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Intestinal absorption of rutin in free and conjugated forms

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Abstract

Quercetin is one of the most common flavonoids in nature, occurring mainly in glycosidic forms such as rutin. Rutin has been reported to exert numerous biochemical and pharmacological activities, though information about its absorption and metabolism is scarce. The aim of this study was to investigate intestinal handling of luminally administered rutin in an isolated preparation of luminally and vascularly perfused rat small intestine. A synthetic perfusate free from blood components was used as vascular medium, with a perfluorocarbon as oxygen carrier. Luminal media consisted of a bicarbonate-buffered sodium chloride solution spiked with rutin (40.5 ± 1.8 μmol/L). Viability was maintained during the entire perfusion; no differences between rutin and control perfusions for perfusion pressure, lactate–pyruvate ratio, oxygen uptake, and acid–base homeostasis were observed. About 10% of the administered rutin appeared at the vascular side, chiefly as free rutin (5.6%), but some rutin sulfate (2.5%) and glucuronide (2.0%) were also detected. The conjugates were preferentially absorbed to the vascular side, while only traces of the glucuronide (0.2%) were found in the luminal perfusate. Minute amounts of the rutin administered were located in the intestinal tissue (1.1%) in the form of unchanged rutin and its glucuronide and sulfate conjugates. The model used serves as a valuable tool for understanding intestinal handling of the bioactive flavonol glycoside rutin, and the obtained results confirm uptake of rutin in the rat small intestine.

Keywords: Intestinal absorption; Intestinal metabolism; Rutin; Flavonol; Rats

1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most common native flavonoids [1], occurring mainly in glycosidic forms such as rutin (3-rhamnosyl-glucosyl-quercetin). In Western diets, the richest sources of quercetin glycosides are onions (347 mg/kg), apples (36 mg/kg), tea (20 mg/kg), and red wine (11 mg/kg) [2]. Quercetin and its glycosides have been reported to exert numerous biochemical and pharmacological activities, such as free radical scavenging [3–5], effects on immune and inflammatory cell functions [6,7], and even anticarcinogenic effects [8–11].

Intestinal uptake is a prerequisite for a possible causal relationship between rutin intake and its proposed chemopreventive action. Most studies, however, have evaluated the absorption of quercetin aglycone [12–15]. Although some reports have claimed intestinal absorption of quercetin [16,17] and the glycoside rutin [18], intestinal handling of rutin requires further investigation in order to appraise the beneficial *in vivo* action.

The present study was designed to investigate intestinal absorption and metabolism of rutin by using an isolated rat small intestine perfusion model [19,20] allowing direct investigation of luminal disappearance and venous appearance of the administered rutin.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (CD rats), 40 days old and weighing about 170 g, were obtained from Charles River. Rats were fed a cornstarch-based rutin- and quercetin-free synthetic diet (Altromin C-1000, Altromin International GmbH) for 7 days. Animals were provided with free access to tap water and food. For perfusions with rutin animals weighed 230.5 ± 10.4 g ($N = 3$), and for control perfusions with basic luminal media 225.1 ± 15.2 g ($N = 3$).

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Abbreviations: ESI^-, electrospray ionization.

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2.2. Vascularly and luminally perfused rat small intestine

The small intestine was prepared in rats as described elsewhere [20–23]. Luminal media consisted of 135 mmol/L of NaCl, 20 mmol/L of $NaHCO_3$ at pH 7.2, and 40.5 ± 1.8 μmol/L of rutin (amount applied 1214.0 ± 55.2 nmol), and no rutin in the case of controls. Oxygen uptake and acid–base homeostasis (Clark pO_2 electrode and pH electrode integrated in an ABL 30 Acid–Base Analyzer, Radiometer) were carefully controlled. Glucose, lactate, and pyruvate were determined photometrically by using enzymatic test kits (Monotest, Boehringer Mannheim). For glucose the MPR3 glucose/GOD-Perid® test kit (glucose oxidase, peroxidase; ABTS®, Boehringer Mannheim), for lactate the MPR3 lactate test kit (lactate dehydrogenase; NAD^+, Boehringer Mannheim), and for pyruvate the MPR1 pyruvate test kit (lactate dehydrogenase; NADH, Boehringer Mannheim) were used. The study was approved by the Regierungspräsidium Stuttgart, Germany.

