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Profound difference in pharmacokinetics between morin and its isomer quercetin in rats

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Abstract

Morin and quercetin are isomeric antioxidant flavonols widely distributed in plant foods and herbs. The pharmacokinetics of both flavonols at two doses were investigated and compared in rats. Parent forms and their glucuronides and sulfates in serum were determined by HPLC before and after enzymatic hydrolysis, respectively. After oral dosing of morin, both the parent form, morin, and its glucuronides and sulfates were present in the bloodstream. The conjugated metabolites predominated at the dose of 25 mg kg^{-1}, whereas the parent form was predominant at the dose of 50 mg kg^{-1}. Moreover, the AUC of morin parent form increased by a factor of 37 when the dose doubled, indicating that morin showed nonlinear pharmacokinetics. On the other hand, quercetin presented only as glucuronides and sulfates in the blood, indicating negligible bioavailability of quercetin, and the metabolites showed linear pharmacokinetics at the two doses studied. When considering the total AUC of parent form with conjugated metabolites, the extent of absorption of morin was 3 fold that of quercetin at the dose of 50 mg kg^{-1}. The results indicated that the difference in hydroxylation pattern on B-ring of flavonol markedly affected their fates in rats.

Introduction

Flavonoids have attracted increasing attention in recent years because of their beneficial biological activity (Cook & Samman 1996; Middleton et al 2000) and ability to modulate both CYP 3A4 and P-glycoprotein (Pgp), the product of *mdr* (multidrug-resistance) genes (Critchfield et al 1994; Scambia et al 1994). Morin (2′,3,4′,5,7-pentahydroxyflavone) (Figure 1) and quercetin (3,3′,4′,5,7-pentahydroxyflavone) are widely distributed as glycosides in plant food and herbs (Liu & Sheu 1989). The flavonoid glycosides are unable to permeate through the gut wall and need to be hydrolysed by intestinal microflora into their aglycones for absorption. The efficacy of flavonoids in-vivo is less documented, presumably because of the limited knowledge on their absorption and metabolism in animals.

Morin has been shown to exhibit beneficial effects, including prevention of coronary artery disease (Elangovan et al 1994), inhibition of tumour proliferation (Tanaka et al 1999) and protection of human erythrocytes, ventricular myocytes and saphenous vein endothelial cells (Wu et al 1995a, b), as well as scavenging of free radicals (Zeng et al 1994). Quercetin has anti-inflammatory (Middleton et al 2000), antihistamine (Murray 1998) antivirus (Ohnishi & Bannai 1993) and antiulcer (Alarcon-de-la-Lastra et al 1994) actions. Moreover, quercetin is a promising candidate for cancer therapy (Ferry et al 1996). Furthermore, much attention has been paid to the strong antioxidant property of quercetin (Vinson et al 1995). It is believed that several pathologies, including cancer, coronary heart disease and chronic inflammation, may be prevented by taking quercetin-rich food or herbs (Hertog et al 1993, 1995; Hollman et al 1996).

Morin and quercetin are structural isomers with close resemblance and possess comparable biological activity. Therefore, how the structure difference on B-ring affects their pharmacokinetics is of great interest. Although the absorption and metabolism of quercetin in man and animals have been extensively discussed (Manach et al 1995; Hollman & Katan 1999; Lanson & Brignall 2000), until now no pharmacokinetic data has been reported for morin. Therefore, the pharmacokinetic behaviour of both

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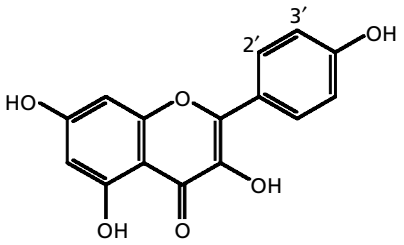

	2′	3′
Morin	OH	H
Quercetin	H	OH

Figure 1 Structures of morin and quercetin.

flavonols and their metabolites were investigated and compared after oral administration to rats in this study. Furthermore, dose-dependent effects on the metabolic pharmacokinetics of both flavonols were investigated.

