Quercetin's Solubility Affects Its Accumulation in Rat Plasma after Oral Administration†

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Absorption of quercetin, a flavonoid regarded as a beneficial dietary factor, was studied in rats. The compound was administered orally in propylene glycol, water/propylene glycol, and water. Quercetin's solubility in vehicles used for quercetin administration was measured. Following administration, tail vein blood was collected as a function of time. Concentrations of quercetin and its metabolites in rat blood plasma were measured by HPLC with electrochemical detection after hydrolysis of respective conjugates with β-glucuronidase and/or sulfatase. Pharmacokinetic profiles of quercetin and isorhamnetin, its 3′-methoxy derivative, were obtained, and on their basis it was concluded that isorhamnetin was present in rat blood plasma mainly as sulfate/glucuronide conjugates. Quercetin's solubility in solvents used for its administration was compared with respective absorption profiles. This comparison revealed that solubilization enhanced quercetin absorption; however, its lack was not an absorption-limiting factor.

Keywords: *Flavonoid absorption; quercetin; isorhamnetin; glucuronide; rat*

INTRODUCTION

The potential physiological activity of dietary flavonoids has recently been the center of interest after it was found in vitro that flavonoids act as antioxidants by lipid peroxidation inhibition, oxygen radical scavenging, and metal ion chelating (Afanas'ev et al., 1989; Cotelle et al., 1992; Terao et al., 1994; Terao and Piskula, 1998) and that this activity is related to the unique flavonoid structure (van Acker et al., 1996; Rice-Evans et al., 1995). Also, epidemiological evidence has emerged showing an inverse association between the intake of dietary flavonoids and coronary heart disease and cancer (Hertog et al., 1995; Knekt et al., 1996).

The average intake of flavonoids was estimated as ranging from 1 g/day (Kuhnau, 1976) to 25 mg/day (Hertog et al., 1993). However, the latter value covers only five aglycons (myricetin, quercetin, kaempferol, apigenin, and luteolin), of which quercetin's 60% share makes this flavonoid one of the most commonly consumed. The main dietary sources of quercetin are onions, apples, wine, berries, and tea (Herrman, 1976; Hertog et al., 1992).

Despite quercetin's abundance in foods, its absorption and metabolism have not been well recognized. There are studies on quercetin's absorption in humans (Holman et al., 1995, 1997; Gugler et al., 1975) and in rats (Manach et al., 1997). Although there is evidence that quercetin is absorbed when administered as a dietary supplement or as an inherent food component (mainly as glycosides) (Hollman et al., 1997), it is still not clear why quercetin was not found in human plasma when administered as a single oral dose with water (Gugler et al., 1975). Moreover, the authors usually did not distinguish between the kinds of quercetin metabolites and measured the amount of total absorbed or excreted compound.

Because quercetin's solubility in a vehicle used for quercetin oral administration might be an absorption-limiting factor, we compared the absorption of quercetin administered to rats in combinations with propylene glycol and water. Following administration, the blood plasma concentrations of quercetin and some of its metabolites (conjugates only) were measured as a function of time by simple HPLC with electrochemical detection.

MATERIALS AND METHODS

Chemicals. Quercetin, β-glucuronidase type VII-A, and sulfatase type H-5 were purchased from Sigma Chemical Co. (St. Louis, MO), and isorhamnetin was purchased from Extrasynthese (Genay, France). Other chemicals were of analytical or HPLC grade.

Animals and Diets. Six 6-week-old Wistar male rats weighing 200−210 g were used for experiment. Animals were supplied by Charles River Japan Inc. (Atsugi, Japan) and kept in a temperatue- (23 ± 1 °C) and light-controlled (7:00 a.m.−7:00 p.m) room in the Institute's animal facility for 4 days before experiments, with free access to tap water and standard MF diet (Oriental Bioservice, Co., Japan). The day before experiment at 8:00 p.m. food was withheld, and the next day at 9:00 a.m. the first group of animals (three rats) was orally adminstered 50 mg/kg quercetin in 2 mL of propylene glycol by direct stomach intubation, the second group was administered 1.5 mL of water followed by immediate quercetin administration, (50 mg/kg in 0.5 mL of propylene glycol), and the third group was administered 50 mg/kg of quercetin in 2 mL of water. Therefore, all groups of rats received the same amount of quercetin in the same total volume. Before (control) and following quercetin administration, samples of tail vein

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blood were collected in heparinized tubes at certain intervals. Blood plasma was prepared by blood centrifugation for 20 min at 4 °C and 800*g*. During experiments animals received humane care in agreement with institutional guidelines.

