Comparison of the Intestinal Absorption of Quercetin, Phloretin and Their Glucosides in Rats

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ABSTRACT Absorption and metabolism of quercetin and isoquercitrin (quercetin 3-O-glucose) were investigated in rats after in situ perfusion of jejunum plus ileum (15 nmol/min) for 30 min and compared with those of phloretin and phloridzin (phloretin 2′-O-glucose). After perfusion of the glucosides, the corresponding aglycone forms and conjugated derivatives appeared in the lumen. The conjugated metabolites were similar to those recovered after intestinal perfusion of the aglycone forms. Regardless of the aglycone or glucoside perfused, only conjugated forms were present in the mesenteric vein blood draining the perfused segment showing the importance of intestinal conjugation. The hydrolysis of glucosides was a prerequisite step before their conjugation by intestinal enzymes and their transport towards the mucosal and serosal sides. In contrast to phloridzin, lactase phloridzin hydrolase activity did not seem to be an essential pathway for isoquercitrin hydrolysis. The 3-O-glucosylation of quercetin improved the net absorption of the aglycone (P < 0.05), whereas phloretin absorption decreased when present as 2′-O-glucoside (P < 0.05). Whatever the perfused compound, the efficiency of the absorption seemed to be linked to the intestinal conjugation process and to the luminal secretion of metabolites. J. Nutr. 131: 2109–2114, 2001.

KEY WORDS: quercetin • phloretin • flavonoid glucosides • rats • in situ perfusion

Flavonoids are widely distributed in edible plants (1) and constitute an integral part of the human diet. The most important groups are anthocyanidins, catechins, flavones, flavonones and flavonols (2). Nevertheless, some minor classes of flavonoids, such as chalcone and dihydroxychalcone, must also be taken into account.

In the flavonol category, quercetin is the most abundant compound in vegetables and fruits and many biological properties are associated to this compound: antibacterial, antiviral, antioxidant, antiproliferative, antiinflammatory and anticarcinogenic effects (3,4). In food, quercetin is present as glycosylated forms, mainly as β-glycosides (5), and the nature of glycosylation markedly influence the efficiency of quercetin absorption. In vivo, it has been shown that absorption and metabolism of quercetin or of quercetin glucosides took place in the small intestine (6,7), whereas rutin (quercetin-3-O-glucose-rhamnose) cannot be absorbed at this level (8–10). Before its absorption, rutin must be hydrolyzed by the cecal microflora and then released quercetin can be directly absorbed or degraded as phenolic acids (11–13).

It appears that the hydrolysis of the glucoside moiety constitutes the first step of the intestinal metabolism of the glucosylated forms. Therefore, using a Caco-2 cells line model, quercetin glucosides were first deglycosylated in the process of intestinal digestion (14). A cytosolic β-glucosidase, located in the small intestine, exhibited activity toward flavonol glucosides (15,16). Another β-glucosidase presents on the brush border of the intestine, lactase phloridzin hydrolase (LPH)2 is characterized by its substrate specificity toward phloridzin (2′-O-glucoside) (17,18). This compound is a classic competitive inhibitor of the intestinal sugar carrier: Na+/glucose cotransporter (SGLT1) (19) and is considered to be an antidiabetic agent (20,21). It is of interest to determine whether flavonol glucosides, which have a structure quite similar to phloridzin (Fig. 1), may also be good substrates for the β-glucosidase site of LPH.

Therefore, in the present study, we compared in rats the intestinal absorption and metabolism of quercetin and isoquercitrin (quercetin 3-O-glucose) and those of phloretin and phloridzin (phloretin 2′-O-glucose) using an in situ intestinal perfusion model. Moreover, to investigate whether LPH plays a crucial role in flavonol glucoside hydrolysis, perfusion experiments were performed in the simultaneous presence of phloridzin (at high concentration) and isoquercitrin. By providing new data about intestinal absorption and metabolism of flavonoid glucosides, this type of study is required to further investigate and understand the biological effects of these compounds.

