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Daidzein is absorbed by passive transport in isolated small intestine of rats

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Abstract

The intestinal absorption of isoflavones is an essential prerequisite for assuring biologic effects. The purpose of this study was to investigate the intestinal transport, metabolism, and tissue uptake of daidzein. To this aim, 3 different tracts of small intestine of rats (proximal, medial, and distal) were isolated, everted, and exposed to physiologic concentrations (mucosal side) of daidzein (25, 50, and 80 μmol/L); perfusion experiments were performed for 60 minutes. Concentrations of total daidzein (at 30, 45, and 60 minutes) and glucuronide daidzein (at 60 minutes) were analyzed on aliquots of serosal solutions, whereas whole intestinal tracts were analyzed to determine daidzein tissue uptake (at 60 minutes). Results show linear relationships between the amounts of aglycone absorbed and taken up by tissue and mucosal daidzein concentrations; both amounts were higher in the distal tracts than in the other intestinal tracts ($P < .05$). The extrapolated total daidzein intestinal absorption was about 6% and total tissue uptake was about 10%. The amounts of conjugate daidzein correspond on average to about 6% of the levels absorbed; results suggest an inverse correlation between conjugation activity and mucosal daidzein concentration. In conclusion, our study suggests that a passive, unsaturable transport is the only mechanism of daidzein absorption in the small intestine of rats, at least within the concentrations tested. Different daidzein permeability of rat intestinal tracts was also demonstrated.

Keywords: Daidzein; Intestinal absorption; Intestinal metabolism; Everted gut; Rats

1. Introduction

Soy isoflavones, such as daidzein and genistein, have recently received great attention for their potential health benefits in decreasing the risk of several chronic pathologies, such as cancer, coronary heart disease [1,2], and osteoporosis [3]. Many in vitro and in vivo investigations have demonstrated their bioactivity in relation to estrogen receptor binding [4,5], natural killer cell activation [6], antiproliferative and growth-inhibiting effects on cancer cells [7,8], and antioxidant activity [9-13]. Nevertheless, the occurrence of any beneficial effects of bioactive compounds is subordinated to their bioavailability and mainly to their absorption in the intestine. Only limited studies on absorption, metabolism, and distribution of isoflavones are available [6,14-18]. Regardless of their chemical form in foods, isoflavones are absorbed as aglycones in the small intestine and partially metabolized to glucuronide conjugates in enterocytes and, consequently, both compounds are secreted in the blood. The isoflavones that escaped the first tract of the intestine are then metabolized by microflora producing various metabolites including equol [19]. However, controversial hypotheses about the mechanism of intestinal isoflavone absorption occur in the literature. For instance, the studies of Setchell et al [20,21] on isoflavone bioavailability in healthy

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women, showing a curvilinear relationship between dietary intake and plasma levels, suggested an intestinal rate-limiting and saturable absorption of isoflavones. Similar conclusions were also drawn by Oitate et al [22] with a study on CaCo-2 cell culture. The aim of this study was to investigate the intestinal absorption, metabolism, and tissue uptake of isoflavone, using a perfusion model of an isolated small intestine of a rat. We focused our attention on daidzein because data regarding absorption of this isoflavone are very limited. Three mucosal daidzein concentrations were tested to discriminate between passive or saturable mechanisms of transport.

2. Methods and materials

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma Chemical (St Louis, Mo) and Merck (Merck Kga A, Darmstad, Germany). The daidzein standard was obtained from LC Laboratories (Woburn, Mass).

2.2. Animal handling

The experimental protocol evaluated transport, metabolism, and tissue uptake of daidzein by using everted small intestine of rats. Twelve male Sprague-Dawley rats, 41 to 43 days old and weighing 150 to 175 g, were purchased from Charles River Laboratories (Wilmington, Mass). All aspects of animal care complied with the ethical guidelines and protocol approved by the Animal Care Committee of the University of Milan. Rats were kept in a room with a 12-h light cycle and controlled humidity and temperature and fed a cornstarch-based soy-free standard diet AIN 76 for 14 days to allow elimination of eventually circulating isoflavones because of previous diet. Rats were provided with free access to tap water and food. After overnight fasting, the animals were anesthetized with diethyl ether and killed by bleeding, and the small intestine was collected and washed with cold isotonic solution (KCl 1.15%, 4°C), as previously reported [23]. The first and the last 10 cm of the intestines were excluded, whereas the successive 12 cm representing the proximal and distal tracts were retained for the experiment. The median 12 cm gut was isolated in the center of the isolated small intestine. All these procedures were performed at 4°C.

