Current Topics in Phytochemistry
Vol. 10, 2011

Original Communication

# Quercitrin is absorbed and metabolized by Caco-2 cells

**Suyun Li[1], Zheng Li[2], Jinglai Li[2], Weina Gao[1], Jianquan Wu[1], Zhenqing Zhang[2], and Changjiang Guo[1,*]**

[1]Institute of Health and Environmental Medicine, Tianjin 300050,
[2]Institute 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 qurecetin 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

*Corresponding author
guo_tj@yahoo.com

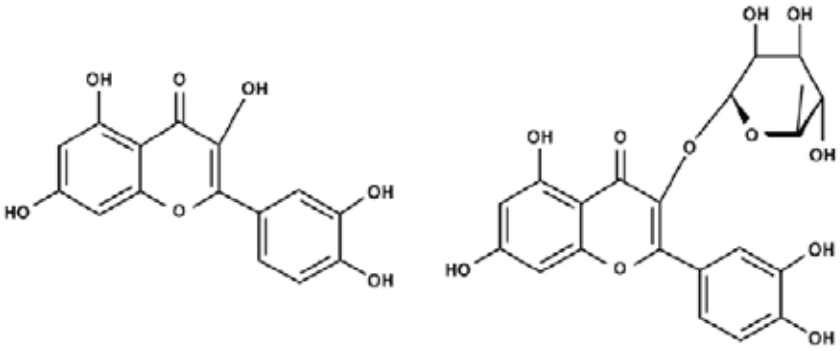


**Figure 1.** Chemical structures of quercetin (left) and quercitrin (right).

by Tian *et al.* revealed that quercitrin could undergo the bilateral transportation in the monolayer model established by Caco-2 cells, suggesting a possibility that quercitrin may be biologically available [12].

In the present study, we investigated the process of absorption and metabolism of quercitrin, using Caco-2 cells as an *in vitro* model. Caco-2 cells are a well-known cell line derived from a human colon adenocarcinoma and spontaneously exhibit various enterocytic characteristics, such as brush-border enzymes, nutrient transporters and intercellular tight junction. It has been extensively used as a tool to study the intestinal absorption of pharmaceuticals and nutrients [13, 14]. The aim of this study was to giving an insight into the cellular absorption of quercitrin and its metabolites formed *in vivo* and providing an experimental basis for further studies.

## MATERIALS AND METHODS

### Chemicals

Quercitrin (quercetin-3-rhamnoside), quercetin, isorhamnetin (3′-methylquercetin), tamarixetin (4′-methylquercetin), L-glutamine, trypsinase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum and nonessential amino acids were obtained from Hyclone (Logan, UT, USA). Methanol and acetonitrile of HPLC grade were purchased from Fisher Scientific (Pittsburgh, PA, USA). Dulbecco's modified Eagle's medium (DMEM) was obtained from GIBCO (Grand Island, NY, USA). All other chemicals used in this study were of analytical grade.

### 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% $CO_2$ 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 $1\times10^6$ 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.,

3.5 mm; Agilent, USA). The mobile phase consisted of water (A) and methanol (B) with 0.1% formic acid. The proportion of A to B was 65:35 (v/v). The flow rate was 0.2 mL/min. The injection volume was 20 μl.

Mass spectroscopy was performed in positive mode using electrospray ion source and selective ion recordings (SIR) for quercetin (m/z 303), quercitrin (m/z 449), isorhamnetin and tamarixetin (m/z 317) and also for other possible metabolites of quercitrin (m/z 383, 397, 493, 479, 543, 559) [16]. The optimized conditions were described as following: capillary voltage 3000 V, ion source temperature 99°C, peak width 0.10 min, pressure of GSI 30 psi, and the flow of drying gas 0.2 ml/min.

The calibration curve for quercetin showed a good linearity in the range of 2 ng/ml to 2000 ng/ml and for quercitrin, isorhamnetin, tamarixetin in the range of 1 ng/ml to 1000 ng/ml, with correlation coefficients of 0.99998, 0.99712, 0.99862, 0.99806, respectively. The intra- and inter-day RSDs were less than 15%.