Materials and Methods

Materials

Quercetin dihydrate, morin, glycofurol, acetic acid (99%) and β-glucuronidase (HP-2, from *Helix pomatia*, containing 89 400 U mL^{-1} of β-glucuronidase and 3300 U mL^{-1} of sulfatase) were purchased from Sigma Chemical Co., USA. Acetonitrile, ethyl acetate and methyl alcohol were LC grade and obtained from Mallinckrodt Baker, Inc., USA. Potassium dihydrogen phosphate was produced by Merck, Germany. L-(+)-Ascorbic acid was purchased from RdH Laborchemikalien GmbH & Co. KG, Germany. 6,7-Dimethoxycoumarin (98%) was purchased from Aldrich, USA. Sodium acetate was obtained from Kohusan Chemical Works Ltd (Japan). Ethyl paraben was purchased from Aldrich, USA. Milli-Q plus Water (Millipore, USA) was used for all preparations.

Instrumentation and HPLC conditions

HPLC apparatus included two pumps (LC-6AD, Shimadzu, Japan), an SLC-6B controller, an UV spectrophotometric detector (SPD-6A, Shimadzu, Japan) and a chromatopac (C-R6A, Shimadzu, Japan). The RP-18 column (Cosmosil, 250 × 4.6 mm) was equipped with a prefilter. The mobile phase was acetonitrile – 0.2% *ortho*-phosphoric acid (27:73 and 28:72 v/v for morin and quercetin, respectively) and the flow rate was 1.0 mL min^{-1} with the detection wavelength set at 250 nm. Nitrogen evaporator (N-Evap 112) was supplied by Organomation Associates, Inc. (USA).

Animal protocol and drug administration

Male Sprague-Dawley rats, 200–300 g, were fasted for 12 h before dosing and continued for another 4 h after dosing. Rats were housed in a 12-h light–dark, constant temperature environment before study. Water was supplied freely. The animal study adhered to The Guide-book for the Care And Use of Laboratory Animals (2002), published by The Chinese Society for the Labora-tory Animal Science, Taiwan, R.O.C..

The oral solutions of quercetin or morin were prepared by dissolving them in a mixed solvent containing dimethylacetamide–PEG 400–water (1:5:4). Rats were dosed with 50 or 100 mg kg^{-1} quercetin and 25 or 50 mg kg^{-1} morin, respectively. Drug administration was carried out via gastric gavage. Blood samples (0.8 mL) were withdrawn via cardiopuncture at 5, 15, 30, 60, 90, 120, 240, 360, 480, 600, 720 and 1440 min post dosing. Blood samples were allowed to clot and then centrifuged to obtain serum, which was stored at −30 °C for later analysis.

Quantitation of morin, quercetin and their glucuronides and sulfates in serum

The assay of free form morin or quercetin and their glucuronides and sulfates were reported in our previous study (Hsiu et al 2001). For the determination of total morin and quercetin, and their conjugates, serum samples (200 μL) were incubated with 100 μL enzyme solution (110.4 U mL^{-1} of β-glucuronidase and 4.2 U mL^{-1} of sulfatase in acetate buffer, pH 5) and 20 μL ascorbic acid (300 mg mL^{-1}) at 37 °C for 4 h followed by partition with 300 μL ethyl acetate (containing ethyl paraben and 6,7-dimethoxycoumarin as internal standards, respectively). The ethyl acetate layer was evaporated under N_2 and reconstituted with 20 μL CH_3OH, and then subjected to HPLC analysis. For the assay of morin or quercetin parent forms, 200-μL serum samples were subjected to the same process but without incubation with β-glucuronidase and sulfatase.

Pharmacokinetic analysis

The peak serum concentration (C_{max}) was recorded as observed. WINNONLIN (version 1.1, SCI software, Statistical Consulting Inc., Apex, NC) was used for the computation of pharmacokinetic parameters. The parameters including area under the serum concentration–time curve to the last point (AUC_{0-t}), terminal elimination half-life ($t_{½}$) and mean residence time (MRT) were calculated by fitting serum data to non-compartment model. The AUC_{0-t} of conjugates was calculated from equation 1.

$$AUC_{conjugates} = AUC_{parent\ form+conjugates} - AUC_{parent\ form} \quad (1)$$

The unpaired Student's *t*-test was used for statistical comparison of pharmacokinetic parameters, with $P < 0.05$ as significant.

