Quercitrin is absorbed and metabolized by Caco-2 cells
Suyun Li¹, Zheng Li², Jinglai Li², Weina Gao¹, Jianquan Wu¹, Zhenqing Zhang², and Changjiang Guo¹,*

¹Institute of Health and Environmental Medicine, Tianjin 300050,  
²Institute of Pharmacology and Toxicology, Beijing 100850, China

ABSTRACT
Quercitrin, one of quercetin glycosides, has been demonstrated to be a powerful antioxidant in vitro. However, its bioavailability is not well defined. In the present study, we investigated the absorption and metabolism of quercitrin on a Caco-2 cell model. The concentrations of quercitrin, quercetin, isorhamnetin and tamarixetin in Caco-2 cells were quantified using a HPLC/MS method after incubation with quercitrin. Meanwhile, other possible metabolites of quercitrin were also detected. The results showed that quercitrin, quercetin, isorhamnetin and tamarixetin were measurable in Caco-2 cells after incubation with quercitrin. Their intracellular concentrations rose up to the peak value 60 min post incubation, and then decreased to the plateau gradually. Furthermore, quercetin monoglucuronide, quercetin trisulphate and quercetin glucuronide sulfate were identified in the Caco-2 cells treated with quercitrin. It is concluded that quercitrin can be uptaken intact by Caco-2 cells and subsequently undergoes metabolic transformations, including methylation, glucuronidation and sulfation.

KEYWORDS: quercitrin, absorption, metabolism, Caco-2 cells

INTRODUCTION
Quercitrin (quercetin-3-L-rhamnoside), a glycosylated form of quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Figure 1), is widely distributed in plant kingdom. Several studies have demonstrated that quercitrin exhibits strong antioxidant, anti-inflammatory, and anti-carcinogenic activities [1-4]. However, the potential actions of quercitrin in vivo are dependent on the intestinal absorption and subsequent interaction with target tissues. Several studies have demonstrated that quercetin and some of its glycosylated derivatives are absorbed considerably in vivo and the sugar moiety in the structure of quercetin glycosides affects their bioavailability significantly in humans. Hollman et al. reported that quercetin glycosides from onions were absorbed by small intestine more efficiently than quercetin aglycone and rutin (quercetin-3- rutinoside), another glycosylated form of quercetin in ileostomy volunteers [5]. It was indicated that rutin was hydrolyzed by intestinal microflora before absorption took place effectively [6]. Walgren et al. found that quercetin aglycone was capable of crossing Caco-2 cell monolayers, whereas quercetin-4′-β-glucoside was not [7]. Subsequently, they demonstrated that quercetin- 4′-β-glucoside was absorbed via the sodium- dependent glucose transporter-1 (SGLT1) and further effluxed from Caco-2 cells by the multidrug resistance associated protein-2 (MRP2) [8, 9]. Olthof et al. found that the bioavailability of quercetin-3-glucoside and quercetin-4′-β-glucoside did not differ in humans [10]. Nevertheless, limited data is available so far in describing the absorption and metabolism of quercitrin. Morand et al. found that quercitrin was not absorbed significantly in the small intestine of rats [11]. In contrast, the study finished recently
Caco-2 cell culture
Caco-2 cells, obtained from American Type Culture Collection (Rockville, MD, USA), were cultured in DMEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37°C. Cells were subcultured at 80% confluence, and used for experiment between passages 42 and 56.

Incubation of Caco-2 cells with quercitrin
Caco-2 cells were seeded at a density of 1×10⁶ cells/mL. Quercitrin was dissolved in 10 mM phosphate buffer saline (PBS, pH 7.4) and added to the culture medium at the final concentrations of 3 µg/ml, 9 µg/ml and 27 µg/ml, respectively. Each treatment was repeated in triplicate. Cells were harvested at the time-points of 0, 30 min, 60 min, 90 min, 120 min, 150 min post incubation and washed twice in PBS. The samples were then mixed with methanol and centrifuged. Aliquots of 60 µl resulting supernatant were diluted with 240 µl methanol for determination of quercitrin, quercetin, isorhamnetin and tamarixetin with HPLC/MS technique described below. Cellular protein content was qualified by Lowry’s assay.