### 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 quercitrin in Caco-2 cells

As shown in Table 1, the intracellular concentration of quercitrin varied in a concentration dependent manner in the Caco-2 cells incubated with quercitrin, indicating that intact quercitrin could be uptaken by Caco-2 cells. The quercitrin 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 quercitrin 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 1.** Change of intracellular concentration of quercitrin in Caco-2 cells.

| Quercitrin | Concentration (ng/mg·protein) | | | | |
|---|---|---|---|---|---|
| (μg/ml) | 30 min | 60 min | 90 min | 120 min | 150 min |
| 3 | 20.0±3.4 | 86.4±10.1 | 26.1±2.7 | 17.5±1.2 | 21.3±6.6 |
| 9 | 38.8±3.7** | 130.6±18.0** | 46.1±4.2** | 32.1±9.5** | 55.5±27.5** |
| 27 | 124.7±10.6** | 608.8±70.9** | 122.9±1.9** | 43.3±4.1** | 51.0±4.3** |

Value are mean±SD (n=3). Caco-2 cells were incubated with quercitrin 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 quercitrin.

**Table 2.** Change of intracellular concentrations of isorhamnetin and tamarixetin in Caco-2 cells.

| | Concentration (ng/mg·protein) | | | | |
|---|---|---|---|---|---|
| | 30 min | 60 min | 90 min | 120 min | 150 min |
| Isorhamnetin | 17.5±2.1 | 38.6±0.7 | 15.9±0.6 | 8.9±0.5 | 9.0±0.7 |
| Tamarixetin | 7.3±1.3** | 20.9±0.4** | 7.6±0.5** | 3.7±0.4** | 4.2±0.8** |

Value are mean±SD (n=3). 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. **P<0.01, compared to isorhamnetin.

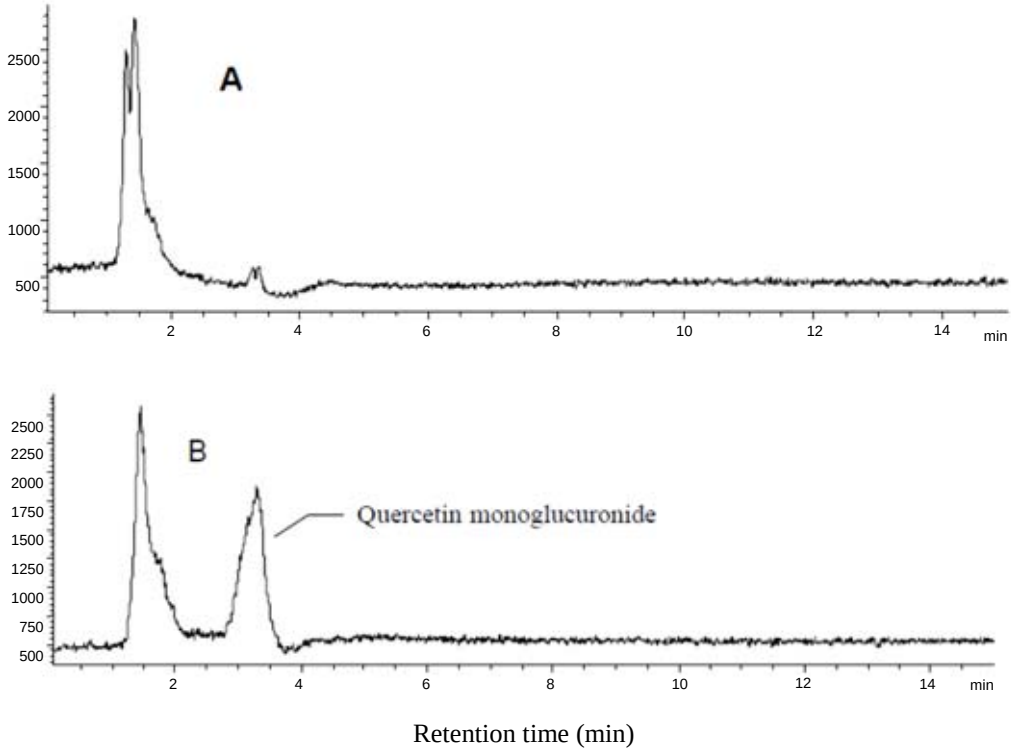


**Figure 2.** HPLC/MS chromatogram of quercetin monoglucuronide (m/z 479).
Caco-2 cells were incubated with quercitrin at the concentration of 27 μg/ml for 60 min and subject to HPLC/MS analysis as described in the Materials and Methods section. A: control; B: quercitrin treated.