Determination of Plasma Quercetin and Isorhamnetin. Quercetin and isorhamnetin (3′-methoxyquercetin) were determined by HPLC after extraction from the blood plasma. To 50 μL of plasma were added 50 μL of 0.2 M sodium acetate buffer, pH 5.0, and 900 μL of methanol/acetic acid (100:5, v/v). The mixture was vortexed for 30 s, sonicated for 30 s, again vortexed for 30 s, and centrifuged for 5 min at 4 °C and 5000*g*. Supernatant was diluted with water (1:1, v/v), and 20 μL was injected onto the HPLC column (TSKgel ODS-80TS, 5 μm, 150 × 4.6 mm, TOSOH, Japan). The flow of mobile phase composed of water/methanol/acetic acid (53:45:2, v/v/v) and 50 mM lithium acetate was 0.9 mL/min. Elution was monitored with amperometric electrochemical detector (ICA-3062, TOA, Japan) with working potential set at +800 mV. Experiments with flavonoid-spiked plasma showed that this procedure ensured a minimum of 95% recovery. Quantitative quercetin and isorhamnetin determination was performed according to an external standard method. The detection limits for quercetin and isorhamnetin were 0.05 and 0.1 μM, respectively, with linear detector response up to 20 μM. When necessary, samples were diluted with mobile phase prior to HPLC analysis.

Enzymatic Hydrolysis of Quercetin and Isorhamnetin Conjugates and Determination of Their Concentration in Plasma. Fifty microliters of rat plasma was mixed with 50 μL of sulfatase type H-5 solution in 0.2 M acetate buffer, pH 5 (500 units of β-glucuronidase and 25 units of sulfatase). The mixture was incubated at 37 °C in a shaking water bath for 50 min. Quercetin and isorhamnetin released during the incubation were extracted and determined as described above. The total conjugated quercetin or isorhamnetin plasma concentration was calculated as the difference between their plasma concentration after and before hydrolysis with sulfatase H-5.

To determine the quercetin and isorhamnetin glucuronides only, 25 units of β-glucuronidase type VII-A was used instead of sulfatase H-5 and the mixture was incubated for 2 h. Quercetin or isorhamnetin glucuronide plasma concentration was the difference between their plasma concentration after and before hydrolysis with β-glucuronidase VII-A.

Quercetin Solubility Test. The amount of quercetin solubilized in each of three vehicles [propylene glycol, water, and 25% propylene glycol in water (v/v)] used for administration was measured. Ten milligrams of quercetin was vortexed for 1 min with 2 mL of propylene glycol or water, sonicated for 1 min, and filtered through a 0.2 μm syringe filter. To check quercetin's solubility in 25% propylene glycol in water (v/v), 10 mg of quercetin was dissolved in 0.5 mL of propylene glycol, mixed with 1.5 mL of water, left for 10 min, and filtrated through a 0.2 μm syringe filter. Quercetin present in filtrates was regarded as solubilized, and its content was measured with the HPLC method as described above.

RESULTS

Figure 1 shows two representative chromatograms of extracts of rat blood plasma taken 6 h after oral administration of a single dose of quercetin, before (A) and after (B) enzymatic hydrolysis of conjugates. The hydrolysis with β-glucuronidase/sulfatase (Figure 1B) released quercetin (t_r = 11.58 min) and isorhamnetin (3′-methoxyquercetin) (t_r = 23.11 min) from their glucuronide and/or sulfate conjugates. The identity of the compounds was confirmed by comparison with authentic standards in HPLC and LC/MS analysis (da Silva et al., 1998).