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2 Abbreviations used: LPH, lactase phloridzine hydrolase; SGLT1, Na+/glucose cotransporter.
HPLC analysis

Sample treatment. Plasma and perfusate samples were spiked with internal standard namely: diosmetin (14 μmol/L) in quercetin and isocleritrin experiments or hesperetin (3.5 μmol/L) for phloretin and phloridzin experiments. They were acidified (to pH 4.9) with 0.1 volume of acetic acid (0.58 mol/L). The samples were treated for 30 min at 37°C in the absence (unconjugated forms) or in the presence (total forms) of 5 × 10^6 U/L β-glucuronidase and 2.5 × 10^5 U/L sulfatase. The reactions were stopped by adding of 2.85 volumes of acetonitrile and the resulting mixtures were centrifuged for 4 min at 14,000 × g. Supernatant (20 μL) was injected and analyzed by HPLC. The concentrations of conjugated derivatives were estimated as the difference between the concentrations of quercetin measured before and after the enzymatic treatment. For the effluent, all the concentrations measured have been corrected by taking into account the intestinal absorption of water as previously described (10).

Chromatographic conditions. The HPLC system used consisted of an autosampler (Kontron 360), an ultraviolet detector (set at 370 nm for flavonols and at 280 nm for dihydroxychalcones) and a software system for data recording and processing. The system was fitted with a 5-μm C-18 Hypersil BDS analytical column (150 × 4.6 mm; Life Sciences International, Cergy, France). The mobile phase consisted of water/H_3PO_4 (99.5: 0.5; solvent A) and acetonitrile (solvent B).

To visualize and separate the conjugated metabolites of flavonoids, the chromatographic conditions were as follows (flow rate: 1 mL/min): 0–2 min: solvent A 85%/solvent B 15%; 2–22 min: linear gradient from solvent A 85%/solvent B 15% to solvent A 60%/solvent B 40%; 22–24 min: solvent A 60%/solvent B 40%; 24–27 min: return to initial mobile phase conditions, then equilibration for 8 min.

Glucose measurements. The glucose concentration was determined by an enzymatic method (22).

Statistics. Values are means ± SEM, and the differences were determined by one-way ANOVA coupled with the Student-Newman-Keuls multiple comparison test. Differences with P < 0.05 were considered significant. The statistical analysis of the glucose absorption was realized by one-way ANOVA coupled with the Tukey post-hoc test.

RESULTS

Intestinal metabolism of isocleritrin and quercetin. When quercetin was perfused for 30 min (at 14.70 ± 0.60 nmol/min) in the intestinal lumen, this flavonol was recovered in the intestinal effluent as the native form (4.90 ± 0.40 nmol/min) and as conjugated forms (7.70 ± 0.40 nmol/min; Fig. 2). When the effluent was hydrolyzed by a β-glucuronidase/sulfatase, 86% of perfused dose was recovered in the effluent at the end of perfusion. Thus, the remaining of perfused quercetin (14%) corresponded to the net absorption of this compound by the intestinal wall.

When isocleritrin (14.20 ± 0.40 nmol/min) was perfused instead of quercetin, the flux of intact glucoside recovered at the end of perfusion was 3.13 ± 0.50 nmol/min, compared with the quercetin perfusion, which the aglycone was found at 4.90 ± 0.40 nmol/min (Fig. 2). This result suggests that the transport through the brush border was more efficient for isocleritrin than for its aglycone. The HPLC profile corresponding to the effluent of isocleritrin perfusion (Fig. 3) was characterized by a peak of isocleritrin, one of free quercetin (1.85 ± 0.50 μmol/L) and four unidentified peaks (noted 1–4). Isocleritrin was stable in the buffer (data not shown); thus, quercetin recovered in the effluent originates from the partial hydrolysis of the glucoside at the level of the intestinal wall. The four unidentified peaks completely disappeared after enzymatic treatment by a β-glucuronidase/sulfatase, indicating that they corresponded to conjugated derivatives. These peaks had retention time similar to those of the conjugated forms of
quercetin found in experiments in which quercetin was perfused (Fig. 3). This similarity suggests that the conjugated forms recovered after isoquercitrin perfusion could correspond to the conjugated forms of quercetin and that isoquercitrin was first hydrolyzed into quercetin before its conjugation by the intestinal enzymes. Taking into account the respective retention times, compared with diosmetin (internal standard), it may be noted that one of the quercetin conjugates (peak 1; Fig. 3) presents a chromatographic behavior close to that of isoquercitrin. In fact, it is difficult to distinguish between glucoside and endogenous conjugated derivatives.

