in the intestine but not in the liver. By using recombinant human UGTs, specific isoforms of UGT have been identified for the glucuronidation of various flavonoids and such information has been accumulated continuously as listed in Table 2. UGT 1A3 was reported to mediate the glucuronidation of flavonoids including naringenin, apigenin, galangin, fisetin, 7-hydroxyflavone, genestein and quercetin.[63] Kaempferol and quercetin were demonstrated to be the substrate of UGT 1A9.[64] UGT 1A9, 1A1, and 2B15 were reported to catalyze the glucuronidation of galangin.[53] UGT 1A1, 1A8, and 1A9 were involved in the glucuronidation of luteolin and quercetin.[65] Moreover, UGT 1A7 displayed differential activities toward flavonoids such as chrysin, apigenin, galangin, fisetin kaempferol, morin, quercetin, etc.[66] UGT 1A10 mainly found in gastrointestinal tract catalyzed the glucuronidation of a number of flavonoids, including apigenin, chrysin, luteilin morin, daidzine genistein naringenin.[67] Our recent study on baicalein was found that it is the substrate of various UGT isozymes including UGT 1A1, 1A3, 1A8, 1A7, 1A9, and 2B15.[54]

In addition to the substrates of various UGTs, some flavonoids were also identified to be the inducers of UGTs. Chrysin and quercetin were reported to induce UGT1A in Caco-2 cells,[68–70] which might be due to the interaction between aryl hydrocarbon receptor and flavonoids.[72] The induction of UGT 1A1 by flavonoids, such as 5,7-dihydroxyflavones luteolin, apigenin, chrysin and baicalein, was demonstrated to be associated with the transactivation of a distal enhancer module, which contained several nuclear receptors (constitutive androstane receptor and pregnane X receptor) and transcription factor (aryl hydrocarbon receptor).[73,74] Such induction of UGTs by flavonoids may enhance the detoxification of exogenic toxins such as carcinogens, mutagens and pesticides, and also accelerate the metabolism of the therapeutic drugs that are substrates of UGTs.

Potential Biological Effects of Metabolites of Flavonoids. Since glucuronides are found to be one of the predominant forms of flavonoids in blood circulation, investigations of their biological activities become the subsequent interests for scientists. A number of studies already demonstrated that the glucuronides of flavonoids also possess biological activities. Quercetin-3-*O*-glucuronide possesses angiogenesis activity through inhibiting extracellular signal-regulated kinases 1/2 phosphorylation elicited by

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Table 2. Glucuronidation of Flavonoids by Various UGTs[a]

		identified human UGT	metabolic formation rate measured at single concentration (nmol/min/mg)	V_{max} (nmol/min/mg)	K_m (μM)
baicalein[54, 67]		1A1	N/A	N/A	N/A
		1A3	–	1.82 ±0.04	6.45 ±0.61
		1A7	–	0.40 ±0.03	9.96 ±2.38
		1A8	–	3.17 ±0.27	19.46±4.80
		1A9	–	6.81 ±0.22	10.71±1.22
		1A10	N/A	N/A	N/A
		2B15	–	0.74 ±0.03	7.09 ±1.22
galangin[53,63,66]	**M1**	1A9	–	0.721	1.1
	M2	1A9	–	3.59	31.8
		1A1	–	0.388	6.3
		2B15	–	0.538	15.7
		1A3	0.610	N/A	N/A
		1A7	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
luteolin[65,67]		1A1	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
		1A9	N/A	N/A	N/A
		1A10	10.3,7.3	N/A	N/A
quercetin[63–67]		1A1	N/A	N/A	N/A
		1A3	0.550	N/A	N/A
		1A7	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
		1A9	N/A	N/A	N/A
		1A10	N/A	N/A	N/A
kaempferol[64,66,67]		1A1	N/A	N/A	N/A
		1A7	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
		1A9	N/A	N/A	N/A
		1A10	5.4,3.9	N/A	N/A
naringenin[63]		1A3	0.612 ±0.059	N/A	N/A
apigenin[63,66,67,71]		1A1	N/A	N/A	N/A
		1A3	0.508 ±0.017	N/A	N/A
		1A7	N/A	N/A	N/A
		1A8	0.574	N/A	N/A
		1A9	N/A	N/A	N/A
		1A10	8.7,6.1	N/A	N/A
fisetin[63,60]		1A1	N/A	N/A	N/A
		1A3	0.276	N/A	N/A
		1A7	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
		1A9	N/A	N/A	N/A
		1A10	N/A	N/A	N/A
7-hydroxyflavone[63]		1A3	0.604	N/A	N/A
genestein[63,67]		1A3	0.131	N/A	N/A
		1A10	10.2,8.9	N/A	N/A
6-hydroxyflavone[67]		1A10	4.4,8.3	N/A	N/A
3′-hydroxyflavone[67]		1A10	3.2,4.4	N/A	N/A
chrysin[66,67,70]		1A1	–	0.360	0.35
		1A6	–	0.157	12.8
		1A7	N/A	N/A	N/A
		1A8	N/A	N/A	N/A
		1A9	–	1.212	1.7
		1A10	7.3,5.7	N/A	N/A