Sensitive electrochemical detection enabled us to measure quercetin metabolites in small 50 μL (or less) portions of rat plasma. This allowed us to obtain a pharmacokinetic profile of plasma quercetin metabolites for each animal and significantly reduced the number of animals used for experiments because it was no longer necessary to kill a group of animals at each time point after administration. However, individual differences resulted in high variations in the concentrations of plasma metabolites, especially during the first hour following administration.

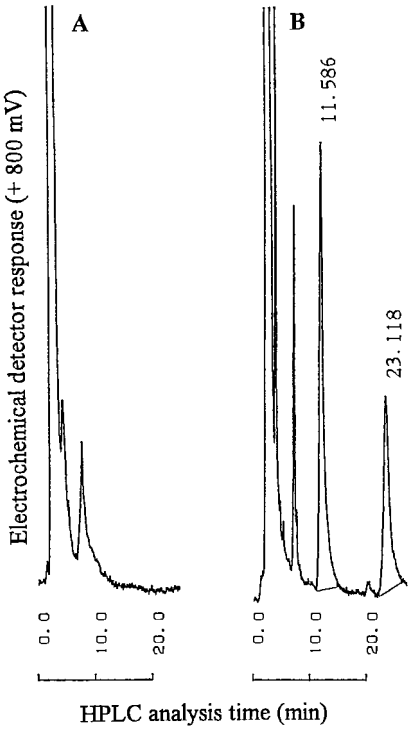

Figure 1. Representative HPLC chromatograms of rat blood plasma extracts 6 h after oral administration of 50 mg/kg qurecetin: (A) no plasma enzymatic hydrolysis; (B) plasma hydrolyzed with sulfatase/β-glucuronidase, peaks with t_r = 11.586 and 23.118 are quercetin and isorhamnetin, respectively.

Quercetin absorption profiles in three groups of animals are presented in Figures 2−4. In the first group, given 50 mg/kg quercetin in 2 mL of propylene glycol (glycol group), the maximum concentration of total plasma quercetin (conjugated and free, quercetin and isorhamnetin) of 70.3 μM was found at 0.5 h following administration (Figure 2). The second group, administered first 1.5 mL of water and subsequently 50 mg/kg quercetin in 0.5 mL of propylene glycol (water/glycol group), had its total maximum quercetin plasma concentration of 15.5 μM at only 0.25 h after administration (Figure 3). In the third group, administered 50 mg/kg of quercetin in 2 mL of water (water group), the plasma concentration of absorbed quercetin continuously rose through the experiment, reaching 5.5 μM at the end of observation. Only in the plasma of the glycol group was free, nonconjugated quercetin found at 0.25, 0.5, and 1 h following administration at the concentrations of 1.2 ± 0.6, 1.7 ± 0.9, and 0.3 ± 0.2 μM, respectively.

In the plasma samples of every animal group, all isorhamnetin was in the conjugated form. Moreover, in contrast to significant quercetin plasma concentration fluctuations, the plasma concentration of isorhamnetin continuously rose and in all groups 8 h after administration exceeded that of quercetin.

