Free Rad. Res., Vol. 31, pp. 569–573
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The Sugar Moiety is a Major Determinant of the Absorption of Dietary Flavonoid Glycosides in Man

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Accepted by Prof. B. Halliwell

(Received 22 March 1999; In revised form 7 April 1999)

Flavonoids are antioxidants present in plant foods. They occur mainly as glycosides, i.e. linked with various sugars. It is uncertain to what extent dietary flavonoid glycosides are absorbed from the gut. We investigated how the nature of the sugar group affected absorption of one major flavonoid, quercetin. Quercetin linked with glucose, i.e. quercetin glucoside and quercetin linked with rutinose, i.e. quercetin rutinoside, both occur widely in foods. When we fed these compounds to nine volunteers, the peak concentration of quercetin (C_{max}) in plasma was 20 times higher and was reached (T_{max}) more than ten times faster after intake of the glucoside ($C_{max} = 3.5 \pm 0.6\,\mu M$ (mean ± SE); $T_{max} < 0.5\,h$) than after the rutinoside ($C_{max} = 0.18 \pm 0.04\,\mu M$; $T_{max} = 6.0 \pm 1.2\,h$). The bioavailability of the rutinoside was only 20% of that of the glucoside. We suggest that quercetin glucoside is actively absorbed from the small intestine, whereas quercetin rutinoside is absorbed from the colon after deglycosylation. Absorption of other food components might also be enhanced by attachment of a glucose group.

Keywords: Absorption, bioavailability, flavonoids, glycosides, sodium–glucose cotransporter

INTRODUCTION

Flavonoids are polyphenolic compounds from plants which potentially are beneficial to human health. Humans take in several hundred milligrams of flavonoids per day from vegetables, fruits, tea and wine. Flavonoids are antioxidants[1] and may prevent lipid peroxidation and the formation of atherosclerotic plaques.[2] Indeed, the intake of the flavonoid quercetin was inversely associated with cardiovascular disease in several,[3–6] though not all[7,8] studies in humans.

Formerly, dietary flavonoids were thought to be poorly absorbed from the intestine[9] because in foods they are mostly present as conjugates of sugars called glycosides. The sugar-flavonol bond is a β-glycosidic bond which is resistant to hydrolysis by pancreatic enzymes.[9] However, we unexpectedly found that human absorption of quercetin-β-glucosides was higher than the absorption of quercetin without its sugar moiety,

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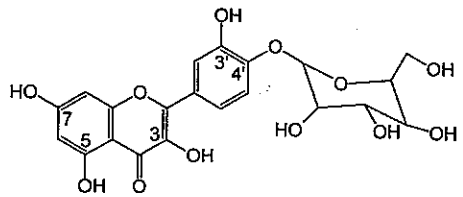
the so-called aglycone, and of quercetin-β-rutinoside.[10] Rutinose is a disaccharide consisting of glucose and rhamnose (Figure 1). In a subsequent study we found that the bioavailability of quercetin glucosides from onions was also superior to that of various quercetin glycosides from apples and to that of pure quercetin rutinoside.[11] These data suggested that the sugar moiety of quercetin glycosides is an important determinant of their absorption, but left open the possibility of matrix effects of the foods. The present study was designed to determine whether it is indeed the sugar moiety which determines quercetin absorption in humans. To that end we compared the time course of the quercetin concentration in plasma after administration of pure quercetin-β-glucoside or pure quercetin-β-rutinoside (Figure 1). The glucoside is a major flavonoid in onions,[12] and the rutinoside occurs in tea and wine.[13,14]

MATERIALS AND METHODS

The study was approved by the Ethical Committee, and participants gave their informed consent.



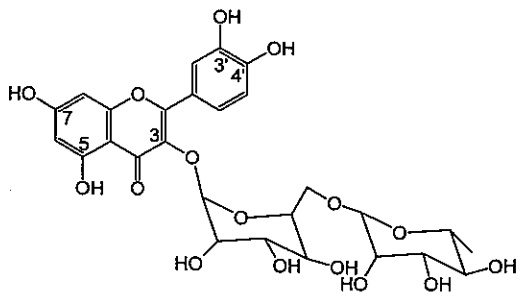


FIGURE 1 Structures of the quercetin glycosides used.

Nine healthy volunteers followed a quercetin-free diet.[10] To ensure a quercetin-free diet, subjects were given a list of vegetables and fruits containing more than 15 mg quercetin/kg and of beverages with more than 4 mg quercetin/l, and were instructed not to consume any of them. After two days we fed four over-night fasting subjects 311 μmol of quercetin-4′-*O*-β-D-glucoside (Spiraeosid 4564, Brunschwig Chemie B.V.) and five days later 311 μmol quercetin-3-*O*-β-rutinoside (Rutosidum DAB, OPG Farma), both dissolved in 10 ml ethanol plus 200 ml water containing 2 g NaCl. The other five received the same treatments in reverse order. Venous forearm blood samples were taken into vacuum tubes containing EDTA at the times depicted in Figure 2, and plasma was prepared and stored at −80°C as described previously.[15]

Quercetin conjugates were hydrolyzed to the aglycone form with HCl/methanol for 5 h at 90°C. Plasma quercetin was determined with HPLC and fluorescence detection after derivatization into a fluorescent quercetin–aluminum complex.[11,16] Peak identity was confirmed by comparing the retention time of plasma quercetin with that of a standard quercetin. Potential co-elution of fluorescent non-flavonol compounds was checked by repeating the HPLC procedure,

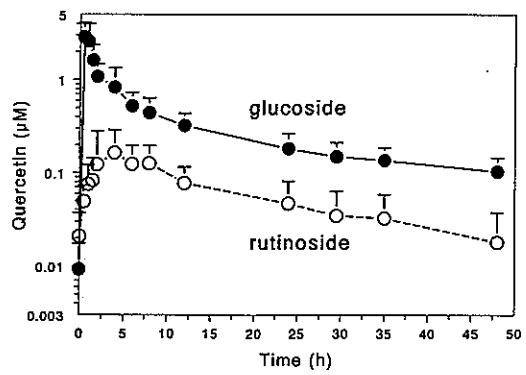


FIGURE 2 Total quercetin concentration (mean ± SD) in plasma of nine subjects after ingestion of 311 μmol quercetin glucoside (●) and 311 μmol quercetin rutinoside (○). Each subject received each supplement in random order.

this time by omitting aluminum. Such compounds were never observed.