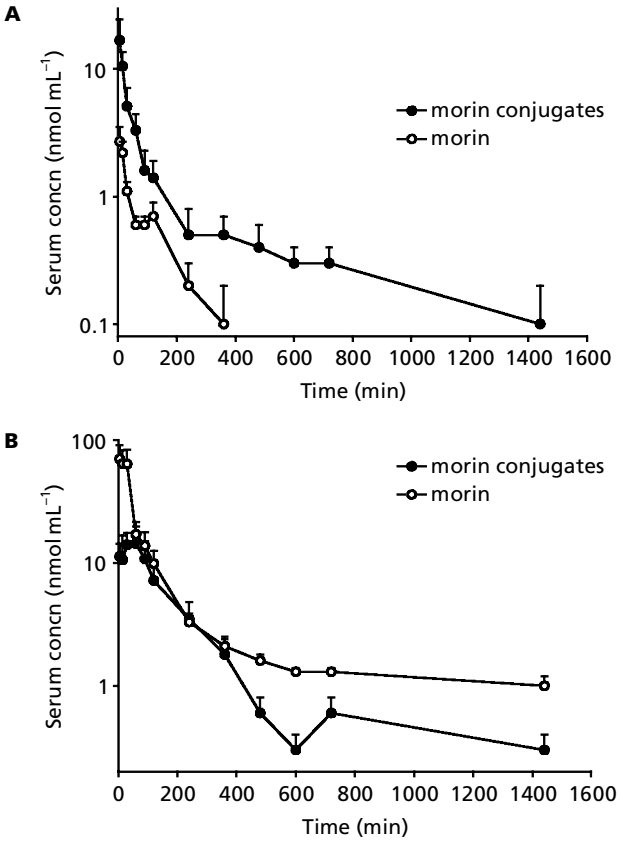

Figure 2 Mean (± s.e.) serum concentration–time profiles of morin ○ and its conjugates (●) after oral administration of 25 mg kg^{-1} (A) or 50 mg kg^{-1} (B) of morin to six rats.

Table 1 Comparison of pharmacokinetic parameters of morin and its conjugates after oral dosing with 25 mg kg^{-1} (83 μmol kg^{-1}) and 50 mg kg^{-1} (165 μmol kg^{-1}) morin to six rats.

Parameters	25 mg kg^{-1}	50 mg kg^{-1}	Difference
Morin			
C_{max}	3.1 ± 0.8	84.9 ± 18.1	2639%**
C_{max}/dose	0.1 ± 0.0	1.7 ± 0.41	317%**
AUC_{0-t}	168.0 ± 41.0	6272.5 ± 1359.0	3634%***
AUC_{0-t}/dose	6.7 ± 1.6	125.4 ± 27.2	1767%***
$t_{½}$	95.7 ± 15.4	271.1 ± 52.1	183%
MRT	71.5 ± 14.2	273.3 ± 67.0	282%*
Conjugates			
C_{max}	18.5 ± 7.2	20.9 ± 6.2	13%
C_{max}/dose	0.7 ± 0.3	0.4 ± 0.1	−41%
AUC_{0-t}	961.1 ± 405.2	2851.1 ± 878.1	197%
AUC_{0-t}/dose	38.4 ± 16.2	58.0 ± 17.0	51%
$t_{½}$	161.2 ± 46.6	305.0 ± 82.6	89%
MRT	157.5 ± 50.7	343.7 ± 99.3	118%
AUC_{total}	1129.1 ± 407.3	9123.6 ± 1618.0	710%**

C_{max} (nmol mL^{-1}), peak serum concentration; AUC_{0-t} (nmol min mL^{-1}), area under the serum concentration–time curve from time zero to the last point; t½ (min), terminal elimination half-life; MRT (min), mean residence time; AUC_{total}, AUC_{0-t} of morin with its conjugates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Results

