

Absorption and metabolism of genistin in the isolated rat small intestine

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Received 15 May 2000

Edited by Barry Halliwell

Abstract Uptake and intestinal metabolism of physiologically active genistin were studied in an ex vivo intestinal perfusion model; lumenally applied concentrations were 5.9, 12.0, and 23.8 $\mu\text{mol/l}$. The intestinal absorption of genistin was 14.9% (± 2.3 , $n=9$), irrespective of the amounts applied. The majority of the absorbed genistin appeared as genistein glucuronide (11.6%), also recovered as the main metabolite on the luminal side (19.5%). Minor amounts of genistin (1.3%) and genistein (1.9%) were found on the vascular side, whereas 15.4% of applied genistin was lumenally cleaved to yield genistein. Sulfate derivatives of genistein or genistin were not observed. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Intestinal absorption; Genistin; Isoflavone; Intestinal metabolism; Rat

1. Introduction

Isoflavones, major dietary components of soybeans [1], have recently received great attention because of their proposed health-related and clinical benefits such as estrogen receptor binding [2] and natural killer cell activation [3]. There have been numerous investigations regarding the antioxidative and anticarcinogenic activity of soy and its bioactive isoflavones, the glycosides genistin and daidzin and the aglycones genistein and daidzein [4–6].

Intestinal absorption of genistin (genistein-7-glucoside) is a prerequisite for its proposed chemopreventive action. Available research on genistin absorption has chiefly been directed to examinations of urinary, biliary and fecal excretion [7–10]. A recent study focused on the absorption of isoflavones in the rat stomach [11]. While aglycones were absorbed from the rat stomach, absorption of genistin has not been observed. The aim of the present study was to evaluate absorption and metabolism of genistin by using an isolated rat small intestine (duodenum, jejunum and ileum) [12,13]. This organ perfusion model facilitates direct investigation of luminal disappearance and venous appearance of administered compounds, thereby allowing the estimation of intestinal absorption.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (CD rats), 30 days old and weighing about 130 g, were obtained from Charles River (Sulzfeld, Germany). Rats were fed a cornstarch-based isoflavone-free synthetic diet (Altromin C-1000, Altromin International GmbH, Lage, Germany) for 14

days to allow elimination of isoflavones from the organism. Animals were provided with free access to tap water and food. For perfusions with genistin animals weighed 216.5 ± 17.2 g ($n=9$) and for control perfusions with basic luminal media 226.0 ± 11.1 g ($n=4$).

2.2. Vascularly and lumenally perfused rat small intestine

The small intestine was prepared in rats as described elsewhere [13–16]. Luminal media consisted of 5.9, 12.0, or 23.8 $\mu\text{mol/l}$ genistin, none in the case of controls, and 135 mmol/l NaCl, 20 mmol/l NaHCO_3 at pH 7.2.

Oxygen uptake and acid–base homeostasis (Clark pO_2 electrode and pH electrode integrated in an ABL 30 Acid–Base Analyzer; Radiometer, Copenhagen, Denmark) were carefully controlled. Glucose, lactate and pyruvate were determined photometrically using enzymatic test kits (Monotest; Boehringer Mannheim, Germany). For glucose the MPR3 Glucose/GOD-Perid[®] test kit (glucose oxidase, peroxidase; ABTS[®]; Boehringer Mannheim, Germany), for lactate the MPR3 lactate test kit (lactate dehydrogenase; NAD^+) and for pyruvate the MPR1 pyruvate test kit (lactate dehydrogenase; NADH) 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 as well as the associated blood vessels were harvested for analyses of genistin, genistein and their conjugates with reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) and mass spectrometry (MS) detection after sample preparation as described below.

2.3.1. Vascular samples. Of each vascular sample, 2 ml was spiked with internal standard 4-nitrophenol (50 μl of a 170 $\mu\text{mol/l}$ solution) and prepared as described earlier [15]. Genistin and genistein exhibited a recovery of $100.1 \pm 3.9\%$ and $97.2 \pm 3.0\%$, respectively (means $\pm$ S.D.).