The flux of conjugated forms secreted into the mucosal side depended on the nature of the perfused compound. It reached 39% for isoquercitrin versus 52% for quercetin \( (P < 0.05; \text{Fig. 2}) \). In parallel, the net absorption of isoquercitrin perfusion was higher than that observed after quercetin perfusion \( (P < 0.05; 25\% \text{ vs. } 14\%, \text{respectively}) \). These data suggest that the 3-O-glucosylation of quercetin improved its net absorption by the intestinal wall. After quercetin or isoquercitrin perfusion, no native forms were present in nonhydrolyzed plasma from mesenteric vein (data not shown). After enzymatic hydrolysis, quercetin appeared in the plasma, indicating that the circulating forms of quercetin were conjugated metabolites.

After a quercetin or an isoquercitrin perfusion, the arteriovenous difference found in quercetin concentration was \( 1.11 \pm 0.50 \text{ nmol/min} \). Nevertheless, because the blood flow was not measured, we did not quantify the flavonol absorption.

**Intestinal metabolism of phloretin and phloridzin.** When phloretin was perfused into the intestinal lumen at a rate of \( 14.40 \pm 0.49 \text{ nmol/min} \), a portion of the aglycone was directly excreted in the effluent at the end of perfusion \( (6.06 \pm 0.62 \text{ nmol/min}; \text{Fig. 4}) \). In parallel to this excretion, the dihydroxychalcon was recovered as conjugated forms \( (4.73 \pm 0.38 \text{ nmol/min}) \), as shown by their disappearance in the presence of \( \beta \)-glucuronidase/sulfatase. As quercetin, phloretin was metabolized by intestinal conjugative enzymes, and some of these conjugated forms (glucuronides and/or sulfates) were secreted into the mucosal side. At the end of the perfusion, the 3.61 ± 0.20 mmol/min of perfused phloretin not recovered in the effluent corresponds to the net absorption of this compound by the intestinal wall.

As phloretin, phloridzin was perfused and found in the effluent as native form \( (2.67 \pm 0.23 \text{ nmol/min}) \) and also as aglycone \( (6.77 \pm 0.50 \text{ nmol/min}; \text{Fig. 4}) \). This result suggests that hydrolysis of phloridzin to phloretin is not a limiting step for its absorption, because 80% of this glucoside was hydrolyzed. After a phloridzin or phloretin perfusion, the secretion of aglycone was not different \( (0.05 < P < 0.1; \text{Fig. 4}) \). Moreover, the analysis of HPLC profiles indicated that the conjugated forms of phloridzin found in the effluent corresponded to those recovered after phloretin perfusion (data not shown). This strongly suggests that the hydrolysis of phloridzin preceded its metabolism by the intestinal conjugative enzymes. When phloridzin was perfused, its absorption by the intestinal wall was markedly lower than that measured when the aglycone was perfused \( (1.11 \pm 0.50 \text{ nmol/min} \text{ vs. } 3.60 \pm 0.20 \text{ nmol/min}) \).
nmol/min; P < 0.05, respectively). Moreover, the flux of the secretion of conjugated metabolites was significantly reduced (P < 0.05) when phloridzin was perfused instead of phloretin (2.87 ± 0.89 nmol/min and 4.73 ± 0.38 nmol/min, respectively).

When phloridzin (phloretin 2'-O-glucose) was present in the perfusion medium, the glucose absorption, measured at the end of perfusion period, was markedly decreased (−42% compared with control; Table 1). By contrast, glucose absorption was not modified in the presence of phloretin (0.05 < P < 0.1).

These data show that the presence of a glucose moiety affects intestinal metabolism of phloretin. Moreover, in contrast to that observed with quercetin 3'-O-glucoside, the 2'-O-glucoylation of phloretin does not favor its net absorption.

**Comparison of intestinal metabolism of isoquercitrin and phloridzin.** After an isoquercitrin or phloridzin perfusion, the levels of the glucosylated forms recovered in the effluent were not different (0.05 < P < 0.1; Figs. 2 and 4). This indicates that the intestinal wall hydrolyzed the two glucosides to the same extent. When phloridzin was perfused, the excretion of the aglycone form was markedly higher than that observed after isoquercitrin perfusion (6.77 ± 0.50 nmol/min vs. 1.85 ± 0.50 nmol/min, respectively; P < 0.05). Moreover, the secretion of conjugated forms and the net absorption of isoquercitrin were more important than were those of phloridzin (P < 0.05; Figs. 2 and 4). This result shows that the net transfer of quercetin from isoquercitrin into the intestinal wall is more efficient than that of phloretin from phloridzin. It should be noted that, in contrast to phloridzin, glucose absorption was not modified by isoquercitrin (Table 1).