Pharmacokinetics of morin and its conjugated metabolites in rats

The relative errors of inter-day and intra-day assay for morin determination by HPLC method were less than 9% and 11%, respectively, in the concentration range of 0.39 ~ 100.00 μg mL^{-1} and the lower detection limit was 0.09 μg mL^{-1} (Hsiu et al 2001). Serum profiles of both morin and its conjugates are shown in Figure 2 after oral dosing of morin at 25 mg kg^{-1} and 50 mg kg^{-1} (Figure 2A and 2B, respectively). The pharmacokinetic parameters of parent-form morin at two doses were listed and are compared in Table 1. The differences in AUC/dose and C_{max}/dose were 18 fold and 13 fold, respectively, between two doses. Moreover, $t_{½}$ and MRT of morin were significantly increased by 183% and 282%, respectively, when the dose was doubled. The difference in AUC of morin glucuronides and sulfates between the two doses was 3 fold, whereas C_{max} did not show marked difference. Furthermore, the early exposure of conjugated metabolites up to 30 min post dosing ($AUC_{0-30\,min}$) did not show substantial difference between the two doses (246.80 ± 111.74 nmol min mL^{-1} at 25 mg kg^{-1} vs 323.0 ± 109.8 nmol min mL^{-1} at 50 mg kg^{-1}). The ratios of AUC of morin conjugates to the total AUC of morin with its conjugated metabolites were 78% and 30% at doses of 25 mg kg^{-1} and 50 mg kg^{-1}, respectively.

Metabolic pharmacokinetics of quercetin in rats

The relative errors of inter-day and intra-day assay for quercetin determination by HPLC method were less than 13% and 7%, respectively, in the concentration range of 0.2 ~ 2.5 μg mL^{-1} and the lower detection limit was 4.9 ng mL^{-1} (Hsiu et al 2001). Serum profiles of quercetin conjugates after oral administration of quercetin at doses of 50 mg kg^{-1} and 100 mg kg^{-1}, respectively, are shown in Figure 3. Because parent-form quercetin was detected only in a few serum samples withdrawn at 5 min following both doses, the pharmacokinetic parameters of quercetin cannot be calculated. After glucuronidase and sulfatase treatment of serum samples, the pharmacokinetic parameters of quercetin glucuronides and sulfates were estimated and shown in Table 2. The AUC of quercetin glucuronides and sulfates obtained at the dose of 100 mg kg^{-1} was significantly higher than that at the dose of 50 mg kg^{-1}, although the difference did not reach two fold. The C_{max} of quercetin glucuronides and sulfates almost doubled when the dose of quercetin was doubled. The calculated MRT for both doses was not different.

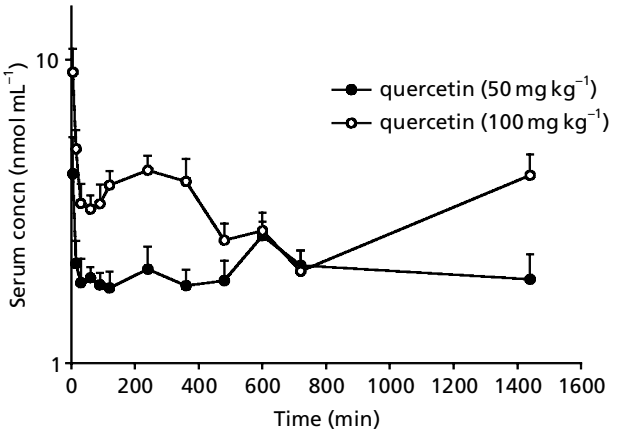

Figure 3 Mean (± s.e.) serum concentration–time profiles of quercetin conjugates after oral administration of 50 mg kg^{-1} (●, n = 5) or 100 mg kg^{-1} (○, n = 6) of quercetin to rats.

Table 2 Pharmacokinetic parameters (mean ± s.e.) of quercetin conjugates after oral dosing with 50 mg kg^{-1} (165 μmol kg^{-1}; n = 5) and 100 mg kg^{-1} (331 μmol kg^{-1}; n = 6) quercetin to rats.

Parameters	50 mg kg^{-1}	100 mg kg^{-1}	Difference
C_{max}	4.9 ± 1.0	9.5 ± 1.6	90%*
AUC_{0-t}	2906.1 ± 230.6	4818.0 ± 385.0	70%**
MRT	707.3 ± 56.7	740.3 ± 38.4	4%

C_{max} (nmol mL^{-1}), peak serum concentration; AUC_{0-t} (nmol min mL^{-1}), area under the serum concentration–time curve from time zero to the last point; MRT (min), mean residence time. *$P < 0.05$, **$P < 0.01$.