2.3.2. Luminal samples. As internal standard 4-nitrophenol (20 μl of a 23.5 mmol/l solution) was added to the luminal effluent of a 10 min period and prepared as described earlier [15]. Genistin and genistein recovery was $100.3 \pm 1.8\%$ and $99.9 \pm 1.2\%$, respectively (means $\pm$ S.D.).

2.3.3. Small intestinal tissue and blood vessels. After lyophilization, the tissue was powdered using a pestle and mortar, and defatted by duplicate extraction with 10 ml hexane. The pellet was extracted three times with 3 ml (1 ml in the case of blood vessels) methanol/acetic acid (3%) (1:1) and centrifuged at $2800 \times g$ for 20 min. The extracts were pooled and the volume adjusted to 10 ml (3 ml in the case of blood vessels). Genistin and genistein recovery was $100.4 \pm 3.9\%$ and $100.0 \pm 4.8\%$, respectively (means $\pm$ S.D.) for small intestinal tissue and $99.4 \pm 4.4\%$ and $96.4 \pm 9.9\%$, respectively (means $\pm$ S.D.) for blood vessels.

2.3.4. Clean-up for LC–MS identification of genistin and genistein conjugates. Twelve milliliter of the vascular perfusate was prepared as described earlier [16].

2.4. Analytical procedures

2.4.1. Gradient HPLC system with UV detection. The HPLC system and the column used were previously described [15]. The eluents were composed of 0.2% acetic acid in H_2O (A) and 0.2% acetic acid in acetonitrile (B). The elution conditions were as follows: 0–2 min 5% B, 2–9 min 5–15% B, 9–20 min 15–60% B, 20–22 min 60–5% B. An injection volume of 25 μl genistin and genistein solution resulted in detection limits of 11 nmol/l and 5 nmol/l and quantitation limits of 20 nmol/l and 9 nmol/l, respectively.

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Table 1

Distribution of genistin, genistein and genistein glucuronide in the luminal, vascular and tissue compartments after perfusion experiments with the isolated rat small intestine

Applied genistin	176.8 ± 1.8		360.1 ± 0.0		713.1 ± 3.6	
	nmol ± S.D.	% ± S.D.	nmol ± S.D.	% ± S.D.	nmol ± S.D.	% ± S.D.
<i>Luminal loss:</i>						
Genistin	70.9 ± 11.0	40.1 ± 6.0	209.8 ± 5.3	58.3 ± 1.5	306.6 ± 58.5	43.0 ± 8.0
Genistein	26.2 ± 7.9	14.9 ± 4.5	56.2 ± 5.0	15.6 ± 1.3	112.9 ± 33.7	15.8 ± 4.7
Glucuronide	42.9 ± 2.3	24.3 ± 1.1	46.3 ± 1.3	12.9 ± 0.4	151.2 ± 37.6	21.2 ± 5.4
Total	140.0 ± 10.3	79.3 ± 5.1	312.3 ± 1.1	86.8 ± 0.3	570.7 ± 52.0	80.0 ± 6.7
<i>Vascular appearance</i>						
Genistin	2.5 ± 0.5	1.4 ± 0.3	5.9 ± 4.0	1.6 ± 1.1	6.1 ± 3.2	0.9 ± 0.4
Genistein	4.1 ± 0.8	2.3 ± 0.5	6.9 ± 4.3	1.9 ± 1.2	10.6 ± 2.4	1.5 ± 0.3
Glucuronide	18.9 ± 4.2	10.7 ± 2.6	33.3 ± 12.0	9.2 ± 3.3	105.9 ± 15.2	14.8 ± 2.2
Total	25.5 ± 3.3	14.4 ± 2.1	46.1 ± 7.7	12.7 ± 2.1	122.6 ± 17.6	17.2 ± 2.6
<i>Intestinal tissue</i>						
Genistin	0.6 ± 0.6	0.3 ± 0.3	0.4 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0
Genistein	3.2 ± 1.3	1.8 ± 0.7	3.4 ± 1.6	0.9 ± 0.4	5.5 ± 2.0	0.8 ± 0.3
Glucuronide	3.0 ± 1.9	1.7 ± 1.1	1.2 ± 0.8	0.3 ± 0.3	3.1 ± 0.9	0.5 ± 0.2
Total	6.8 ± 1.7	3.8 ± 1.0	5.0 ± 0.9	1.3 ± 0.3	9.2 ± 2.9	1.4 ± 0.4
<i>Blood vessels</i>						
Genistin	traces	traces	0.08 ± 0.02	0.02 ± 0.01	0.06 ± 0.00	0.01 ± 0.01
Genistein	0.05 ± 0.03	0.03 ± 0.01	0.09 ± 0.02	0.03 ± 0.01	0.08 ± 0.05	0.01 ± 0.01
Glucuronide	0.53 ± 0.28	0.30 ± 0.16	0.13 ± 0.02	0.04 ± 0.01	0.73 ± 0.24	0.10 ± 0.03
Total	0.58 ± 0.28	0.33 ± 0.17	0.30 ± 0.04	0.09 ± 0.01	0.87 ± 0.26	0.12 ± 0.04
Recovery	172.9 ± 8.1	97.8 ± 3.6	363.7 ± 8.4	100.9 ± 2.3	703.4 ± 31.9	98.7 ± 3.9