2.3. Absorption experiment

Isolated gut sections (about 12 cm) were immediately everted on a metal rod as previously reported [23]. The ends of the intestinal segments were cannulated and the tissue transferred into a bath at 37°C. A buffer solution, named serosal solution, heated to 37°C (in millimoles per liter: 136 NaCl, 2.7 KCl, 1.4 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 0.4 NaH_2PO_4, 16 glucose, pH 7.2) was flowed inside the everted gut. Perfusion was carried out with a peristaltic pump to guarantee a constant flow (1 mL/min) of the serosal solution inside the everted intestine throughout the 60 minutes of the experimental time. Outside the gut, the mucosal solutions contained 25, 50, or 80 μmol/L daidzein (previously dissolved in tetrahydrofuran) in the same buffer solution, heated to 37°C. The absorption experiment started when the everted tracts of intestine were dipped in mucosal solution. The intestinal tissue was kept oxygenated by a mixture of 95% O_2/5% CO_2. Aliquots (1 mL) of the serosal solution were collected at 30, 45, and 60 minutes and stored at −80°C until analysis.

2.4. Serosal samples

Serosal samples, collected at 30, 45, and 60 minutes, were thawed out, vortexed, and injected (20 μL) into high-performance liquid chromatography (HPLC)-photodiode array detector (DAD) to determine the absorption of free aglycone. Conjugated daidzein was analyzed as daidzein after enzymatic cleavage [24]. Briefly, to 1 mL of the serosal sample collected at 60 minutes was added 440 IU of β-glucuronidase type VII in potassium phosphate buffer (0.2 mol/L, pH 6.8). After 1 hour incubation at 37°C, the sample was stored at −80°C until HPLC analyses. Amounts of conjugated isoflavone were obtained from the difference between aglycone concentration measured with and without enzymatic hydrolyses.

2.5. Small intestinal tissue

The procedure was applied as described by Andlauer et al [25]. Briefly, after freeze drying, the tissue was powdered with a mortar and pestle and defatted twice by extraction with 10 mL hexane. The supernatants were combined and extracted with methanol to rule out any loss of daidzein. The pellet was extracted 3 times with methanol/water (1:1) and centrifuged at 2800g for 20 minutes. The extracts were pooled and adjusted to 10 mL. The samples were then stored at −80°C until analysis.

2.6. Analytical procedure

The samples were analyzed by HPLC following the Andlauer method with minor modification [25]. The HPLC system consisted of a model 1525 binary HPLC pump (Waters, Milford, Mass) equipped with a Rheodyne injector (loop, 20 μL) connected with a Photodiode array detector model 2996 (Waters). Chromatograms were analyzed by the Empower software (Waters). Separation of isoflavones was obtained with a YMC pack ODS-AQ, 5-μm (250 × 4.6 mm) column (YMC, Kyoto, Japan) eluting at a flow rate of 0.5 mL/min for 40 minutes with a linear gradient composed of acetonitrile (A) and formic acid buffer (0.05 mol/L, pH 4) (B). The elution conditions were as follows: 0 to 3 minutes, 90% B; 3 to 12 minutes, 90% to 80% B; 12 to 17 minutes, 80% to 70% B; 17 to 22 minutes, 70% to 65% B; 22 to 30 minutes, 65% to 60% B; 30 to 35 minutes, 60% to 50% B; 35 to 40 minutes, 50% to 90% B. The eluted compounds were detected by their absorbance at 260 nm and quantified in relation to a standard curve (0.4-4 μmol/L).

