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Bioavailability and Metabolic Pharmacokinetics of Rutin and Quercetin in Rats

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ABSTRACT

Rutin and quercetin, a flavone glycoside and its aglycone, are the flavonoids most widely and abundantly present in herbs and plant foods. The aim of this study was to characterize and compare the bioavailability and metabolic pharmacokinetics of rutin and quercetin in rats. Quercetin was administered intravenously (33 μmol/kg) and orally (165 μmol/kg), while rutin was administered only orally (328 μmol/kg) to rats. Blood samples were withdrawn via cardiopuncture at specific time points. An HPLC method was used to determine the concentrations of quercetin before and after hydrolysis using β-glucuronidase and sulfatase, respectively. The pharmacokinetic parameters were calculated using noncompartment model of WINNONLIN. The results showed that after intravenous administration of quercetin, 93.8% of the dose was circulating as its sulfates and glucuronides. After oral administration of quercetin, the glucuronides and sulfates of quercetin were exclusively present in the bloodstream, whereas the parent form of quercetin was not detected. The oral absorption rate of quercetin was 53% compared to intravenous administration after dose correction. When rutin was orally administered, sulfates and glucuronides of quercetin were exclusively present in the bloodstream, whereas rutin and quercetin were not detected. Quercetin showed higher oral absorption rate than rutin. In conclusion, quercetin sulfates and quercetin glucuronides represent the major metabolites either rutin or quercetin was administered to rats.

Key words: rutin, quercetin, conjugated metabolites, sulfates, glucuronides, pharmacokinetics

INTRODUCTION

Flavonoids are polyphenolic compounds containing a unique C_6-C_3-C_6 structure (diphenyl propane structure). More than 4,000 varieties of flavonoids have been found in herbs, vegetables, fruits and beverages[(1)]. Recently, flavonoids have attracted increasing interest because of their various beneficial biological activities to human health[(2)] although some epidemiological studies reported a negative correlation between flavonoid intake and the occurrence of cardiovascular diseases and possibly cancer[(3,4,5)].

Rutin and quercetin (structures shown in Figure 1), a flavone glycoside and its aglycone, are the flavonoids most widely and abundantly present in herbs and plant foods. Quercetin has been reported to exert numerous pharmacological activities, such as free radical scavenging[(6)], TNF-alpha inhibition[(7)], and anticarcinogenic effects[(8,9)]. In addition, quercetin markedly enhanced the absorption of digoxin[(10)], but profoundly decreased the oral bioavailability of cyclosporine[(11)]. Quercetin is mainly present in nature as its glycosides in which one or more sugar groups are bound to phenolic groups by glycosidic linkage. Rutin, a very common quercetin glycoside, was recognized to decrease the permeability of capillaries since 1946[(12)]. It has been reported to scavenge free radical, to lower hepatic and blood cholesterol levels, and showed antiplatelet activity[(13,14)].

In recent years, the biological fates of flavonoid glycosides are gradually understood that they are generally hydrolyzed by intestinal and/or bacterial enzymes to corresponding aglycones which are absorbable by the gut[(15)]. Rutin was found being hydrolyzed to quercetin in the intestine, then absorbed as quercetin and presented as conjugated metabolites of quercetin in the circulation[(16)]. Many studies on the biological fates of quercetin and rutin were reported, including metabolism of quercetin[(17,18)] and pharmacokinetics of quercetin and rutin[(19,20)]. However, detailed information concerning the individual pharmacokinetics of quercetin glucuronides and quercetin sulfates still remained limited. Therefore, in this study we attempted to compare the bioactivities and metabolic pharmacokinetics of oral quercetin with rutin in rats.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

Rutin hydrate (purity 95%), quercetin dihydrate (purity 95%), glycofurol, acetic acid, β-glucuronidase (type B-1 from bovine liver) and sulfatase (type H-1 from *Helix*