2.3. Sampling and sample preparation

Vascular (50 mL) and luminal (5 mL) aliquots were obtained, and the entire isolated small intestine was harvested for analyses of rutin, quercetin, and their conjugates with reversed-phase HPLC with electrochemical detection after sample preparation as described below.

2.3.1. Vascular samples

One milliliter of each vascular sample was centrifuged at 10,000 × *g* for 7 min. The supernatant was separated and the pellet resuspended with 0.1 mL methanol and centrifuged again at 10,000 × *g* for 5 min. The combined supernatants were analyzed by HPLC. Rutin showed a recovery from vascular media of 95.7 ± 6.0% (means ± SD).

2.3.2. Luminal samples

After centrifugation at 700 × *g* for 20 min, the supernatant was separated. The pellet was extracted with ethanol and centrifuged again at 700 × *g* for 20 min. The volume of the combined supernatants was defined and the solution was analyzed by HPLC. Rutin recovery from luminal media was 99.0 ± 0.7% (means ± SD).

2.3.3. Small intestinal tissue

After lyophilization of the entire small intestine, the tissue was defatted by extracting twice with 10 mL hexane and powdered using a mortar and pestle. The pellet was extracted three times with 4 mL methanol/water (1:1) and centrifuged at 2800 × *g* for 10 min. The extracts were pooled and the volume adjusted to 25 mL. Rutin exhibited a recovery of 94.5 ± 10.2% (means ± SD).

2.4. Analytical procedures

2.4.1. Isocratic HPLC system with amperometric detection

The HPLC system (Sykam) consisted of an S 1100 solvent delivery system, an S 2000 HPLC controller, an S 8110 low-pressure gradient mixer, a Marathon Basic$^+$ autoinjector (Spark) with a 100-μL loop and a Biometra Electrochemical Detector at 600 mV (Biometra). Continuous on-line monitoring and data quantitation was performed with a Chromatopac C-R6A data processor (Shimadzu). Separation was carried out on a Hypersil ODS-2 column (125 × 4.6 mm i.d., 3 μm, Muder & Wochele), with a flow rate of 1.0 mL/min. Isocratic elution of rutin with an elution time of about 6 min was achieved with tetrahydrofuran: water:formic acid (8:82:10) as eluent; quercetin eluted at 6 min with tetrahydrofuran:water:formic acid (20:70:10). An injection volume of 10 μL (30 μL in the case of quercetin) resulted in a detection limit of 30 nmol/L and a quantitation limit of 90 nmol/L, respectively.

2.4.2. Gradient HPLC system with mass spectrometric detection (LC–MS)

For the identification of rutin and rutin conjugates, we used a clean-up procedure and gradient HPLC system combined with an MS detector in the electrospray ionization mode (ESI^-) as described earlier [23].

2.4.3. Cleavage of rutin and quercetin conjugates

Conjugates such as glucuronides and sulfates were analyzed as rutin and quercetin after enzymatic cleavage according to Sfakianos *et al*. [24], with modifications as described below. For cleavage of glucuronides, 0.06 mL of a sodium phosphate buffer (0.1 mol/L, pH 6.8) and 0.06 mL glucuronidase solution (210 Fishman units; *Escherichia coli*, Sigma-Aldrich) were added to 0.5 mL sample solution. Cleavage of sulfate conjugates was performed with 0.05 mL glucuronidase–sulfatase solution (97 units; *Helix pomatia*, Sigma-Aldrich) and 0.4 mL sample solution in an acetate buffer (0.05 mL, 0.2 mol/L, pH 4.5). Mixtures were incubated for 45 min at 37°. (Glucuronidase: β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31; sulfatase: aryl-sulfate sulfohydrolase, EC 3.1.6.1).

The applicability of the enzymatic cleavage in cleaned-up fluorocarbon emulsion (see vascular samples) was confirmed by the conversion of 4-nitrophenol glucuronide and 4-nitrophenol sulfate with β-glucuronidase and β-glucuronidase–sulfatase, respectively. The cleavage of 4-nitrophenol glucuronide resulted in 4-nitrophenol recovery of 96.6 ± 1.8% (means ± SD, N = 3), while the cleavage of 4-nitrophenol sulfate resulted in a recovery of 102.0 ± 0.5% (means ± SD, N = 3).