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

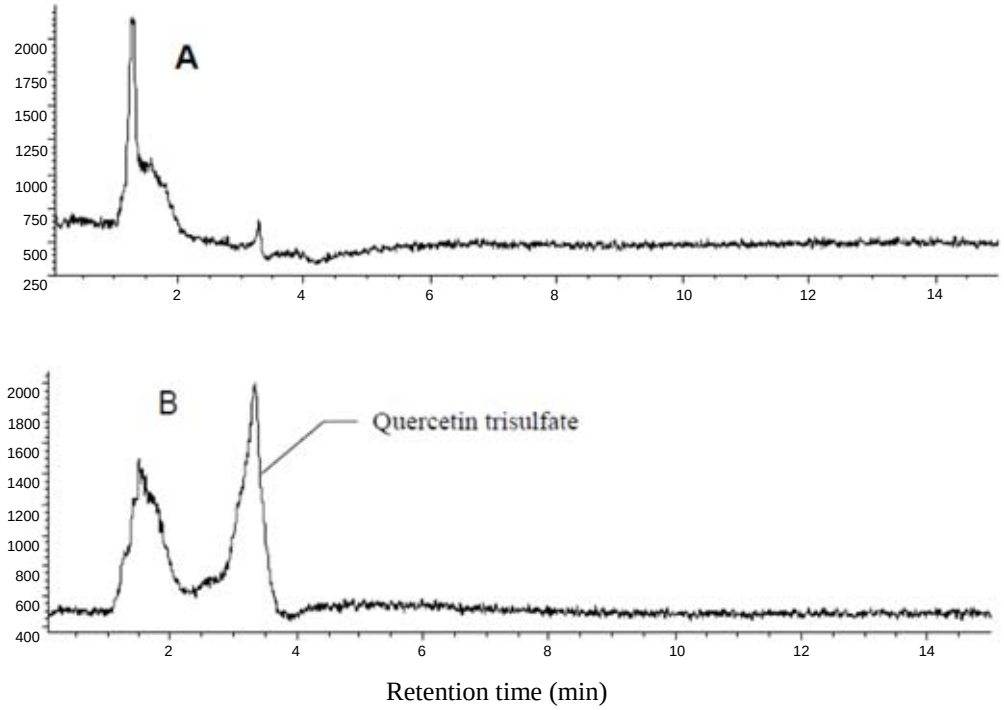


**Figure 3.** HPLC/MS chromatogram of quercetin trisulfate (m/z 543).
Caco-2 cells were incubated with quercitrin at the concentration of 27 μg/ml for 60 min and subject to HPLC/MS analysis as described in the Materials and Methods section. A: control; B: quercitrin treated.

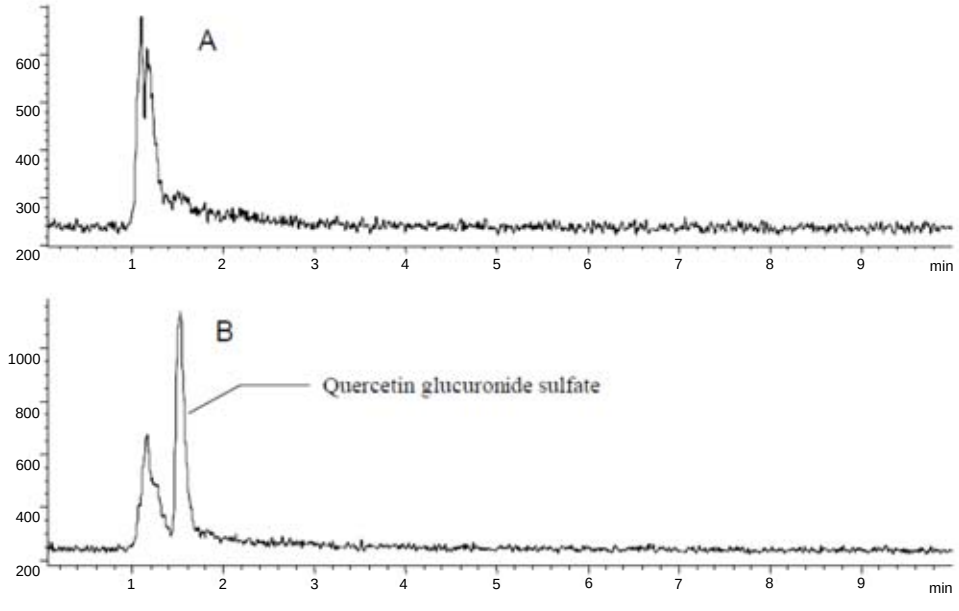


**Figure 4.** HPLC/MS chromatogram of quercetin glucuronide sulfate (m/z 559).
Caco-2 cells were incubated with quercitrin at the concentration of 27 μg/ml for 60 min and subject to HPLC/MS analysis as described in the Materials and Methods section. A: control; B: quercitrin treated.

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-glucuronsyltransferase, 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 metabolites 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

This work was supported by a grant from the Science and Technology Program for the Eleventh Five Years of China (No. 2008BAI58B06).

## 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

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