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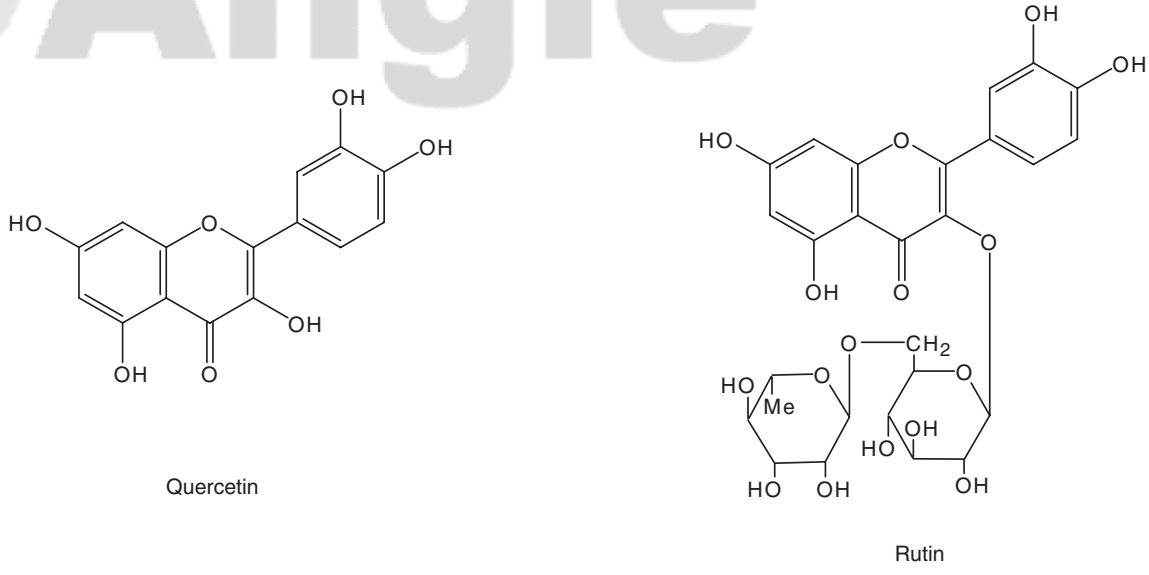

Figure 1. Chemical structures of quercetin and rutin.

pomatia) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, ethyl acetate and methyl alcohol obtained from Mallinckrodt Baker, Inc. were of LC grade (Phillipsburg, USA). Polyethylenglycol 400, potassium dihydrogen phosphate were products of Merck (Darmstadt, Germany). L (+)-Ascorbic acid was purchased from RdH Laborchemikalien GmbH & Co. KG (Seelze, Germany). 6,7-Dimethoxycoumarin (98%) was purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Sodium acetate was obtained from Kohusan Chemical Works, Ltd. (Kyoto, Japan). Milli-Q plus water (Millipore, Bedford, USA) was used for all preparations.

II. *Instrumentation and HPLC Conditions*

HPLC system was equipped with a Shimadzu SIL-10AD VP automatic sample injector, a Shimadzu SPD-10A VP Detector and two Shimadzu LC-10AT VP pumps. Reversed-phase separation was carried out using a RP-18 column (Cosmosil, 150 × 4.0 mm, 5 μm) equipped with a prefilter. The mobile phase consisted of acetonitrile and 0.01% phosphoric acid (24:76) and the flow rate was 1.0 mL/min. The detection wavelength was set at 370 nm.

III. *Drug Administration and Blood Collection*

Male Sprague-Dawley rats weighing 300-380 g were fasted for 12 hr before drug administration and continued for another 3 hr. Water was supplied *ad libitum*. The animal study adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" (published by The Chinese Society for the Laboratory Animal Science, Taiwan, ROC).