HPLC/MS analysis
An Agilent 1100 LC/MSD SL quadrupole mass spectrometer system (Agilent Technologies, CA, USA) was used for HPLC/MS analysis. A method described previously by Matsumoto et al. was followed with slight modification [15]. Briefly, Chromatographic separation was achieved on an Agilent C18 column (100 mm×2.1 mm I.D.,
Quercitrin and Caco-2 cells

As shown in Table 1, the intracellular concentration of quercitin varied in a concentration dependent manner in the Caco-2 cells incubated with quercitin, indicating that intact quercitin could be uptaken by Caco-2 cells. The quercitin concentration rose up to the peak value 60 min after incubation, and then decreased to the plateau gradually.

**Change of intracellular concentration of quercetin in Caco-2 cells**

Quercetin was detectable in the Caco-2 cells incubated with quercitrin. At 60 min post incubation, intracellular concentration of quercetin was as high as 12.8 ng/mg protein in the Caco-2 cells exposed to 27 µg/ml of quercitrin. However, the concentration of quercetin was generally lower than 5.2 ng/mg protein when the Caco-2 cells were incubated with 3 µg/ml or 9 µg/ml of quercitrin (data not shown).

**Change of intracellular concentration of isorhamnetin and tamarixetin in Caco-2 cells**

As seen in Table 2, isorhamnetin and tamarixetin were measurable in the Caco-2 cells treated with quercitrin at the concentration of 27 µg/ml, but hardly detectable at the concentration of 3 µg/ml or 9 µg/ml. The changes of intracellular concentration of these two metabolites was time and dose dependent. First, they increased up to the peak 60 min after incubation, and then decreased down gradually to the plateau. It was noted that the concentration of isorhamnetin was significantly higher than that of tamarixetin.

**Other metabolites of quercitin in Caco-2 cells**

Based on the results of HPLC/MS analysis using SIR, quercetin glucuronide sulfate (m/z 559), quercetin monoglucuronide (m/z 479), quercetin

<table>
<thead>
<tr>
<th>Quercitrin (µg/ml)</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>20.0±3.4</td>
<td>86.4±10.1</td>
<td>26.1±2.7</td>
<td>17.5±1.2</td>
<td>21.3±6.6</td>
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<tr>
<td>9</td>
<td>38.8±3.7**</td>
<td>130.6±18.0**</td>
<td>46.1±4.2**</td>
<td>32.1±9.5**</td>
<td>55.5±27.5**</td>
</tr>
<tr>
<td>27</td>
<td>124.7±10.6**</td>
<td>608.8±70.9**</td>
<td>122.9±1.9**</td>
<td>43.3±4.1**</td>
<td>51.0±4.3**</td>
</tr>
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</table>

Table 1. Change of intracellular concentration of quercitin in Caco-2 cells.

Value are mean±SD (n=3). Caco-2 cells were incubated with quercitin at the concentrations of 3 µg/ml, 9 µg/ml, 27 µg/ml for 30 min, 60 min, 90 min, 120 min, 150 min, respectively and subject to HPLC/MS analysis as described in the Materials and Methods section. **P<0.01, compared to 3 µg/ml of quercitin.

Statistical analysis

Data are presented as means±SD. Statistical analysis were carried out using one way ANOVA. The significant level was set at P<0.05.

RESULTS

**Change of intracellular concentration of quercetin in Caco-2 cells**

As shown in Table 1, the intracellular concentration of quercetin varied in a concentration dependent manner in the Caco-2 cells incubated with quercitrin, indicating that intact quercitin could be uptaken by Caco-2 cells. The quercitin concentration rose up to the peak value 60 min after incubation, and then decreased to the plateau gradually.

**Change of intracellular concentration of isorhamnetin and tamarixetin in Caco-2 cells**

As seen in Table 2, isorhamnetin and tamarixetin were measurable in the Caco-2 cells treated with quercitrin at the concentration of 27 µg/ml, but hardly detectable at the concentration of 3 µg/ml or 9 µg/ml. The changes of intracellular concentration of these two metabolites was time and dose dependent. First, they increased up to the peak 60 min after incubation, and then decreased down gradually to the plateau. It was noted that the concentration of isorhamnetin was significantly higher than that of tamarixetin.

**Other metabolites of quercitin in Caco-2 cells**

Based on the results of HPLC/MS analysis using SIR, quercetin glucuronide sulfate (m/z 559), quercetin monoglucuronide (m/z 479), quercetin
trisulfate (m/z 543) were identified. However, other possible metabolites, such as quercetin sulfate (m/z 383), methylquercetin sulfate (m/z 397) and methylquercetin glucuronide (m/z 493) were not detected (Figures 2, 3, 4).