Table 2. Continued

	identified human UGT	metabolic formation rate measured at single concentration (nmol/min/mg)	V_{max} (nmol/min/mg)	K_m (μM)
morin[66,67]	1A1	N/A	N/A	N/A
	1A7	N/A	N/A	N/A
	1A8	N/A	N/A	N/A
	1A9	N/A	N/A	N/A
	1A10	1.0,0.2	N/A	N/A
myricetin[67]	1A10	N/A	N/A	N/A
biochanin A[66,67]	1A1	N/A	N/A	N/A
	1A7	N/A	N/A	N/A
	1A8	N/A	N/A	N/A
	1A9	N/A	N/A	N/A
	1A10	14.3,13.6	N/A	N/A
daidzein[66,67]	1A1	N/A	N/A	N/A
	1A7	N/A	N/A	N/A
	1A8	N/A	N/A	N/A
	1A9	N/A	N/A	N/A
	1A10	7.9,7.0	N/A	N/A
formononetin[66,67]	1A1	N/A	N/A	N/A
	1A7	N/A	N/A	N/A
	1A8	N/A	N/A	N/A
	1A9	N/A	N/A	N/A
	1A10	9.8,9.8	N/A	N/A
2-hydroxyflavanone[67]	1A10	1.0,0.3	N/A	N/A
4-hydroxyflavanone [67]	1A10	8.9,6.9	N/A	N/A
6-hydroxyflavanone[67]	1A10	15.7,12.4	N/A	N/A
hesperetin[67]	1A10	11.9,12.9	N/A	N/A
hesperidin[67]	1A10	1.1,0.1	N/A	N/A
naringenin[66,67,71]	1A1	N/A	N/A	N/A
	1A7	N/A	N/A	N/A
	1A8	1.534	N/A	N/A
	1A9	N/A	N/A	N/A
	1A10	11.2,8.3	N/A	N/A
pinostrobin[67]	1A10	0.5,0.1	N/A	N/A

[a] Key: N/A, not available; –, not applicable.

vascular endothelial growth factor.[75] Quercetin glucuronides could also inhibit the growth of human lung cancer cell line NCI-H209 through G2/M arrest and induce apoptosis via caspase-3 cascade.[76] (−)-Epicatechin 5-*O*-glucuronide was reported to possess a high potency of antioxidative activities like its parent compound.[77] Both quercetin and quercetin 3-*O*-glucuronide were found to inhibit peroxynitrite-induced consumption of lipophilic antioxidants in human plasma low-density lipoprotein.[78]

More interestingly, it was reported that the position at which glucuronidation takes place might influence the biological activities of flavonoids. For instance, the chemical redox potential of the B-ring is lower than that of the A-ring; thus, the antioxidative effect of flavonoid aglycons with –OH at B ring would be more potent that that in A ring. Therefore, glucuronidation at the B-ring, such as 3′- or 4′-*O*-glucuronide much more profoundly impairs the antioxidative effect of

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the corresponding aglycons.[79] Another study also demonstrated that the potency of inhibition of xanthine oxidase by quercetin glucuronides depended on the position of -*O*-glucuronidation with the order of 4′-*O*- > 3′-*O*- > 7-*O*- > 3-*O*-glucuronide of quercetin.[55] However, most of the activity studies on metabolites of flavonoids were conducted in the *in vitro* system, the actual *in vivo* beneficial effects of the phase II metabolites of flavonoids, especially flavonoid glucuronides, and to what extent the parent flavonoids and their metabolites contribute to the overall effect in the body are largely unknown and warranted for further investigations.