For intravenous administration (iv), quercetin was dissolved in PEG 400 and filtered through a 0.22 μm membrane. The iv bolus were given to 6 rats via the tail vein at a dose of 33 μmol/kg. Blood samples (0.8 mL) were withdrawn via cardiopuncture prior to dosing and at 5, 10, 15, 30, 60, 90, 120, 240, 360, 480 and 720 min post-dosing. Water was supplied at 2-hr intervals via gastric gavage during the experiment.

For oral administration (po), quercetin was dissolved in PEG 400 and rutin was dissolved in glycofurol. Quercetin and rutin were given at 165 μmol/kg and 328 μmol/kg, respectively, via gastric gavage. Blood samples were withdrawn via cardiac puncture at 5, 15, 30, 60, 120, 240, 360, 480, 1440, 2160 and 2880 min post dosing of quercetin, and at 5, 15, 30, 60, 120, 240, 420, 600, 1440, 2040, 2880, 3480, 4440 and 4920 min post dosing of rutin. All blood samples were centrifuged at 9860×g for 15 min and the serum obtained were stored at -30°C until analysis.

IV. *Determination of Quercetin Conjugated Metabolites in Serum*

The concentrations of quercetin glucuronides and quercetin sulfates in serum were determined after β-glucuronidase and sulfatase treatments, respectively. For enzymolysis, 200 μL of serum sample was mixed with 100 μL of β-glucuronidase (500 units/mL in pH 5 acetate buffer) or sulfatase (1000 units/mL in pH 5 acetate buffer), 50 μL of ascorbic acid (200 mg/mL) and incubated at 37°C for 2 hr and 1 hr, respectively, under anaerobic condition by sucking air with syringe, and protected from light by wrapping with aluminum foil. After hydrolysis, the serum was acidified with 20 μL of 0.1 N HCl and partitioned with 350 μL of ethyl acetate (containing 2.0 μg/mL of 6,7-dimethoxycoumarin as the internal standard). The ethyl acetate layer was evaporated under N_2 to dryness and reconstituted with an appropriate volume of methanol prior to HPLC analysis.

V. *Validation of Assay Method for Serum*

The system suitability was evaluated through intra-day and inter-day analysis of precision and accuracy. The accuracy of this method was further assessed with recovery studies by spiking methanolic quercetin standard solution into blank serum and water in triplicates to afford 0.2, 12.5 and 100.0 μg/mL, respectively. Afterwards, the concentrations obtained in blank serum to the corresponding ones in water were compared. The LLOQ (lower limit of quantitation) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy, whereas LOD (limit of detection) represents the lowest concentration of analysis in a sample that can be detected with signal/noise greater than 3.

VI. *Data Analysis*

The peak serum concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained from experimental observation. The pharmacokinetic parameters were analyzed by noncompartmental method with the aid of the program WINNONLIN (version 1.1 SCI software, Statistical Consulting, Inc., Apex, NC, USA). The area under the serum concentration-time curve (AUC_{0-t}) was calculated using trapezoidal rule to the last point. The other pharmacokinetic parameters were calculated using the following relationships:

Oral absorption rate of Q = $AUC_{0-t\ (Q\ conjugates)}$ of Q po / $AUC_{0-t\ (Q\ free\ form\ +Q\ conjugates)}$ of Q iv × 5

(Q: quercetin)

VII. *Statistical Analysis*

The paired Student's *t*-test was used to compare the difference of pharmacokinetic parameters between sulfates and glucuronides of quercetin, and $p < 0.05$ was considered significant.

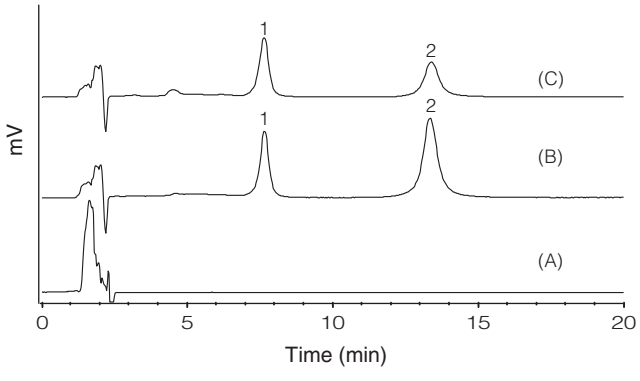

Figure 2. Chromatograms of (A) blank serum; (B) serum spiked with quercetin (12.5 μg/mL) and internal standard; and (C) a serum sample hydrolyzed with sulfatase (quercetin: 8.3 μg/mL); 1: internal standard (6,7-dimethoxycoumarin); 2: quercetin.