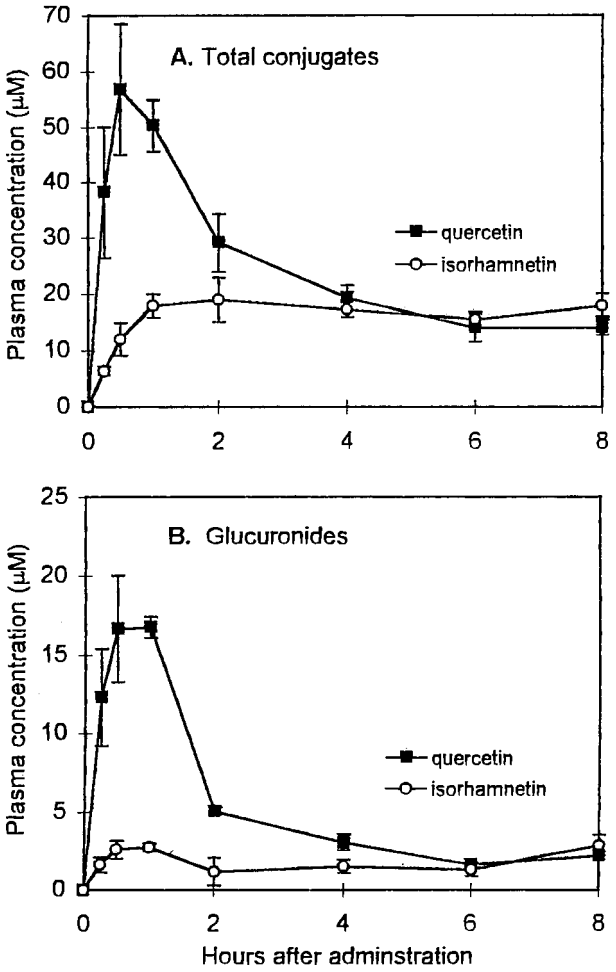

Figure 2. Quercetin metabolite concentration in rat plasma after oral administration of 50 mg/kg quercetin in 2 mL of propylene glycol. Values are the mean ± SEM ($n = 3$).

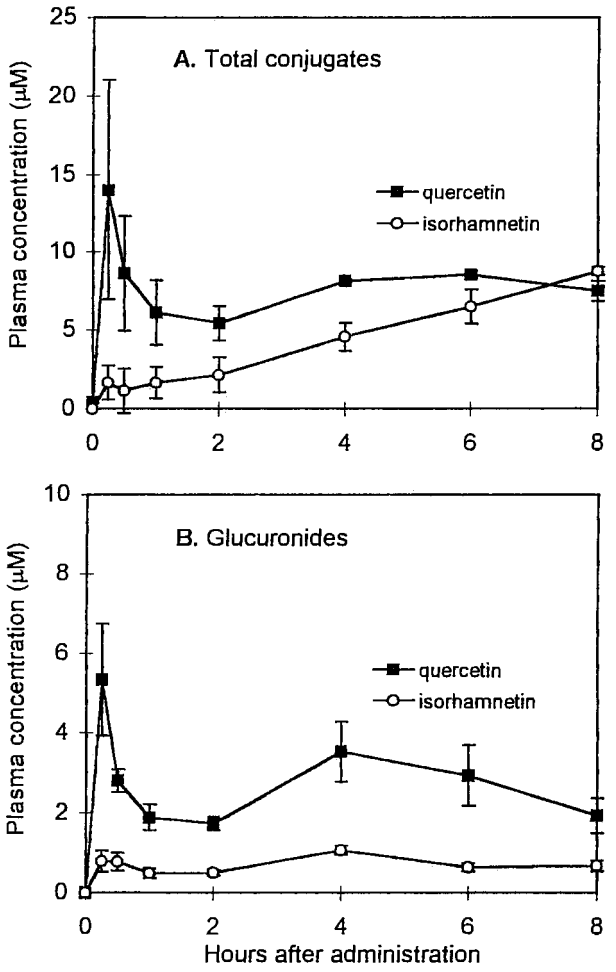

Figure 3. Quercetin metabolite concentration in rat plasma after oral administration of 1.5 mL of water followed by 50 mg/kg quercetin in 0.5 mL of propylene glycol. Values are the mean ± SEM ($n = 3$).

Quercetin's solubility in tested solutions was significantly different. If quercetin's solubility in propylene glycol is taken as 1 (complete solubilization), the relative quercetin solubility in 25% propylene glycol was found to be 3.8×10^{-3} and that in water 1.6×10^{-5}.

There were big differences in plasma concentrations between the total conjugated quercetin and especially isorhamnetin and their respective glucuronides (Figures 2−4). Because we could not find sulfated-only quercetin or isorhamnetin, those differences consist most probably for the mixed, sulfate/glucuronide, quercetin, and isorhamnetin conjugates.