On the other hand, it is worth to be noted that some metabolic reactions of flavonoids probably generate potential toxic intermediates (metabolites), although limited information on the toxic metabolisms of flavonoids is available. For instances, it has been demonstrated that in both human intestinal Caco-2 cells and hepatic Hep G2 cells, quercetin was oxidized by peroxidases to quinone/quinine methide intermediates, which then reacted with macromolecules to form protein and/or DNA adducts and might lead to toxicity.[80,81] Another study reported that incubation of glabridin with CYP3A4 in the presence of NADPH resulted in the generation of the reactive metabolites, which then covalently bound to P-450 monooxygenases and thus inactivated the enzyme activities.[82]

Position Preferences in the Glucuronidation of Flavonoids. As the positions of glucuronidation can significantly influence the biological activities of flavonoids, studies on the regioselectivity of glucuronidation of flavonoids by UGTs become essential and various research groups have conducted a series of investigations in this area. Due to the complicated structural nature of multihydroxylated flavonoids, reactivity of the flavonoids can be influenced by steric hindrance, intramolecular hydrogen bond, and electron-donating and -withdrawing effects on different rings by substituted groups. Several research groups developed systematic approaches to study the relationships between the flavonoid structures and their glucuronidation activities. Glucuronidation of luteolin and quercetin was preferentially occurred at 7-, 3-, 3′-, or 4′-hydroxyl groups in human liver and intestine microsome preparations.[65] K_m for the formation of quercetin glucuronides by human liver homogenates followed the order of 4′- > 3′- > 7- > 3-hydroxyl groups while hydroxyl group on 5- position did not seem to be a site for glucuronidation.[55] The reported study on the structure–reactivity relationship of glucuronidation by UGT 1A10 with various structural diverse flavonoids suggested that glucuronidation at A-ring occur preferentially on the positions at 6-OH of flavones and 7-OH of isoflavones, while at B-ring conjugation at C4′-OH seemed to be more favorable than the hydroxyl groups at other places. Similarly, hydroxyl group at 5 position was reported as a nonreactive site for glucuronidation.[67] To evaluate the regioselectivity of glucuronidation of flavonoids in human jejunum S9, a systematic study on monohydroxyflavones (HF) was conducted by our group using seven HFs covering all the possible positions of the monohydroxyl substitution.[83] The results demonstrated that glucuronidation activity of 6- and 3′-HF was much greater than that of 3-, 4′-, 7-, and 2′-HF with 5-HF as the lowest. It is well established that the UGT mediated glucuronidation occurred via S_N2 reaction with hydroxyl group acting as a nucleophile to attack C-1 of pyranose acid ring of UDPGA.[60,84] Thus, the nucleophilicity, steric property of the hydroxyl group of the nucleophile may affect the overall rate of this S_N2 reaction. The electron density on the oxygen at 5-OH, 7-OH, 2′-OH, and 4′-OH but not at 3-OH, 6-OH, and 3′-OH could be delocalized into the conjugated system of flavone, resulting in reduction of nucleophilicity of hydroxyl group and would thus reduce its overall glucuronidation activity.[83] The reactivity of 5-HF was found to be the lowest and as suggested to be due to both the adjacent carbonyl group at the C-4 as steric hindrance and unfavorable nucleophilicity of 5-OH. As limited knowledge on the 3D conformation of UGTs and UGT-substrates are available so far, further studies are warranted to investigate the role of the UGTs in the position preference of glucuronidation of flavonoids.