**Intestinal metabolism of isoquercitrin in presence of phloridzin.** When perfused at 146.40 ± 3.50 nmol/min, phloridzin markedly inhibited glucose absorption (−83%; Table 1). When phloridzin and isoquercitrin were perfused together, the concentration of isoquercitrin recovered in the effluent was not different (0.05 < P < 0.1) from that observed with isoquercitrin alone (3.74 ± 0.30 nmol/min vs. 3.13 ± 0.50 nmol/min, respectively; Fig. 5). This indicates that the presence of phloridzin did not affect the capacity of isoquercitrin.
to enter the intestinal cells and its hydrolysis. Moreover, as shown in the Figure 5, the excretion of quercetin consecutive to isoquercitrin perfusion was not modified by phloridzin (0.05 < P < 0.1). By contrast, phloridzin markedly decreased the secretion of conjugated forms of quercetin (3.33 ± 0.30 nmol/min vs. 5.55 ± 0.40 nmol/min for isoquercitrin alone; P < 0.05). This phenomenon led to a 61% increase in the net absorption of isoquercitrin (5.81 ± 0.26 nmol/min vs. 3.61 ± 0.13 nmol/min for isoquercitrin alone; P < 0.05).

**DISCUSSION**

The aim of this work was to study the absorption and the intestinal metabolism of quercetin and of one of its glucosides, isoquercitrin. The metabolism of these compounds has been compared with that of phloretin and its glucoside, phloridzin. After intestinal infusion of quercetin or phloretin, some conjugated forms were recovered in the effluent. Because the biliary duct was cannulated at the beginning of the perfusion, these conjugated forms were necessarily of intestinal origin. Conjugative enzymes are present in the enterocytes (23,24) and this enzymatic equipment allows intestinal cells to glucuronidate, sulfate and methylate flavonoids (14,25,26). Moreover, using Caco-2 cells, Plumb et al. (27) have shown that when quercetin was added in the apical side, some conjugated quercetin were recovered on this side, indicating that quercetin had been absorbed and metabolized in the cells and then transported back to the apical side. These data are consistent with our results and confirm that enterocytes constitute an effective site for flavonoids conjugation.

After an isoquercitrin or phloridzin infusion, the conjugated forms recovered in the effluent were similar to those found after perfusion of the corresponding aglycone. This result is corroborated by a previous study (28), performed with everted intestine, and showing that the conjugated derivative recovered on the mucosal side was identical in experiments using phloretin or phloridzin. Nevertheless, it could not be excluded that microflora present into the intestinal lumen contribute to glucoside hydrolysis and to conjugation of the aglycones (29,30). In our experiments, because the rats were deprived of food and because the first 20 min of the perfusion step washed the intestinal contents, the contribution of the intestinal microflora to the metabolism of flavonoids should be quite limited.

Some studies have reported that quercetin glucosides are present in human plasma without metabolic conversion (31,32). However, in the present study, we did not detect any intact aglycone or glucosylated forms in the mesenteric vein, showing that all the glucosides were necessarily hydrolyzed and conjugated before their transfer on the serosal side. Indeed, recently, using a multielectrode coulometric detection, together with suitable chromatographic conditions, we have shown that the circulating metabolites present in the plasma of rats fed an isoquercitrin meal or a quercetin meal were identical and corresponded to conjugated derivatives of quercetin, such as glucuronidated, sulfated and methoxylated forms (7,33).

The present study clearly demonstrated that the hydrolysis of the glucosyl moiety constitutes a crucial step in the intestinal metabolism of flavonol and dihydroxychalcone glucosides. When glucosides were perfused, the corresponding aglycone form was recovered in large amount in the effluent. The hydrolysis of phloridzin to phloretin is performed by the β-glucosidase site of LPH, present on the brush border of the enterocytes (18). Phloridzin is considered to be a competitive inhibitor of the glucose/Na⁺-dependent carrier (19,34), but the dihydroxychalcone moiety is not transported by SGLT1 (35). Furthermore, our data show that a large proportion of the phloretin released from phloridzin hydrolysis diffused directly into the effluent, and we observed that the magnitude of the secretion of quercetin from isoquercitrin was considerably lower. The high secretion of phloretin into the lumen could be related to the extracellular hydrolysis of the glucoside and/or to differences between the activities of intestinal conjugation of each compound.