Pharmacokinetic parameters were calculated with nonlinear regression analysis on assumption of a two-compartment open model.

RESULTS

After intake of the glucoside, the concentration of quercetin in plasma increased much more rapidly and to a higher level than after intake of the rutinoside (Figure 2). The mean peak plasma concentration of quercetin was about 20 times higher after the glucoside than after the rutinoside (Table I). Peak concentrations were reached within 0.5 h after ingestion of the glucoside, whereas after the rutinoside the peak was reached much slower (Figure 2, Table I). In seven out of nine subjects the quercetin concentration had reached its maximum already at the first blood sampling, 0.5 h after ingestion of the glucoside supplement; therefore the actual maximum may have been even higher and may have been reached earlier than suggested by Figure 2. The quercetin concentration in plasma decreased similarly for the two sources: half-lives of elimination were about 24 h (Table I). By comparing the areas under the plasma concentration-time curves (Table I), we calculated that the bioavailability of the rutinoside was 20% of that of the glucoside.

TABLE I Pharmacokinetic parameters (mean ± SE) of quercetin glycosides after one-time ingestion of 311 μmol quercetin-4′-*O*-*β*-D-glucoside and 311 μmol quercetin-3-*O*-*β*-rutinoside in nine subjects. Each subject received each supplement in random order. Data were fitted to a two-compartment open model

Parameter	Supplement	
	Quercetin-4′-*O*-*β*-D-glucoside	Quercetin-3-*O*-*β*-rutinoside
C_{max} (μM)	3.5 ± 0.6	0.18 ± 0.04
T_{max} (h)	< 0.5	6.0 ± 1.2
Elimination half-life (h)	21.6 ± 1.9	28.1 ± 6.4
$AUC_{0-\infty}$ (μM · h)	18.8 ± 2.4	3.7 ± 0.7

$AUC_{0-\infty}$ = Area Under the plasma Concentration-time curve extrapolated to infinity.

DISCUSSION

We found that the glucoside of quercetin was absorbed much more efficiently than the rutinoside. The glucoside produced its peak quercetin concentration in plasma after a time interval similar to that for D-glucose.[17] We therefore speculate that the glucoside is absorbed from the small intestine, whereas the rutinoside might transit the small intestine without absorption and might be absorbed from the colon. The position of the sugar moieties differs between the two molecules. However, our preliminary studies (Olthof *et al.*, in preparation) showed that the 3-glucoside was absorbed as efficiently as the 4′-glucoside. Thus it is the nature and not the position of the sugar moiety which controls absorption. If indeed the glucoside is absorbed from the small intestine while the rutinoside is not then this implies that the intact quercetin glucoside is able to pass across the endothelial membrane for the following reasons. One reason is that these *β*-glycosides are resistant to hydrolysis by HCl in the stomach.[18] Another reason is that *β*-glycosidases are not secreted into the small intestine.[9] A third reason is that the broad-specificity *β*-glucosidases needed to hydrolyze quercetin glucoside are not bound to the brush border membrane.[19] The presence of quercetin-4′-glucoside in human plasma[20] also suggests that the intact glucoside may be absorbed.

We therefore suggest that flavonoids conjugated with glucose are carried as such into the small-gut enterocyte. As a speculation we suggest that this may involve active transport, for instance via the intestinal Na^+-glucose cotransporter. Studies using everted intestinal sacs or mucosal cell preparations from rodents suggest that glucose can be transported by the Na^+-glucose cotransporter even if attached to bulky ligands. Nitrophenyl-*β*-D-glucoside, nitro phenyl-*β*-D-galactoside, naphthol-*β*-D-glucoside and naphthol-*β*-D-galactoside were transported across the intestinal wall of rats by the

Na^+-glucose cotransporter[21,22] as were methylazoxymethanol-β-D-glucoside[23] and sulphamethazine-D-glucoside.[24] Those phenyl-β-D-glucosides that were actively transported shared common three-dimensional structures.[25] So far, two *in vitro* studies on absorption mechanisms of quercetin glucosides have been published. In everted sacs of rat jejunum, quercetin glucosides were capable of binding to the Na^+-glucose cotransporter in a sodium dependent way.[26] However, no evidence for active transport of quercetin glucosides was found in human intestinal epithelial Caco-2 cells.[27] Thus, *in vitro* evidence for active transport of quercetin glucosides is still conflicting.

Active transport of β-D-glucosides would explain why quercetin is extensively absorbed from onions – in which it occurs as glucosides – and why free quercetin is only poorly absorbed.[10] Quercetin-3-rutinoside is probably absorbed poorly because rutinose cannot be transported by the putative transporter. This active glucoside transport also would explain that the bioavailability of quercetin glucosides from onions was superior to that of various quercetin glycosides from apples and of pure quercetin rutinoside.[11]

Acknowle dgment s

This work was supported by the Foundation for Nutrition and Health Research and the Netherlands Heart Foundation (94.128).

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