Table 1
Viability parameters (means ± SD, N = 3) of control perfusion and rutin experiments

	Control	Rutin
Oxygen consumption (μmol × min^{-1} × g^{-1})	3.8 ± 0.3	3.8 ± 0.3
Lactate formation (μmol × min^{-1} × g^{-1})	9.2 ± 5.85	7.0 ± 2.8
Glucose consumption (μmol × min^{-1} × g^{-1})	9.9 ± 2.5	6.9 ± 1.9
Arterial pressure (mm Hg)	64 ± 7	62 ± 4
Arterial pH	7.5 ± 0.1	7.5 ± 0.0
Venous pH	7.3 ± 0.1	7.3 ± 0.1

2.5. Chemicals and solvents

All chemicals used were of analytical grade. Solvents for HPLC analysis were of HPLC grade. Rutin was purchased from Extrasynthèse, quercetin from Fluka.

2.6. Calculations

Fluxes [nmol × min^{-1} × (g dry intestine)$^{-1}$, means ± SD] were calculated from concentration differences (ΔC, arterio-venous, and proximo-distal), the corresponding flow rates and the dry weight (DW) of the entire small intestine used in the experiment according to the following equation:

$$\text{Flux} = \frac{\Delta C[\text{nmol mL}^{-1}] \times \text{flow [mL min}^{-1}]}{\text{DW[g]}}$$

For calculation of the flux we used the actual luminal and vascular flow rates, which were somewhat different from the theoretical flow rates of 0.5 (luminal) and 5 mL/min (vascular).

Statistical differences of fluxes were determined using ANOVA and subsequent Tukey's range test for paired observations at a procedure-wise error rate of 5%. Viability parameters were compared using Student's *t*-test for unpaired observations. *P* values less than 0.05 were considered to indicate significant differences.

3. Results

In control perfusion experiments with rutin-free basic perfusion media, no flavonoids were detectable. Stability of rutin and quercetin in the luminal and vascular perfusate was confirmed for 2 hr at 37°. Viability of the organ preparation was confirmed in all perfusion experiments by repeatedly measuring oxygen uptake, glucose–lactate handling, and acid–base homeostasis. Indeed, viability was maintained over an experimental period of 60 min. No significant differences were observed when viability data from control and rutin perfusion experiments were compared (Table 1).

The vascular appearance rate of rutin increased during perfusion (significantly for the first 30 min, Fig. 1). Approximately 10% of rutin administered appeared at the vascular side, chiefly as free rutin (5.6%) but also as rutin glucuro-