Role of Efflux Transporters, Enterohepatic Circulation, and Enteric Cycling in the Disposition of Flavonoid Glucuronides. In addition to the first-pass metabolism, the role of efflux transporters in contributing to the low oral bioavailability of drugs has been well acknowledged. Among the efflux transporters located in the intestine and the liver, ATP-binding cassette (ABC) superfamily, including several members such as P-glycoprotein (P-gp), multidrug resistance associated proteins (MRPs)m and breast cancer resistance protein (BCRP/MXR), is well investigated for their roles in intestinal and hepatic disposition of drugs. Increasing evidences have demonstrated the interactions of flavonoid aglycons and glycosides with ABC transporters. For example, ginkgo flavonols, quercetin, kaempferol, and isorhamnetin were demonstrated to be the substrates of P-gp in Bacap37/

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MDR1 transfected cell model.[85] Biochanin A and silymarin were found to increase the intestinal absorption of P-gp substrates digoxin and vinblastine.[86] MRP2 was reported to play important roles in reducing oral bioavailabilities of genistein-7-glucoside, epicatechin, quercetin-4′-glucoside.[87–89] Apigenin, biochanin A, chrysin, genistein, kaempferol, hesperetin, naringenin, and silymarin increased the accumulation and cytotoxicity mitoxantrone of in MCF-7 MX100 cells overexpressing BCRP due to their competitive interaction with the same efflux transporter.[90] Genistein, naringenin, acacetin, and kaempferol potentiated the cytotoxicity of SN-38 and mitoxantrone in BCRP-transduced K562 (K562/BCRP) cells.[91] The interaction of flavonoid alycones and glycosides with ABC transporters may greatly influence their absorption, disposition and excretion in the body, and also alter pharmacokinetic profiles of the concurrently administrated drugs acting on the same transporters, thereby probably leading to significant impacts in the therapeutic outcomes.

The interaction of flavonoid glucuronides and efflux transporters in the intestine and the liver has also been investigated. *In vitro* absorption model such as Caco-2 cell monolayer model has been proven to express a series of efflux transporters such as MDR1 (P-gp),[92] MRPs,[93] and MXR (BCRP)[94] as well as various metabolizing enzymes,[95] and thus became popular in the investigation of the mechanism of drug absorption.[96] Chrysin glucuronides and sulfates formed in the Caco-2 cells were actively transported to both apical and basolateral sides of Caco-2 cell monolayer model, and their active transport were effectively modulated by MRP inhibitors.[97] It was also speculated that the low oral bioavailability of chrysin found in human was mainly due to extensive phase II metabolism and efflux of the generated metabolites back into the intestine.[98] Similarly, glucuronide and sulfate conjugates of apigenin, baicaleinn and various isoflavonoids including genistein, formononetin, daidzein were also formed in Caco-2 cells followed by active efflux in both apical and basolateral sites. The transport of these flavonoids in the presence of MRP inhibitors such as MK 571 and LTC4 significantly decreased the efflux of glucuronide and sulfate conjugates. [25,99,100] MRPs, also named as "conjugate transporters", usually mediate the transport of organic anions and phase II metabolites including glutathione, glucuronidated and sulfated conjugates,[101] and thus MRPs may also be important transporters involved in the disposition of glucuronide of flavonoids. The members of the MRP family such as MRP2 and MRP3 were reported to locate at the apical and basolateral sides of the gut respectively.[101,102] The apical and basolateral efflux of glucuronides of flavonoids may be mediated by the MRPs resides at the respective sides of Caco-2 cells. Unfortunately, the commonly used nonselective MRP inhibitors, such as MK571,