When isoquercitrin was perfused, the magnitude of its hydrolysis into quercetin was of the same order of magnitude as that of the dihydroxychalcone glucoside. In the presence of a high flux of phloridzin (150 nmol/min), the excretion of isoquercitrin and the secretion of quercetin from isoquercitrin were not affected. These results suggest that the hydrolysis of isoquercitrin by LPH did not constitute a limiting step for its intestinal metabolism and support the hypothesis that isoquercitrin could be hydrolyzed by a cytosolic β-glucosidase (15,16). Such a process could contribute to decrease the secretion of the aglycone into the lumen as observed in our study. Phloridzin did not influence the hydrolysis of isoquercitrin and it is not excluded that the lactase site of LPH could participate in its hydrolysis. Recently, it has been shown in vitro that the lactase site of LPH was responsible for the hydrolysis of different glucosides and particularly, that of quercetin glucosides (36).

It has been previously suggested that SGLT1 was involved in the transport of flavonol glucosides into the intestinal cells (6). Using the technique of the everted intestine, it has been shown that quercetin glucosides are able to interact with the sodium-dependant glucose transport (37). However, no evidence for an active transport of these compounds was found in experiments performed on human intestinal epithelial Caco-2 cells (38). In our study, when phloridzin was perfused, the activity of SGLT1 was affected, as reflected by the marked inhibition of glucose absorption. However, the absorption of isoquercitrin was not significantly modified by the presence of phloridzin. These whole data do not account for the involvement of SGLT1 in the intestinal transport of isoquercitrin and raise questions about the nature of the transporter responsible for isoquercitrin entry into intestinal cells in vivo.

After a quercetin infusion, the secretion of conjugated forms was higher than after an isoquercitrin infusion. By contrast, the net absorption increased when isoquercitrin was perfused instead of its aglycone form. This result, obtained in situ, is in accordance with the in vivo studies reporting that, in humans, glucosylated forms of quercetin were more efficiently absorbed than quercetin itself (6,39). In our experiments, the direct perfusion of quercetin could result in a high concentration of this compound in the enterocyte, leading to an enhancement in the rates of conjugation and transfer of conjugated forms across the brush border into the intestinal lumen. For isoquercitrin, this phenomenon could be limited by the prerequisite step of hydrolysis to quercetin. In the same way, the secretion of conjugated metabolites seems to be more important for phloretin than for phloridzin. Thus, the O-glucosylation of quercetin and phloretin improved the secretion of conjugated metabolites in the lumen.

We did not detect any trace of perfused compounds in intestinal mucosa extract (data not shown), indicating the absence of binding to the enterocytes. In such conditions, the difference between the concentrations measured in the original perfusate and in the remaining perfusate corresponded to the real net absorption.

The comparison of the net absorption of quercetin and phloretin indicated that the absorption of the flavonol was
better, whereas the secretion of conjugated metabolites was lower for phloretin than for quercetin. This suggests that quercetin enters the cells more readily than phloretin, possibly because of the planarity of the quercetin molecule. The higher net absorption of isoorientin, compared with that of phloretin, may be due to differences in the hydrolysis step. The extracellular hydrolysis of phloridzin may have elicited a production of the aglycone directly in the lumen, leading to a decrease of its net absorption. By contrast, the intracellular hydrolysis of isoorientin could limit the excretion of quercetin on the mucosal side and favor its net absorption.

The presence of phloridzin decreased the intestinal conjugation of isoorientin. The decreased secretion of quercetin conjugation in the lumen led to an improvement in the net absorption of isoorientin. This result could be explained by a saturation of the intestinal capacity of conjugation and/or to a limitation of the secretion into the mucosal side when the two flavonoid glucosides are simultaneously present in the lumen. In a previous study of the interactions between phlorizin and β-p-nitrophenol at the intestinal level, it was shown that the inhibition of the glucuronidation process improved intestinal absorption of the drug (40). Thus, the intestine could limit the net absorption by an extensive secretion of conjugates.

In conclusion, the present study clearly shows that the absorption of flavonoid glucosides was highly dependent on the mechanisms involved in their hydrolysis and on the activity of their intestinal conjugation. However, additional investigations should be performed to determine the nature of the carrier involved in flavonol glucoside transport at the intestinal level.

LITERATURE CITED