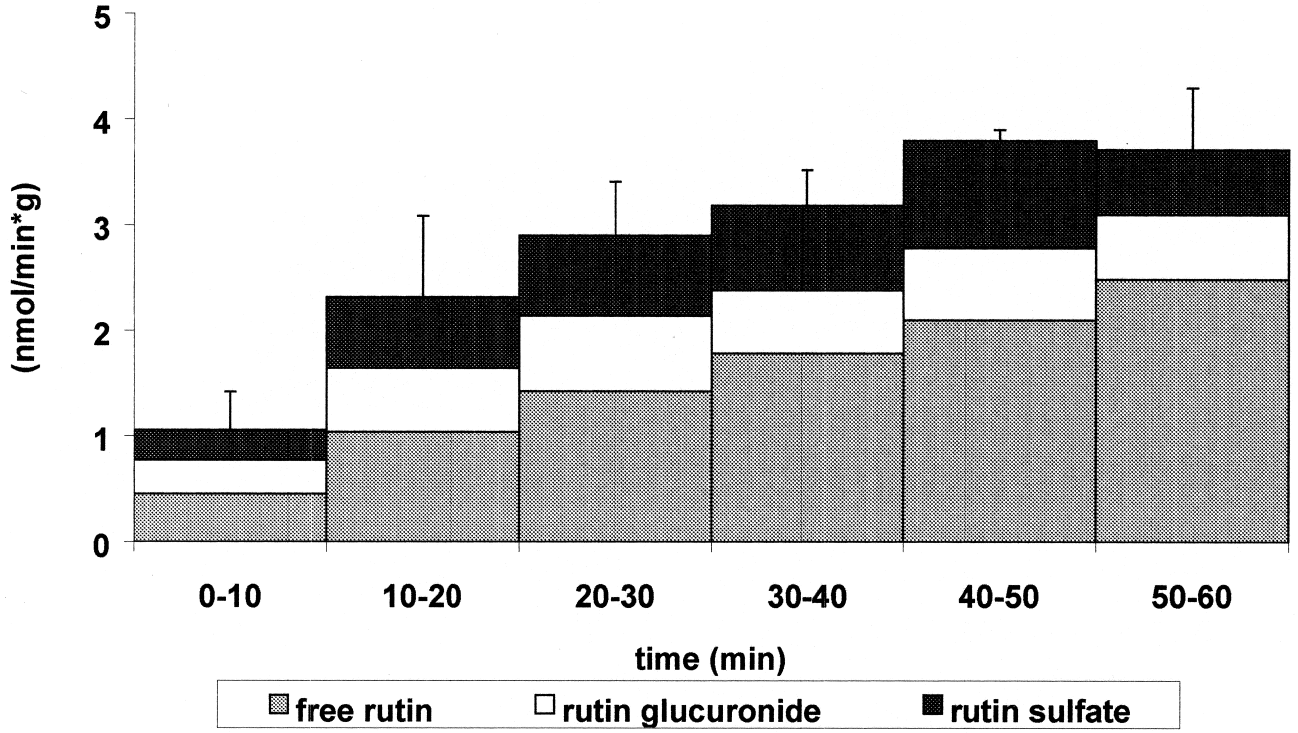

Fig. 1. Vascular appearance rates (nmol × min^{-1} × g^{-1}, means ± SD) of rutin and rutin conjugates in perfused rat small intestine. Rutin (1214.0 ± 55.2 nmol) was applied in three perfusion experiment of 60 min each. Vascular perfusates were pooled every 10 min and analyzed for rutin and its conjugates.

Table 2
Distribution of rutin and rutin conjugates in the luminal, vascular, and tissue compartments after perfusion experiments of rutin with isolated rat small intestine

	Luminal effluent		Vascular side		Intestinal tissue	
	nmol	%[a]	nmol	%[a]	nmol	%[a]
Rutin	1046.0 ± 48.6	86.2 ± 1.9	68.9 ± 15.6	5.6 ± 1.0	11.3 ± 4.8	0.9 ± 0.4
Glucuronide	2.5 ± 0.1	0.2 ± 0.0	24.7 ± 6.0	2.0 ± 0.5	0.9 ± 0.4	0.1 ± 0.03
Sulfate	ND		29.8 ± 7.4	2.5 ± 0.7	1.1 ± 0.6	0.1 ± 0.05
Total	1048.5 ± 48.7	86.4 ± 1.9	123.4 ± 10.0	10.1 ± 0.4	13.3 ± 5.5	1.1 ± 0.4

Rutin (1214.0 ± 55.2 nmol) was applied in three perfusion experiments of 60 min. Recoveries are given as means ± SD. Mean recovery over three experiments was 1185.3 ± 62.7 nmol (97.6 ± 2.2%). ND, under the detection limit.
[a] Based on the dosage of 1214.0 nmol.

nide (2.0%) and rutin sulfate (2.5%). Rutin conjugates were quantified as rutin after enzymatic incubation of luminal and vascular effluents and tissue extracts. Additionally, rutin sulfate in the vascular effluent was verified by LC–ESI$^-$–mass spectrometry. Characteristic fragment ions of rutin sulfate were the molecular ion $[M-H]^-$ at *m/e* 689 and the sodium adduct ion $[M-2H+Na]^-$ at *m/e* 711. With LC–ESI$^-$–mass spectrometry, however, we observed no mass spectrum of rutin glucuronide. The transfer of the rutin conjugates from the gut tissue to the vascular side revealed an increase until about 20 min, when an apparent steady state was established (Fig. 1). This increase was significant for the glucuronide but not for the sulfate conjugate.

Most of the luminally administered rutin left the organ preparation via luminal efflux (Table 2). The main compound in the luminal effluent was rutin, accompanied by minute amounts of rutin glucuronide. The glucuronide was identified and quantified by enzymatic hydrolysis with glucuronidase and subsequent HPLC analysis. Minute amounts of the rutin were located in the intestinal tissue in the form of unchanged rutin (0.9%) and its glucuronide (0.1%) and sulfate conjugates (0.1%) (Table 2). Traces of quercetin and quercetin conjugates at concentrations close to the detection limits were found both at the luminal and vascular sides. Mean total recovery of rutin and metabolites of all three experiments was 97.6%.