RESULTS

Typical chromatograms of blank serum and quercetin in serum are shown in Figure 2. Good linear relationship was obtained for quercetin within the concentration range of 0.2-100.0 μg/mL (Y = 0.57 X – 0.02, r = 0.999). The intra-day and inter-day precision and accuracy analysis of quercetin in serum indicated that the coefficients of variations and the relative errors were below 10.1 and 6.7%, respectively, as shown in Table 1. The recoveries of quercetin from serum were 90.9 ± 6.1, 89.3 ± 2.2 and 105.3 ± 1.4% for the concentrations of 0.2, 12.5 and 100.0 μg/mL, respectively. The LLOQ was 0.2 μg/mL and LOD was estimated as 0.05 μg/mL.

Mean serum concentration - time profiles of quercetin and its conjugates after iv administration of quercetin are shown in Figure 3. After iv bolus, no parent form was detected after 60 min and the blood levels of quercetin sulfates and quercetin glucuronides were much higher than their parent form during the experiment. The half-life of quercetin parent form was 21 min which was shorter than those of quercetin sulfates (371 min) and quercetin glucuronides (561 min). The AUC_{0-60} of quercetin parent form and its conjugates listed in Table 2 indicated that the conjugated metabolites accounted for 93.8% of the total quercetin including the parent form and its conjugates in the circulation. The mean AUC_{0-t} of quercetin sulfates was

Table 1. Intra-day and inter-day analytical precision and accuracy of quercetin in serum (n = 3)

	Intra-day		Inter-day	
Conc. (μg/mL)	Precision Mean ± S.D. (C.V.%)	Accuracy (%)	Precision Mean ± S.D. (C.V.%)	Accuracy (%)
100.0	106.7 ± 0.6 (0.6)	6.7	106.0 ± 0.3 (0.3)	6.0
50.0	52.6 ± 0.4 (0.8)	5.3	52.5 ± 0.0 (2.2)	5.0
25.0	25.8 ± 0.1 (0.2)	3.3	25.8 ± 0.2 (0.9)	3.2
12.5	12.6 ± 0.0 (0.3)	0.5	12.5 ± 0.1 (0.5)	0.2
6.3	6.1 ± 0.0 (0.3)	-2.6	6.1 ± 0.0 (0.6)	-2.5
3.1	3.0 ± 0.0 (0.4)	-5.6	2.9 ± 0.0 (0.8)	-6.2
1.6	1.5 ± 0.0 (0.7)	-3.1	1.5 ± 0. 0 (1.5)	-2.7
0.8	0.8 ± 0.0 (1.2)	-3.9	0.8 ± 0.0 (1.8)	-4.3
0.4	0.4 ± 0.0 (5.2)	-3.9	0.4 ± 0.0 (3.7)	0.8
0.2	0.2 ± 0.0 (1.6)	6.5	0.2 ± 0.0 (10.1)	4.5

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Table 2. Comparison of pharmacokinetic parameters of quercetin sulfates and glucuronides in serum after intravenous administration of 10 mg/kg (33 μmol/kg) quercetin to 6 rats