Concentrations of 5.9, 12.0, and 23.8 µmol/l were applied, each in three experiments, corresponding to genistin amounts of 176.8, 360.1, and 713.1 nmol, respectively. 'Traces' are under the quantitation limit.

2.4.2. Gradient HPLC system with MS detection (LC-MS). For the identification of genistin and genistein conjugates we used a gradient HPLC system as described earlier [16].

2.4.3. Cleavage of isoflavone conjugates. Genistin and genistein conjugates were analyzed as genistein after enzymatic cleavage according to Sfakianos et al. [17], with some modifications as described earlier [15].

2.5. Chemicals and solvents

All chemicals used were of analytical grade. Solvents for HPLC analysis were of HPLC grade. Genistin was purchased from Extrasynthese (Genay, France), genistein and the enzymes β-glucuronidase and arylsulfatase from Sigma-Aldrich (Deisenhofen, Germany).

2.6. Calculations

Fluxes (nmol min⁻¹ (g dry intestine)⁻¹, means ± S.D.) were calculated from arterio-venous and proximo-distal concentration differences (ΔC), respectively, the corresponding flow rates and the dry weight (DW) of the entire small intestine used in the experiment according to the following equation:

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

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 genistin-free basic perfusion media, neither genistin nor genistein was detected. Genistin and genistein stability in the luminal and vascular perfusate was confirmed for 10 h at 37°C. Viability of the organ preparation was confirmed in all perfusion experiments by repeatedly measuring oxygen uptake, glucose-lactate han-

dling and acid-base homeostasis. No significant differences in viability data could be observed between genistin and control perfusion experiments¹.

Most of the luminally administered genistin left the organ preparation via luminal efflux (Table 1). Main compounds in the luminal effluent were genistin, followed by genistein and genistein glucuronide. Genistein glucuronide was verified via LC-MS by the molecular ion [M-H]⁻ at *m/e* 445 and the signal for the aglycone fragment [M-glucuronic acid-H]⁻ as base peak at *m/e* 269. 14.9% of genistin administered (mean of all nine experiments) appeared on the vascular side, chiefly as genistein glucuronide but also as genistein and as unmetabolized genistin. Genistein glucuronide and genistin in the vascular effluent were also verified by LC-MS. Characteristic fragment ions of genistin were the molecular ion [M-H]⁻ at *m/e* 431 and the aglycone ion [genistein-H]⁻ at *m/e* 269. Genistein glucuronide was preferentially secreted to the luminal side. Minute amounts of the administered genistin were located in the intestinal tissue and the blood vessels as unchanged genistin, genistein and genistein glucuronide.

Luminal disappearance and vascular appearance rates of genistin as well as vascular and luminal appearance rates of metabolites increased proportionally to increasing luminal genistin concentrations applied (Table 2). Fig. 1 shows the time dependence of the fluxes in experiments with 713.1 nmol genistin applied.