4. Discussion

To assess the intestinal absorption and metabolism of rutin, we used an isolated preparation of a vascularly and luminally perfused rat small intestine, characterized by fully maintained tissue viability. Intact mucosal morphology without loss of villous tip cells after a 120-min perfusion was previously demonstrated in histologic specimens of isolated intestinal preparations perfused in the same way as in the present study [25]. As in earlier studies, the viability and functional integrity of the intestinal preparation were carefully and continuously controlled and the viability confirmed [20–23]. Oxygen uptake of the preparations (3.8 $\mu mol \times min^{-1} \times g^{-1}$) conforms closely to previously published data (3.7–8.6 $\mu mol \times min^{-1} \times g^{-1}$ [26]). Oxygen consumption of the small intestine *in vivo* was apparently higher, corresponding to 10 $\mu mol \times min^{-1} \times g^{-1}$ [27]. Glucose consumption (6.7 $\mu mol \times min^{-1} \times g^{-1}$ [28]) was somewhat lower than in the control perfusions in the present study (9.9 $\mu mol \times min^{-1} \times g^{-1}$), but fit well to rutin experiments (6.9 $\mu mol \times min^{-1} \times g^{-1}$). Lactate formation (7.0 and 9.2 $\mu mol \times min^{-1} \times g^{-1}$) was not so different from that reported by Hanson and Parsons (7.0 $\mu mol \times min^{-1} \times g^{-1}$, [28]). Arterial pressure in the gut of the narcotized rat varied from 80 to 100 mm Hg [27], slightly higher than in the present perfused intestine (62 and 64 mm Hg) and highly dependent from the viscosity of the vascular medium. In studies with isolated small intestinal preparations, 58–90 mm Hg were reported [21].

Control perfusions without rutin with basic media confirmed that the small intestine of the experimental animals did not contain flavonoids sequestered from food. It should be pointed out that the amount of rutin administered (1214.0 nmol = 2.0 mg) corresponded to the physiological range of its usual nutritional intake with food (about 20 g of apple [29], 15 g of buckwheat [30,31], or 1 g of tea [32]).

The steady increase in the vascular appearance of free rutin and total rutin reflects the transport and metabolic activity of the small intestinal tissue (Fig. 1). The absorption rate of total rutin compounds in our study was about 10%, in contrast to a previous study in an isolated rat small intestine model that was unable to show rutin absorption [14], but in agreement with other studies demonstrating rutin absorption in rats [16,17] and in healthy ileostomy subjects [33].

It is generally thought that flavonoid glycosides are hydrolyzed to the corresponding less polar aglycones prior to gastrointestinal absorption [34–37], and the ability of mammals to hydrolyze flavonoid glycosides to the corresponding aglycones has been repeatedly reported [38–41]. In the present study, we found only traces (at the detection limit) of quercetin aglycone in the perfusates. This is not surprising, considering that rutin has been shown to be a poor substrate for β-glucosidase prepared from rat and human small intestinal tissues [40,41].

About 44% of the rutin absorbed was conjugated with

glucuronic acid (20%) and sulfate (24%), which is in contrast to observations recently made in a study with the rat small intestine, where no conjugates were found [42]. For quercetin, however, sulfate and glucuronic acid conjugates were reported in several studies [14,42–45]. Glucuronide and sulfate conjugates were analyzed as rutin after enzymatic cleavage. It should be pointed out that neither of the two enzymes, i.e. the glucuronidase from *E. coli* or the glucuronidase–sulfatase from *Helix pomatia*, were able to cleave the rhamno-glucosid rutin, despite an apparent β-glucosidase activity of these enzymes [46].

It is generally accepted that glucuronic acid and sulfate conjugates are water-soluble detoxification products intended for excretion. The conjugates formed in the small intestinal tissue were preferentially absorbed to the serosal side, with only minute amounts of the glucuronide secreted into the luminal perfusate (Table 2).

As rutin remains stable in the luminal effluent and the recovery was nearly complete (97.6%), a microbial cleavage during perfusion is unlikely. Cleavage more likely occurs in the colon [47,48], yielding ring fission products such as phenolic acids. Hence, rutin found in the luminal effluent is not lost for the organism. Microorganisms of the colon might be able to cleave rutin to absorbable phenolic acids of considerable biological activity [38,49].

Recent observations derived from experiments with human intestinal cells (Caco-2) [50] suggest a secretion rather than an absorption of quercetin glycosides. In contrast, our present results clearly indicate an absorption of rutin in the rat small intestine.

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