**DISCUSSION**

Two different mechanisms have been hypothesized for the absorption of quercetin glycosides. One is that quercetin glycosides are hydrolyzed initially by lactose phlorhizin hydrolase (LPH) at the brush border membrane of intestinal epithelium or the β-glucosidases excreted by gut microflora and the resulting aglycone diffuses passively across the apical membrane of epithelial cells [17-20]. The other is that the absorption begins with the uptake of intact quercetin glycosides by the epithelial cells via SGLT1 and is followed by hydrolysis under the action of cytosolic β-glucosidases. The quercetin...
Quercitrin and Caco-2 cells

Figure 3. HPLC/MS chromatogram of quercetin trisulfate (m/z 543). Caco-2 cells were incubated with quercitrin at the concentration of 27 µg/ml for 60 min and subject to HPLC/MS analysis as described in the Materials and Methods section. A: control; B: quercitrin treated.

Figure 4. HPLC/MS chromatogram of quercetin glucuronide sulfate (m/z 559). Caco-2 cells were incubated with quercitrin at the concentration of 27 µg/ml for 60 min and subject to HPLC/MS analysis as described in the Materials and Methods section. A: control; B: quercitrin treated.
aglycone released is further methylated, glucuronidated or sulfated and eventually enter the bloodstream. Some of quercetin glycosides and possibly quercetin glucuronides are transported back into the intestinal lumen by MRP2 [8, 9, 19, 20]. In the present study, we demonstrated that intact quercitrin could be taken up by Caco-2 cells and underwent hydrolysis, methylation and other metabolic processes. It is unclear whether SGLT1 or MRP2 is involved in the absorption process, because we do not use any inhibitors specifically for SGLT1 or MRP2 in this study. Nevertheless, our results were consistent with the data reported by Tian et al., in which they demonstrated that quercitrin could transport across the monolayers developed by Caco-2 cells. The apparent permeability coefficient was 0.79 ± 0.07 from apical to basolateral and 0.91 ± 0.05 from basolateral to apical. Therefore, it was postulated that the process of quercitrin absorption was accomplished partly by passive diffusion [12]. However, we are not able to conclude whether part of quercitrin is hydrolyzed first and absorbed in the form of quercetin aglycone because there is no data supporting that LPH or β-glucosidases are expressed at the apical membranes of Caco-2 cells. Further studies should be carried out to investigate the exact mechanisms involved in the absorption of quercitrin.

It is well recognized that quercetin or its glycosides is present exclusively as conjugated metabolites, mainly in methylated, glucuronidated and sulfated forms after absorption and cannot be detected significantly in aglycone form or as the glycosides originally present in various food sources [19, 20]. Cermak et al. demonstrated that main metabolites of quercetin in vivo were two methylated derivatives, isorhamnetin and tamarixetin [21]. In this study, we also found that two methylated metabolites, isorhamnetin and tamarixetin could be detected in a dose-dependent manner in the Caco-2 cells after incubation with quercitrin. The concentration of isorhamnetin was about 2 times higher than that of tamarixetin, indicating that methylation of quercetin at C-3' position (isorhamnetin) is more favoured than the methylation at C-4' position (tamarixetin). Similar findings had been reported by Ader et al., when they investigated the metabolism of quercetin in the pig [22]. It should be pointed out that, though the methylation is the major metabolic pathway of quercetin aglycone in vivo, other conjugation processes also happen to the quercetin aglycone [22, 23-25]. In this study, the quercetin monoglucuronide, quercetin trisulphate and quercetin glucuronide sulfate were identified in the Caco-2 cells treated with quercitrin. It is not surprising because the activities of UDP-glucuronosyltransferase, phenol sulfotransferase and catechol-O-methyltransferase have been detected in Caco-2 cells [8, 18, 20]. Currently, it is not certain which one of quercetin metabolite is more active biologically in vivo. Further study is needed to pay more attention to the roles played by different metabolites of quercetin in vivo.

In summary, it is concluded that quercitrin can be uptaken intact by Caco-2 cells and is further deglycosylated and undergoes metabolic transformations, including methylation, glucuronidation, and sulfation. However, whether the cellular internalization of quercitrin is dependent on the SGLT1 or LPH and β-glucosidases are initially involved needs to be confirmed by further investigation.

ACKNOWLEDGEMENT

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ABBREVIATIONS

DMEM, Dulbecco’s modified Eagle’s medium; HPLC/MS, high performance liquid chromatography/mass spectrometry; LPH, lactose phlorhizin hydrolase; MRP2, multidrug resistance associated protein-2; PBS, phosphate buffer saline; SGLT1, sodium-dependent glucose transporter-1; SIR, selective ion recordings

REFERENCES