Parameter \ Quercetin	Free form	Sulfates	Glucuronides
$AUC_{0\text{-}60}$[a] (nmol•min•mL^{-1})	114.1 ± 11.9	1606.8 ± 187.6	311.2 ± 63.8[e]
$AUC_{0\text{-}t}$[b] (nmol•min•mL^{-1})	450.1 ± 65.3	6662.3 ± 923.6	1931.8 ± 524.0[f]
$T_{1/2}$[c] (min)	20.7 ± 5.9	371.2 ± 17.2	560.7 ± 235.9
MRT[d] (min)	223.4 ± 50.4	473.4 ± 24.7	716.8 ± 335.1

Data expressed as means ± S.E.
[a]Area under the serum concentration-time curve from time zero to 60 min.
[b]Area under the serum concentration-time curve from time zero to the last point.
[c]Half-life.
[d]Mean residence time.
[e]$p < 0.001$.
[f]$p < 0.01$.

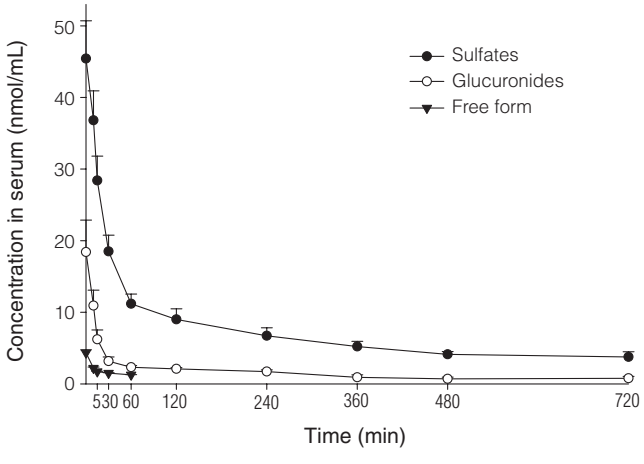

Figure 3. Mean (± S.E.) serum concentration - time profiles of quercetin and its conjugated metabolites after intravenous administration of quercetin (33 μmol/kg) to 6 rats.

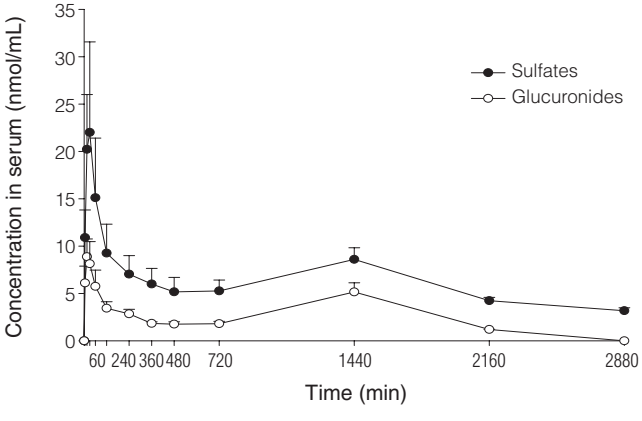

Figure 4. Mean (± S.E.) serum concentration - time profiles of conjugated metabolites of quercetin after oral administration of quercetin (165 μmol/kg) to 6 rats.

significantly higher than that of quercetin glucuronides by 245% ($p < 0.01$). The mean residence times of quercetin sulfates and glucuronides were longer than quercetin.

After oral dosing of quercetin, no parent form of quercetin was detected in serum. The serum profiles of quercetin conjugates are shown in Figure 4. The concentrations of quercetin sulfates were markedly higher than quercetin glucuronides at each time point. The pharmacokinetic parameters of quercetin conjugates are listed in Table 3. The mean C_{max} of quercetin sulfates was significantly higher than that of quercetin glucuronides by 129% ($p < 0.01$). Similarly, the mean $AUC_{0\text{-}t}$ of quercetin sulfates was significantly higher than that of glucuronides by 161% ($p < 0.001$). Because the oral dose of quercetin was 5 times of intravenous dose in this study, after dose correction, the oral absorption rate of quercetin was about 53% based on comparing the $AUC_{0\text{-}t}$ of quercetin conjugates after oral dosing with that of the total quercetin including quercetin and its conjugates after iv bolus.