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LTC4, and estradiol-17-β-glucuronide, could not distinguish different members in the MRP family. The transfected transporter systems were thus utilized to provide unambiguous evidence on which transporters mediate efflux of glucuronides of flavonoids. The transport study of baicalein 7-*O*-glucuronide was performed using membrane vesicles overexpressing MRP1, -2, and -3, BCRP, and K562MDR cells overexpressing P-gp. The results indicated that baicalein 7-*O*-glucuronide was preferentially transported by MRP1, -2, -3 and BCRP but not P-gp.[100] Consistently, the intestinal secretion of baicalein 7-*O*-glucuronide in Eisai hyperbilirubinemic rats, the MRP2 hereditarily defective animal model, was significantly lower than that in normal SD rats, demonstrating the role of MRP 2 in excreting baicalein 7-*O*-glucuronide to intestinal lumen.[103] The role of BCRP in the intestinal efflux of flavonoids glucuronides should also be acknowledged. By using *in situ* intestinal perfusion model, it was found that quercetin glucuronide was efficiently transported to the gut lumen by BCRP rather than MRP2, which was further confirmed in BCRP and MRP2 transfected MDCKII cell model.[104] Furthermore, it was found that through aryl hydrocarbon receptor-dependent pathway chrysin and flavone up regulated the mRNA level of BCRP while epicatechin suppressed the BCRP expression.[105]

A series of mechanistic studies suggested that the coupling of UGT-efflux transporters was the critical process entailing the enteric recycling of flavonoids.[50,99,106] As illustrated in Figure 3, the flavonoid aglycons converted to glucuronides by UGT would be excreted out of the enterocytes to both the serosal and luminal sides of intestinal epithelium via ABC transporters or other efflux transporters. The glucuronides transported to the mesenteric blood would enter the systemic circulation after hepatic extraction. On the other hand, appreciable amounts of glucuronides were secreted to the gut lumen, where they may not be readily and directly reabsorbed due to their high polarity.[54,106,107] Although poor absorption of glucuronides of flavonoids has been demonstrated using rat intestinal perfusion model and Caco-2 cell model,[54,100] as reported for baicalein 7-*O*-glucuronide,[108] the reabsorption of flavonoid aglycon in the intestinal might still occur after it was released from the corresponding glucuronide via hydrolysis by intestinal glucuronidase or intestinal microflora. Another impact of the coupling of UGT-efflux transporters is that the efflux transporter may influence the phase II metabolism. It was found that during the absorption of high concentration apigenin in Caco-2 cells, the excretion rates of apigenin sulfate and glucuronide were much slower than its formation rates of conjugations,[109] indicating that efflux transport may be the rate-limiting step for the disposition of apigenin. It is suggested that the less efficient transport of flavonoid conjugate would probably result in a

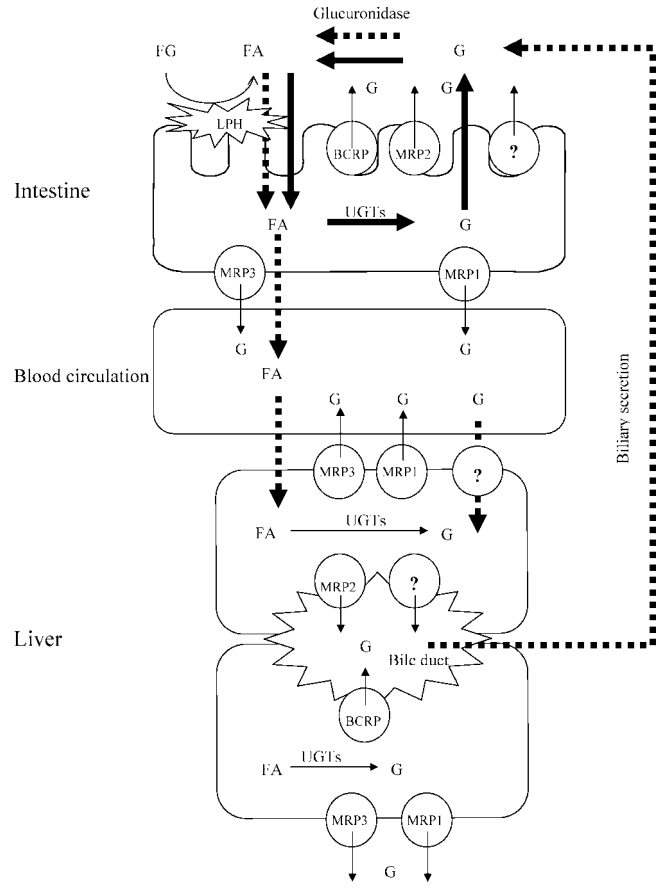

Figure 3. Diagram of enterohepatic circulation (dashed line) and enteric recycling (solid line) of flavonoids: FG, flavonoid glycosides; FA, flavonoid aglycons; G, flavonoid glucuronides; LPH, lactase phlorizin hydrolase; ?, other possible transporters. All the indicated transporters refer to the transport of G.