¹ Viability parameters (means ± S.D.): control perfusion (*n*=4) and **genistin** (*n*=9) experiments: oxygen consumption: 3.8 ± 0.3 (**5.0 ± 0.6**) µmol min⁻¹ g⁻¹, lactate-pyruvate ratio: 27.4 ± 5.3 (**30.8 ± 5.0**); glucose consumption: 6.9 ± 1.5 (**6.0 ± 1.6**) µmol min⁻¹ g⁻¹; arterial pressure 79.5 ± 21.0 (**74.8 ± 12.1**) mm Hg; arterial pH: 7.5 ± 0.1 (**7.5 ± 0.0**); venous pH: 7.3 ± 0.0 (**7.4 ± 0.0**).

Table 2

The average fluxes of genistin and its metabolites over the perfusion time of 60 min ($\text{nmol min}^{-1} \text{g}^{-1}$, means $\pm$ S.D.)

Applied genistin ($\text{nmol} \pm \text{S.D.}$)	176.8 $\pm$ 1.8	360.1 $\pm$ 0.0	713.1 $\pm$ 3.6
Luminal disappearance	2.0 $\pm$ 0.1	3.2 $\pm$ 0.3	8.4 $\pm$ 1.0
Luminal appearance (secretion)	0.9 $\pm$ 0.3	2.1 $\pm$ 0.3	5.3 $\pm$ 0.3
Vascular appearance	0.5 $\pm$ 0.0	0.9 $\pm$ 0.1	2.5 $\pm$ 0.3

In none of the luminal and vascular perfusates or gut tissue extracts were mixed glucurono-sulfoconjugates, sulfate conjugates of genistein and glucuronide or sulfate conjugates of genistin detectable with LC-MS.

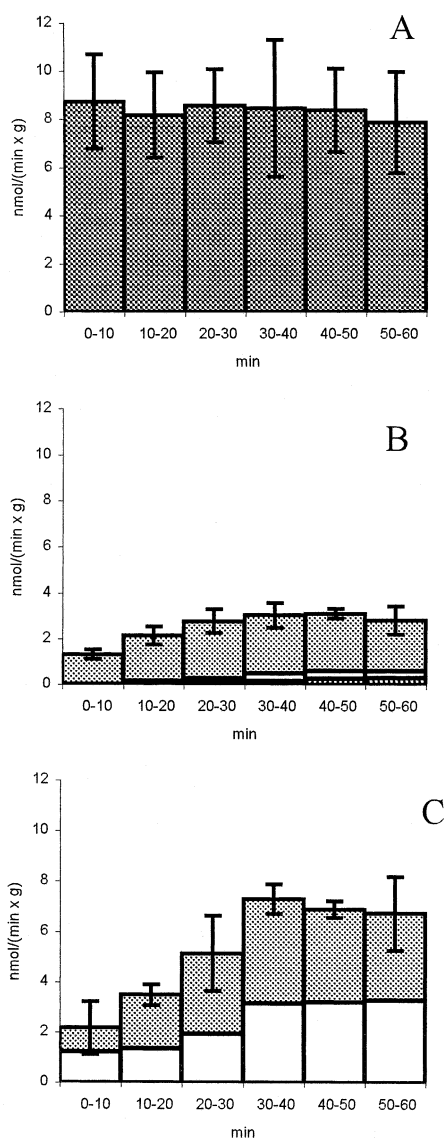


Fig. 1. Fluxes ($\text{nmol min}^{-1} \text{g}^{-1}$, means $\pm$ S.D.) of genistin and genistin metabolites in the perfused rat small intestine. A concentration of $23.8 \mu\text{mol/l}$, corresponding to a genistin amount of 713.1 nmol , was applied in three perfusion experiments each of 60 min. Luminal and vascular perfusates were pooled every 10 min and analyzed for genistin and its conjugates. A: Luminal disappearance rate of genistin. B: Vascular appearance rates of genistin, genistein and genistein glucuronide (gray, white and hatched bars, respectively). C: Luminal appearance rate of genistein and genistein glucuronide (gray and white bars, respectively) (back transport).