After oral dosing of rutin, no rutin or quercetin was detected. The serum profiles of quercetin conjugates are shown in Figure 5. The serum level of quercetin sulfates was markedly higher than that of quercetin glucuronides at each time point. The pharmacokinetic parameters of quercetin conjugates are listed in Table 4. The mean C_{max} of quercetin sulfates was significantly higher than that of quercetin glucuronides by 126% ($p < 0.01$). The mean $AUC_{0\text{-}t}$ of quercetin sulfates was significantly higher than

Table 3. Comparison of pharmacokinetic parameters of quercetin sulfates and glucuronides in serum after oral administration of 50 mg/kg (165 μmol/kg) quercetin to 6 rats

Parameter \ Quercetin conjugates	Sulfates	Glucuronides
C_{max}[a] (nmol•mL^{-1})	27.3 ± 8.1	11.9 ± 1.3
$AUC_{0\text{-}t}$[b] (nmol•min•mL^{-1})	17756.4 ± 2879.1	6803.8 ± 1000.3[d]
MRT[c] (min)	1265.1 ± 71.6	1063.7 ± 15.8[e]

Data expressed as means ± S.E.
[a]Peak serum concentration.
[b]Area under the serum concentration - time curve from time zero to the last point.
[c]Mean residence time.
[d]$p < 0.01$.
[e]$p < 0.05$.

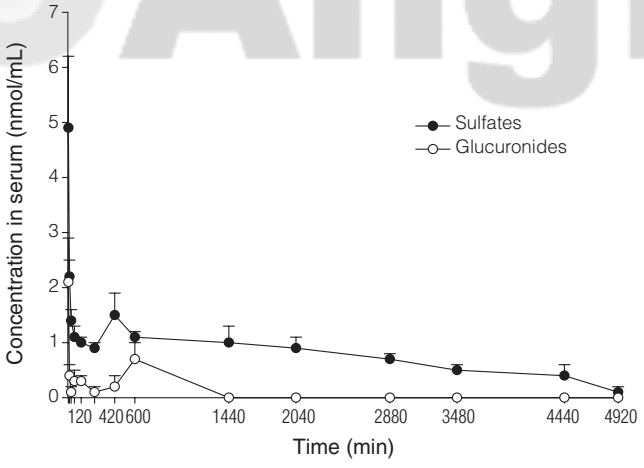

Figure 5. Mean (± S.E.) serum concentration - time profiles of conjugated metabolites of quercetin after oral administration of rutin (328 μmol/kg) to 6 rats.

Table 4. Comparison of pharmacokinetic parameters of quercetin sulfates and glucuronides in serum after oral administration of 200 mg/kg (328 μmol/kg) rutin to 6 rats

Parameter \ Quercetin conjugates	Sulfates	Glucuronides
C_{max}[a] (nmol•mL^{-1})	5.2 ± 1.2	2.3 ± 0.7[d]
AUC_{0-t}[b] (nmol•min•mL^{-1})	3533.0 ± 750.4	161.0 ± 63.1[d]
MRT[c] (min)	1644.5 ± 148.2	272.9 ± 84.1[d]

Data expressed as means ± S.E.
[a]Peak serum concentration.
[b]Area under the serum concentration-time curve from time zero to the last point.
[c]Mean residence time.
[d]$p < 0.01$.

that of quercetin glucuronides by 2094% ($p < 0.01$) and the mean residence time of quercetin sulfates was longer than quercetin glucuronides.

DISCUSSION

The quercetin sulfates and quercetin glucuronides in serum cannot be determined directly due to the lack of authentic standard. Therefore, the concentrations of quercetin in serum sample were determined before and after respective treatment with β-glucuronidase or sulfatase in order to calculate the concentrations of quercetin sulfates and glucuronides. The conditions for enzymolysis were optimized, including sufficient reaction time, protection from light and addition of antioxidant. As a result, ascorbic acid was added to the serum for antioxidation and the incubation was conducted anaerobically and protected from light to prevent the potential decomposition of quercetin liberated from its sulfates and glucuronides. Validation indicated that the present assay methods were precise and accurate, and thus applicable in pharmacokinetic studies of quercetin and its conjugated metabolites.