DISCUSSION

Almost all of the quercetin metabolites found in rat plasma were in the form of glucuronide and sulfate/glucuronide conjugates (Figures 2−4), and the presence of nonconjugated quercetin during the first hour following administration in the glycol group was probably due to animals' overdosing. The presence of quercetin metabolites in the common blood circulation of animals in all groups as soon as 0.25 h after administration suggests that quercetin absorption started from the beginning of alimentary tract. Although the overall pattern of total conjugated quercetin plasma concentration changes was similar to that of quercetin glucuronides (Figures 2−4), there were significant differences among the groups, especially when the extent of absorption and the absorption profile during the first 2 h after quercetin administration were compared. In the glycol and water/glycol groups an initial rapid increase of quercetin plasma concentration followed by its also rapid decrease was observed. This phenomenon took place within the first 2 h in the glycol group and within the first hour in the water/glycol group (Figures 2 and 3). Probably, at least in part, those quercetin plasma concentration fluctuations were the reflection of changes in quercetin absorption rate combined with a very effective process of plasma quercetin elimination. The decrease of the quercetin absorption rate could be attributed to the proceeding of a quercetin precipitation process in the alimentary tract developed by stomach secretions (glycol group) or water with stomach secretions (water/glycol group), which lowered the amount of quercetin available for absorption. Indeed, quercetin solubility tests showed a decrease in the amount of solubilized quercetin in 25% propylene glycol in water by almost 3 orders and in water by 5 orders as compared to propylene glycol. In contrast, despite its low solubility, when administered as a water suspension, quercetin was absorbed and accumulated in rat plasma at a constant rate for 8 h (Figure 4). The lack of initial increase of quercetin plasma concentration followed by its rapid decrease in the water group supports the idea that those quercetin plasma changes found in the glycol and water/glycol groups were caused by quercetin

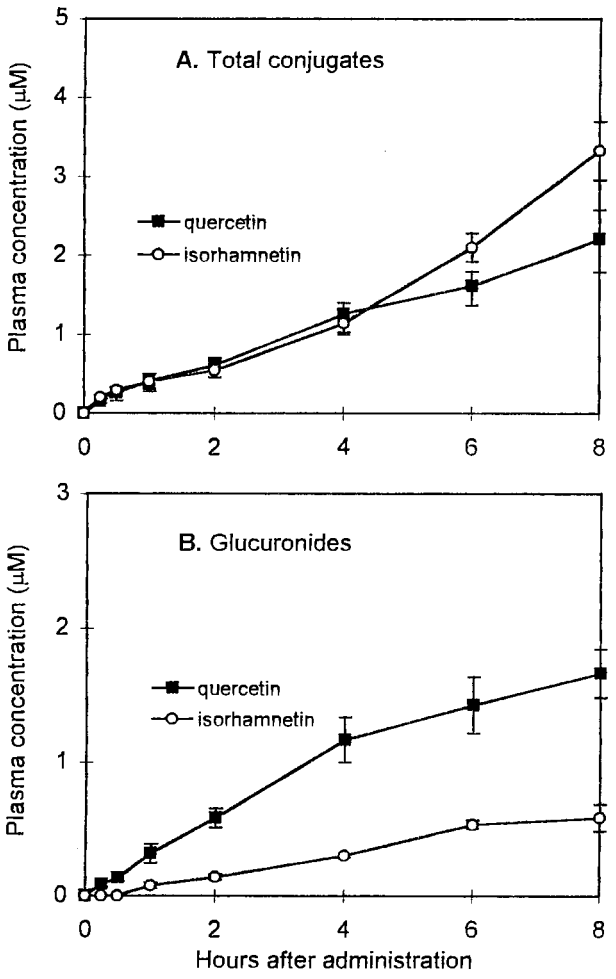

Figure 4. Quercetin metabolite concentration in rat plasma after oral administration of 50 mg/kg quercetin in 2 mL of water. Values are the mean ± SEM ($n = 3$).

precipitation in the alimentary tract. However, comparison of absorption profiles among groups with quercetin's solubility in vehicles used for administration shows that there was no direct correlation between the extent of absorption and quercetin's solubility. For instance, when the glycol and water/glycol groups were compared, despite the 3 orders lower quercetin solubility in the water/glycol group, the maximum plasma concentration decreased only ~4-fold. This indicates that apart from solubility there must also be other factor(s) influencing the quercetin absorption, such as propylene glycol itself or a certain threshold in particle size of the compound to be absorbed.