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product inhibition of phase II metabolism. However, the *in vivo* significance of this hypothesis needs further verification.

In addition to efflux to serosal side and luminal sides of intestinal epithelium, biliary excretion has also been reported as an important disposition pathway of flavonoid glucuronides (Figure 3). Approximately 40% of oral dose of baicalein was recovered in rat bile as conjugates including baicalein 6-*O*-glucuronide, 6-*O*-methylbaicalein 7-*O*-glucuronide, baicalein 7-*O*-glucuronide, 6-*O*-glucopyranuronosylbaicalein 7-*O*-sulfate, and baicalein 6,7-di-*O*-glucuronide.[110] High concentrations of conjugated metabolites of quercetin were observed in the bile and about 35% of quercetin metabolites, mainly glucuronide and sulfate conjugates, was found to be excreted via bile after intravenous administration of quercetin.[52,111] Appreciable amounts of biliary secretion of glucuronides and/or sulfates of biochanin A, formononetin, apigenin, and genistein have also been reported in rats.[106,107] High concentration of chrysin conjugates, mainly chrysin glucuronide, was secreted via bile in rats after oral administration of chrysin.[98] Since the efflux transporters such as P-gp and MRPs expressed in the intestine are also found in the liver, the role of these transporters in the hepatic disposition was also investigated. The efflux transport of quercetin conjugates in HepG2 cells and human liver cancer cells was effectively inhibited by MK 571 but was not altered by verapamil, a P-gp inhibitor.[112] The significant biliary excretion of glucuronides of flavonoids implies the possible occurring of enterohepatic circulation. Enterohepatic circulation of quercetin was investigated in linked-rat model.[52] To establish this model, the donor rats was administrated intravenously with quercetin and their bile was guided to the duodenum of the recipient rats. The result showed that no significant absorption of quercetin and its conjugates occurred in the recipient rats indicating the absence of enterohepatic circulation for quercetin. However enterohepatic circulation was observed for baicalein 7-*O*-glucuronide in the similar linked-rat model,[113] which might be due to the intestinal reabsorption of baicalein aglycon released from hydrolysis of baicalein 7-*O*-glucuronide mediated by intestinal microflora.[108]

How Effective Would Flavonoids Be *in Vivo*?

It is a general impression that the *in vivo* concentrations of the parent flavonoids may not be high enough to reach the effective levels as demonstrated in various biological activities tested *in vitro*. However, the UGT-efflux transporter coupling may enable potential enterohepatic circulation and enteric recycling that are generally believed to prolong the elimination of flavonoids. In addition, taking the ingestion habits into consideration, repeated daily intake might also result in accumulation of high concentrations of the parent flavonoids in the body. Therefore, the accumulative amounts of flavonoids *in vivo* may be adequate to exert their beneficial activities.

Concluding Remarks

To date, it is well recognized that orally administered flavonoids would undergo extensive presystemic first-pass metabolism. Substantial intestinal and hepatic glucuronidation have been found in a number of structurally diverse flavonoids. Various UGT isozymes, expressed in the liver and the intestine, have been identified to catalyze the glucuronidation of flavonoids. Although the oral bioavailabilities of flavonoids are low, the concentrations of their phase II metabolites, in particular glucuronides in the body are still appreciable and some of these metabolites are also demonstrated to be bioactive. UGT isoforms exhibited their selectivity toward different flavonoids and position preferences in flavonoid glucuronidations. UGTs-efflux transporters coupling in both the intestine and the liver may result in enterohepatic circulation and enteric recycling, leading to longer lasting and thus accumulation of flavonoids in the body, in particular with repeated intake. Therefore, despite low oral bioavailabilities, flavonoids and some of their bioactive phase II conjugates may accumulate adequate amount in the body to produce their pharmacological activities. Further investigation on the correlation between activities and accumulated concentrations of flavonoids and their metabolites after the repeated oral administration is warranted.

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