The serum profiles of quercetin parent form and its conjugated metabolites after iv bolus of quercetin indicated that conjugation metabolism of quercetin occurred very rapidly and extensively. In addition, the half-lives of quercetin sulfates and glucuronides were longer than their parent form by 2~3-fold, possibly due to their enterohepatic circulation[21,22]. Because rutin was not absorbed *per se*, intravenous pharmacokinetics of rutin can not afford any information about its bioavailability. Therefore, intravenous administration of rutin was not conducted.

When quercetin and rutin were administered orally, the glucuronides and sulfates of quercetin appeared at the first time point (5 min post dosing) and were exclusively circulating in the bloodstream, indicating very rapid absorption of quercetin and simultaneous sulfation and glucuronidation during absorption. Additional peaks of both quercetin conjugates were observed during the apparent elimination phase in Figures 4 and 5, implying enterohepatic circulation of these conjugated metabolites. The parent forms of quercetin and rutin were not detected, indicating that the absolute systemic bioavailabilities of quercetin and rutin were essentially zero. Together with the finding of the null absolute systemic bioavailability of baicalein[23], these results indicated that extensive conjugation metabolism of polyphenols occurred during the first pass through gut and liver. These facts are in good agreement with previous polyphenol studies[24,25], which stated that glucuronidation/sulfation was central to the flavonoid metabolism and absorption. The absorption rate of quercetin (53%) was higher than that of baicalein (40%) in our previous study[23]. This could be accounted for by the fact that the lipophilicity of quercetin was greater than baicalein from the observation of its longer retention time on the reversed phase HPLC chromatogram (data not shown).

After dose correction, much lower C_{max} and AUC_{0-t} of quercetin sulfates and quercetin glucuronides were shown for oral rutin than quercetin, indicating that the rate and extent of quercetin absorption was much higher than its glycoside - rutin. One of the reasons for poor absorption of rutin is that it is too hydrophilic to diffuse through cell plasma membrane and it was absorbable only after being hydrolyzed into quercetin which is absorbable. Therefore, the hydrolysis of rutin was indispensable for its absorption and this can account for the null bioavailability of rutin.

Recently, two quercetin metabolites, quercetin 3-*O*-β-D-glucuronide and quercetin 3'-*O*-sulfate in human plasma were reported after consumption of onions[26]. Another study also identified glucuronides and sulfates of quercetin from plasma after oral dosing of quercetin 4'-*O*-β-glucoside to rats[27]. In this study, oral administration of rutin or quercetin resulted in predominant presence of quercetin sulfates in the circulation. Therefore, the conjugated metabolites of quercetin, in particular quercetin sulfates, might be responsible for the *in vivo* effects of quercetin and rutin. Nowadays, there is proliferation of *in vitro* studies of polyphenols. Nonetheless, the bioactivies of parent form of

quercetin and rutin investigated *in vitro* could not explain the *in vivo* effects of oral quercetin and rutin. In addition, recent study on quercetin 3-glucuronide (Q3GA) reported that Q3GA was more effective than quercetin aglycone in the inhibition of H_2O_2-induced intracellular ROS production in mouse fibroblast cultured cells, although little Q3GA diffused into the cytoplasm or cell nucleus compartment[28]. A recent study on morin and its sulfates/glucuronides also demonstrated that the conjugated metabolites of morin were 1000-fold more effective than morin aglycone in anti-inflammation activity[29].

In conclusion, quercetin and rutin were transformed into sulfates and glucuronides of quercetin *in vivo*. Therefore, various bioactivities of quercetin sulfates and glucuronides are worthy for further investigations.

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