Discussion

As a positional isomer of quercetin, morin attracted our interest for investigating the metabolic pharmacokinetics to make a comparison with quercetin. After oral administration of 25 mg kg^{-1} morin to rats, both the parent-form morin and its conjugated metabolites were present in serum samples up to 120 min, and the profile of parent-form morin was much lower than that of the conjugated metabolites. On the other hand, when the dose of morin was doubled, the profile of parent form was much higher than that of the conjugated metabolites. By comparing the fates of morin between the two doses, a clear indication of nonlinear metabolic pharmacokinetics of morin was demonstrated in rats. Dose-dependent pharmacokinetic behaviour of morin was further demonstrated by the augmentation in the AUC_{0-t} and C_{max} of morin by 37 and 27 fold, respectively, when the dose was only doubled. The extraordinary increase in AUC_{0-t} and C_{max} of morin might be, in part, explainable by a marked interaction of morin at higher dose with the cell membrane interface like egg yolk, to result in significant leakage which made the paracellular absorption of morin possible (Ollila et al 2001). The increase in AUC_{0-t} of morin conjugated metabolites was about 3 fold when the dose was doubled, indicating that the level of metabolites was higher than expected. The much greater enhancement in AUC_{0-t} of morin conjugates based on dose increment might arise from the huge increase in morin absorption. The absorption extent of morin expressed by the total AUC_{0-t} of morin with its conjugated metabolites at 50 mg kg^{-1} was about 8 fold that at 25 mg kg^{-1}. The MRT significantly increased by 282% when the dose was doubled. Moreover, the ratio of conjugated metabolites to the total exposure, including parent form and its conjugates, decreased from 78% to 30%, indicating that conjugation metabolism was saturated at the higher dose. Significant rise in C_{max} was shown for the parent-form morin but not observed for its conjugated metabolites. The comparable C_{max} of morin conjugates between the two doses was not in agreement with the marked difference in morin levels, also indicating that conjugation metabolism was saturated. It can therefore be presumed that possibly enhanced membrane leakage and substantial metabolism saturation of morin may synergistically account for the dose-dependent pharmacokinetic behaviour.

A very different fate of oral quercetin was found when compared with morin in rats. After an oral dose of 50 mg kg^{-1} or 100 mg kg^{-1}, negligible parent form of quercetin was detected in serum throughout the investigation and the major molecules found circulating in the bloodstream were quercetin glucuronides and sulfates. The results indicated that quercetin was extensively transformed by conjugation metabolism. When the dose was doubled, the C_{max} and AUC_{0-t} of quercetin glucuronides and sulfates increased almost proportionally with dose.

The fast emergence of glucuronides and sulfates of morin and quercetin after oral dosing indicated that morin and quercetin were rapidly absorbed and instantaneously transformed into conjugated metabolites through gut wall or liver metabolism. The almost exclusive presence of quercetin conjugated metabolites was in good agreement with a previous study (Spencer et al 1999). In contrast to the fate of morin at 50 mg kg^{-1}, which presented mainly parent form in the blood, quercetin showed nearly no parent form at the same dose, and even at a higher dose (100 mg kg^{-1}), indicating that more extensive metabolism of quercetin occurred. When the total exposure of morin and quercetin were compared at the dose of 50 mg kg^{-1}, based on the total AUC of parent forms with their respective conjugated metabolites, the absorption of morin was about 3 fold that of quercetin. Due to the possible interaction of morin with biomembrane to cause leakage, it is speculated that morin could enhance the oral absorption of quercetin or other small drug molecules. More interaction studies will be carried out in our laboratory to support this presumption in the near future.

Conclusions

Morin and quercetin exhibited profound difference in metabolic pharmacokinetics in rats when administered

orally. Quercetin glucuronides and sulfates existed exclusively in the circulation, whereas morin co-existed with its glucuronides and sulfates. Moreover, quercetin exhibited linear metabolic pharmacokinetics for the doses studied, whereas the pharmacokinetics of morin were nonlinear. It is suggested that conjugated metabolites of quercetin be more focused than quercetin for in-vitro researches to provide an accurate model of in-vivo systems, whereas either morin or its conjugated metabolites was important for in-vitro pharmacological studies of morin.

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