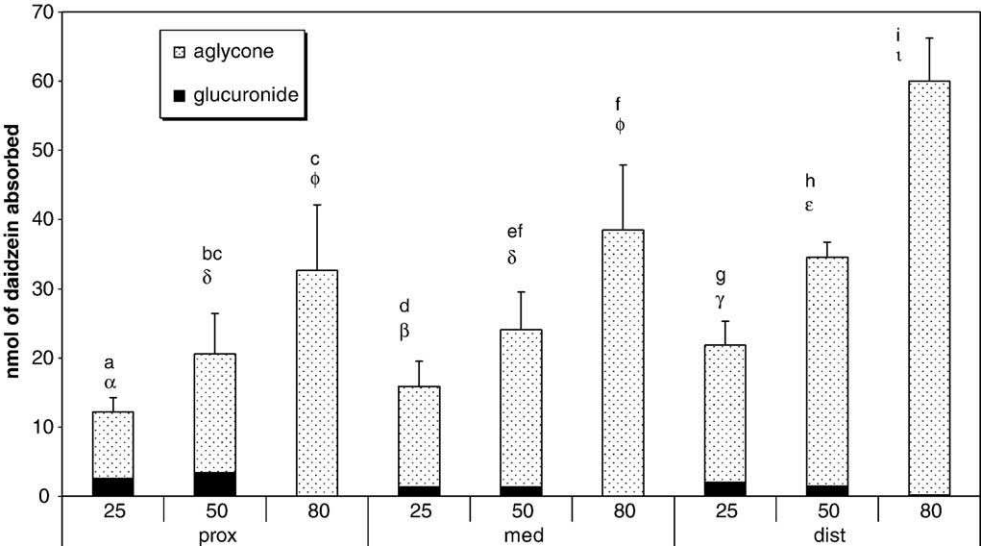

Fig. 1. Daidzein intestinal absorption. Final amounts of daidzein (nanomoles) transported as aglycone (⊡) and conjugated compound (■) through proximal, medial, and distal intestinal tracts at 25, 50, and 80 μmol/L daidzein mucosal concentrations. The values are expressed as mean ± SD, n = 4. Data were analyzed by Student t test (see text). Different letters mean significant differences within the same segment of the intestinal tract ($P < .05$). Different Greek symbols mean significant differences within the same mucosal daidzein concentration ($P < .05$).

2.7. Statistical analysis

Statistical evaluations were performed by Student t test of the paired observations to analyze the difference between the intestinal section, and Student t test of the unpaired observations to analyze the different concentrations of daidzein. P values < .05 were considered to indicate significant differences. Data are expressed as means ± SD (n = 4).

3. Results and discussion

The time courses of the intestinal daidzein transport followed a linear trend in all the experimental conditions tested, suggesting the integrity of tissue along the experimental time, and the rate of absorption results linearly correlated with the daidzein mucosal concentration (data not shown).

Within the same daidzein mucosal concentration, the isoflavone absorption increased from the proximal to the distal tract (Fig. 1), but only the amounts absorbed through the distal tracts were all significantly different ($P < .05$).

Considering the same intestinal tract, the amounts of aglycone transported increased linearly with the daidzein concentrations of the mucosal solutions in all tracts, although only in the distal tracts were all the data significantly different ($P < .05$).

Extrapolating the absorption from the analyzed tracts to the entire small intestine (approximately 100 cm), the percentage of absorption was about 6% of the doses administered. With respect to the total isoflavone absorbed, the amounts of glucuronide in the serosal solutions were low and approximately equivalent to 6%. However, the conjugation activity decreased with the increase of the mucosal daidzein concentration: 12%, 6%, and not detectable for 25, 50, and 80 μmol/L, respectively (Fig. 1). In fact, regardless of the intestinal tracts, the conjugation activity with 80 μmol/L daidzein mucosal concentration was significantly lower than those with 25 and 50 μmol/L concentrations ($P < .05$).

Similarly with absorption, the tissue uptakes showed linear increases in relation to the mucosal daidzein concentrations, although they seem less related to the intestinal

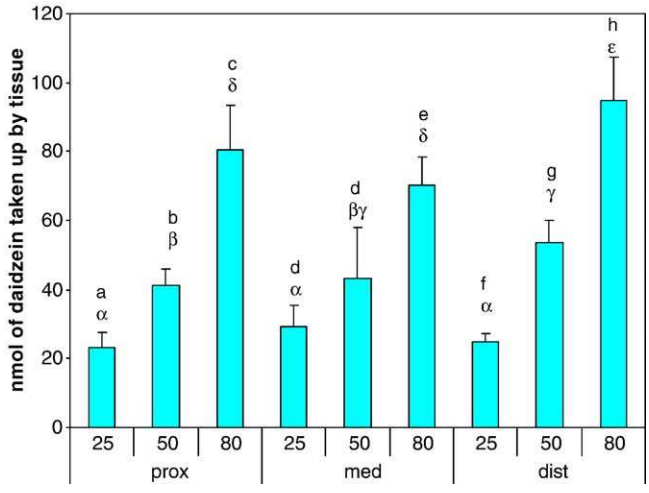

Fig. 2. Tissue daidzein uptake. Final amounts of daidzein (nanomoles) taken up by proximal, medial, and distal intestinal tracts at 25, 50, and 80 μmol/L daidzein mucosal concentrations. The values are expressed as mean ± SD, n = 4. Data were analyzed by Student t test (see text). Different letters mean significant differences within the same segment of the intestinal tract ($P < .05$). Different Greek symbols mean significant differences within the same mucosal daidzein concentration ($P < .05$).

tract; for instance, the distal tract showed a significant higher daidzein tissue uptake with respect to the other 2 tracts, with only 80 μmol/L daidzein mucosal concentration (Fig. 2). About 10% of the daidzein administered was calculated to have been taken up by the entire intestinal tissue.