In a number of studies, including the present one, quercetin was administered to subjects (humans and animals) after overnight fasting. When the compound was administered as a food additive (1.5−45 mg/kg) (Hollman et al., 1995), the absorption was observed. On the other hand, when 4 g of quercetin in gelatin capsules together with 250 mL of water was administered to humans (~60 mg/kg), neither quercetin nor its conjugates were found in plasma or urine (Gugler et al., 1975). Nevertheless, quercetin was well absorbed when administered in solubilized form in DMSO (Ueno et al., 1983) or, as here, in propylene glycol. It seems that in addition to species differences or vehicles used for oral administration, bile plays an important role in the absorption of flavonoids from the alimentary tract. Its absorption-enhancing function was demonstrated in an experiment with 3-palmitoyl-(+)-catechin in rats (Hackett and Griffiths, 1982). Therefore, an alternative for better flavonoid absorption would be coadministration with some emulsifying agents. Moreover, catechin absorption was shown to be enhanced when catechin was administered along with phospholipids (P. G. Pietta and P. Simonetti, presented at the International Symposium on Antioxidant Food Supplements in Human Health, October 16−18, 1997, Kaminoyama, Japan; unpublished data).

From Figures 2−4 it is clear that a significant part of absorbed quercetin was in the form of isorhamnetin, the quercetin 3′-methoxy derivative. Despite the methylation of quercetin having been previously reported (Ueno et al., 1983) and found also for epicatechin, another flavonoid with a catechol-like structure in the B ring (Shaw and Griffiths, 1980), the isorhamnetin plasma concentration was never shown as a function of time following single oral quercetin administration, especially with discrimination of isorhamnetin glucuronides from its total plasma content. This discrimination enabled us to reveal that isorhamnetin (methylated quercetin), analogically to methylated epicatechin (Piskula and Terao, 1998), was present in rat blood plasma mainly in the form of mixed sulfate/glucuronide conjugates.

In all groups the ratio of isorhamnetin/quercetin concentration in plasma increased during the experiment; 8 h following administration of isorhamnetin, plasma concentration exceeded that of quercetin, especially in the water group. It was demonstrated that the ratio of methylated/nonmethylated quercetin in the plasma of rats fed 0.25% quercetin diet for 14 days was 5, compared to 1.7 in bile and urine (Manach et al., 1996). This suggests preferential excretion of nonmethylated quercetin or very effective quercetin methylation, which leads to the increase of plasma concentration of isorhamnetin.

The previously mentioned biological activity of flavonoids, and quercetin among them, is based on epidemiological studies and on in vitro experiments. To verify those findings in vivo, more research is needed on quercetin metabolites rather than on quercetin itself. After absorption, quercetin is present in the common blood circulation in the form of various metabolites for a time sufficiently long to affect the organism. Especially during the first period after absorption when quercetin is not yet fully metabolized, it can probably exhibit some of its in vitro activities. It is clear from this study that the extent of quercetin absorption in part depends on the solubility in the vehicle used for administration. However, it is important to realize that after oral administration, there is secretion of stomach fluids, which might have impact on the absorption process via a decrease of the administered compound's solubility. Using propylene glycol as the vehicle for quercetin administration enables us to obtain a very high quercetin plasma concentration in a short time, which might be very useful for studying the biological activity of quercetin in vivo. However, it must be emphasized that the proportion of particular metabolites, that is, quercetin/isorhamnetin, during initial high absorption is different from the situation when quercetin is ingested with diet.

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