4. Discussion

To assess the intestinal absorption and metabolism of genistin, we used an isolated preparation of a vascularly and lumenally perfused rat small intestine, characterized by fully maintained tissue viability. Intact mucosal morphology without loss of villous tip cells after 120 min perfusion has previously been demonstrated in histologic specimens of the isolated intestinal preparations perfused in the same way as in the present study [18]. As in earlier studies, viability and functional integrity of the intestinal preparation were carefully and continuously controlled [13,14]. The functional integrity of the intestinal preparation was monitored and the viability confirmed in agreement with data from earlier studies using the same model [15,16].

Control perfusions with basic media without genistin confirmed that the small intestine of the experimental animals did not contain genistin sequestered from food. It must also be emphasized that in the present study the administered amount of genistin ($176.8\text{--}713.1 \text{ nmol}$) was in the physiological range corresponding well to its usual nutritional intake with food ($1\text{--}4 \text{ g}$ from tofu or $1.5\text{--}6 \text{ g}$ from tempeh [1]).

The absorption rate of total genistein compounds of about 15% is in good agreement with earlier observations gained from human feeding experiments with soymilk corresponding to 14.6% [19], 10% and 37% [8], respectively. In feeding experiments with rats, bioavailability of genistin calculated from the urinary excretion of genistein and genistein derivatives was about 22% [20], indicating that other parts of the digestive tract might be involved in the absorption processes.

The ability of mammals to hydrolyze flavonoid and isoflavonoid glycosides to the corresponding aglycones has been repeatedly claimed [21–23]. Microorganisms of the rat and human intestine are able to cleave β -glucosides [24]. However, since genistin remains stable in the luminal effluent, a microbial cleavage to genistein during perfusion is unlikely and genistein appearing lumenally was more likely hydrolyzed in the small intestinal tissue and subsequently secreted.

It was proposed that flavonoid glycosides are hydrolyzed to the corresponding less polar aglycones prior to gastrointestinal absorption [25–27]. In the present study, however, genistin was partly absorbed without previous cleavage which contradicts the above assumption. Supporting our observation, phloretin and quercetin have recently been shown to occur as glycosides in human plasma [28].

Transepithelial transport of naphthol and 4-nitrophenol monoglucoside conjugates by the sodium-dependent glucose transporter SGLT1 was confirmed in experiments with everted intestinal sac preparations [29,30]. From human studies it was concluded that quercetin absorption is enhanced by conjugation with glucose, possibly by absorption via the glucose carrier [31]. Genistin, as genistein-7-monoglucoside, might also be absorbed by this transport system. Yet, the absorption rate of genistein [15] was not enhanced due to conjugation

with glucose. An increase in luminal genistin disappearance rates was seen in proportion to the concentrations applied luminally, thus rendering a transepithelial transport of genistin by diffusion more likely than an active transport by a carrier system.

Regardless of the concentrations applied we observed an efficient cleavage of genistin to genistein and a conjugation of the aglycone with glucuronic acid (Table 1). Several investigators have shown this phase II biotransformation to occur in the gut tissue [30,32,33]. Genistein glucuronide was preferentially secreted into the luminal perfusate, while only about a third was transported to the vascular side. The highly hydrophilic, charged glucuronides ($pK_a = 2-3$, [34]) are unlikely to pass cell membranes by passive diffusion, however, specialized transport carrier systems for phenol glucuronides in the brush border and the basolateral membrane may explain our observations [32,35].

Genistein glucuronides were reported to be weakly estrogenic and are activators of human natural killer cells at nutritionally relevant concentrations [3]. In experiments with everted rat intestine, it was recently shown that secreted glucuronides are reabsorbed in the colon, and thus, not lost to the organism [36].

The implication of the intestinal model as presented facilitates direct ex vivo investigations of intestinal handling of phytochemicals, thereby allowing future research on their absorption, transport, distribution and metabolism.

Acknowledgements: We are indebted to R.-P. Franke and W. Röhlke, Central Institut für Biomedical Technique, Department Biomaterials, University of Ulm, Germany, for the production of the perfluorocarbon emulsion and we thank W. Armbruster, Institute for Food Chemistry, University of Hohenheim, Stuttgart, Germany for performing the LC-MS analyses.

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