Previous in vivo studies [20,21] on isoflavone pharmacokinetics and bioavailability in healthy women pointed out a curvilinear relation between dietary intake and plasma levels. These results suggested that the intestinal absorption of isoflavones could be rate limiting and saturable. To verify this hypothesis, we investigated the relation between levels of aglycone transported across everted small intestines of rats and mucosal isoflavone concentrations, focalizing our study on daidzein. The range of daidzein concentrations of mucosal solutions was chosen to be physiologic. Taking into consideration that the mean isoflavone intake of Asian people ranges between 50 and 100 mg/d, we calculated that after a meal the concentration reached in the intestinal lumen could correspond to approximately 50 μmol/L. Consequently, the other concentrations tested were the first lower to investigate the absorption occurring in nonhabitual consumers of isoflavone-rich food, and the second higher to verify the hypothesis formulated.

The rates of absorption of daidzein across the proximal, medial, and distal tracts are consistent with data previously found for genistein at similar mucosal concentration [26], suggesting that an analogous mechanism of transport for these isoflavones, at least in aglycone chemical form, exists in the small intestine.

At the same concentration of isoflavone in the mucosal solution, daidzein absorption increased from the proximal to the distal tract. This may be related to the higher distal permeability because of the presence of more permeable pores in this region or to an increase in paracellular permeability as suggested by Pantzar et al [27]. For other nutrients, such as minerals, when the luminal concentrations are high and passive transport overcomes saturable transport [28], there was a higher absorption in the distal as compared with the proximal tracts of the small intestine. In an in vitro study, higher transport was also found in the ileum than in the jejunum due to the quercetin glycoside whose absorption is suggested to occur by simple diffusion via tight junction [29]. Considering the same intestinal tract, the amounts of aglycone absorbed increased linearly with the daidzein concentration of mucosal solutions in all tracts, suggesting that the main mechanism of aglycone absorption across the small intestine is a passive transport. These data seem to contrast with the hypothesis of Setchell et al [20,21] of a rate-limiting and saturable uptake of isoflavones. However, this contradictory evidence could be due to the different methodological approaches used, such as ex vivo vs in vivo models. In effect, with in vivo models many factors can affect the absorption of bioactive compounds and plasma kinetics such as the effect of the diet matrix, the contribution of the large intestine or endogenous metabolism, transport and excretion rates. The study of Sfakianos et al [24], using perfusion of the short intestinal segment of duodenum of rats, suggested that the initial absorption of genistein occurs by passive mechanism, in agreement with our own results.

Extrapolating the results to the entire small intestine, the percentage of daidzein absorption was about 6% of the mucosal doses. Our results are lower than previous data presented in the literature: in particular, Andlauer et al [26], in their study with everted small intestines of rats, indicated that the intestinal absorption of luminally administered genistein (12 μmol/L) was 40.6%, and similar recovery was also found in the absorption experiment of pure isoflavones with Caco-2 cell culture, approximately 35% of the initial dose (10 μmol/L) [30]. Different experimental procedures could account for these different results. In effect, our data of isoflavone absorption of the entire small intestine of the rat are extrapolated considering that the isolated tracts tested could be representative of the small intestine. We cannot in fact exclude that in perfusing the whole small intestine, as Andlauer and coworkers did, the percentage of absorption should have been higher than the one calculated. Inconsistent results from in vitro experiments are present in the literature regarding the percentage of conjugated isoflavones secreted in the serosal side: data from studies using everted intestine of rats ranged between 80% and 18% of the amount of isoflavone absorbed, whereas in cell cultures, it ranged between 31% and 12% [26,30,31]. We found an average of glucuronide derivates of 6% in the serosal solution with respect to the daidzein absorbed, with a trend of conjugation activity inversely correlated to the isoflavone concentrations in a mucosal solution. It is generally accepted that the secretion into the serosal side of conjugated isoflavone is the result of the intestinal UDP glucuronosyltransferase activity on aglycones, followed by transport of conjugates across the basolateral membrane of enterocytes possibly by a specific carrier transport. Consequently, we can hypothesize that the 80 μmol/L daidzein mucosal concentration had negatively affected enzyme activity or affinity with the carrier; nevertheless, this unexpected result needs further investigation. Despite the lower percentage of isoflavone absorption and metabolism, this study found a higher daidzein tissue uptake than that previously reported for genistein (10% vs ~1.5% [26]). This result could be ascribed to the different chemical structures of the 2 isoflavones: because of the lack of the hydroxyl group in position 5, an interaction with membrane phospholipids for daidzein seems to be easier than for genistein, as sustained by a study with liposomes [32]. This different behavior was also observed in our previous study in Jurkat T-cell culture that demonstrated a protective effect of daidzein, but not of genistein, against induced oxidative damage to membrane lipids [13].

In conclusion, the present study on isolated small intestines of rat suggests that the only mechanism of daidzein absorption is a passive and unsaturable transport at least within the concentrations tested in this study. Moreover, the distal tract of the small intestine presents a

higher ability to absorb daidzein than the other intestinal tracts probably because of a lower tissue selectivity.

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