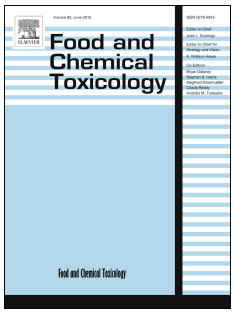
Accepted Manuscript



Transmembrane Transport of Steviol Glucuronide and Its Potential Interaction with Selected Drugs and Natural Compounds

Meiyu Wang, Huixin Qi, Jiajun Li, Yunting Xu, Hongjian Zhang, Ph.D.

PII: S0278-6915(15)30081-8

DOI: 10.1016/j.fct.2015.10.011

Reference: FCT 8417

To appear in: *Food and Chemical Toxicology*

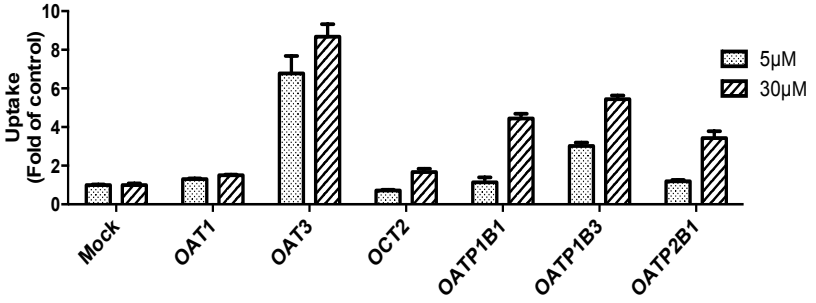
Received Date: 2 September 2015

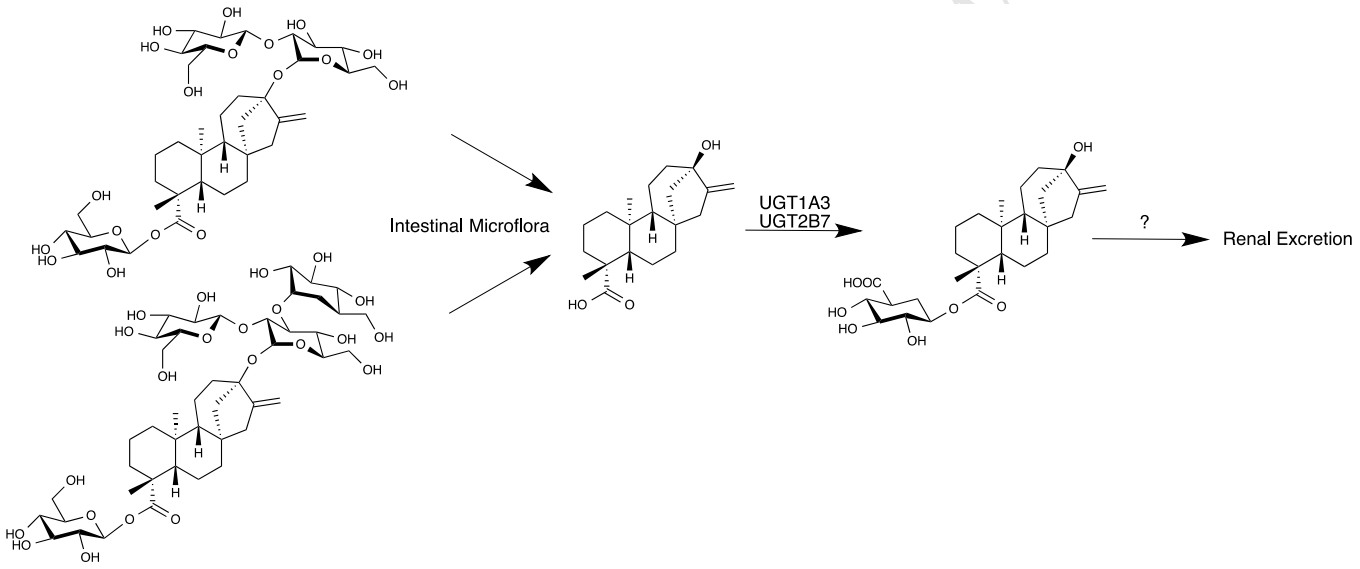
Revised Date: 17 October 2015

Accepted Date: 20 October 2015

Please cite this article as: Wang, M., Qi, H., Li, J., Xu, Y., Zhang, H., Transmembrane Transport of Steviol Glucuronide and Its Potential Interaction with Selected Drugs and Natural Compounds, *Food and Chemical Toxicology* (2015), doi: 10.1016/j.fct.2015.10.011.

Proposed mechanism and involvement of uptake transporters in the transmembrane transport of steviol glucuronide





TITLE PAGE

Transmembrane Transport of Steviol Glucuronide and Its Potential Interaction with Selected Drugs and Natural Compounds

Meiyu Wang[1], Huixin Qi[1], Jiajun Li, Yunting Xu, Hongjian Zhang[2]

College of Pharmaceutical Sciences

Soochow University

Suzhou, China

[2]Corresponding author:

Hongjian Zhang, Ph.D.

College of Pharmaceutical Sciences

Soochow University

199 RenAi Road, Suzhou Industrial Park

Suzhou, China 215123

Tel +86 (512) 6588-2659

Fax +86 (512) 6588-2089

zhanghongjian@suda.edu.cn

Footnote:

[1] Meiyu Wang and Huixin Qi contributed equally to this work.

Abbreviations:

BCRP, breast cancer resistant protein; MATE, multidrug and toxin extrusion protein; MRP, multidrug resistant protein; OAT, organic anion transporter; OATP, organic anion transport protein; OCT, organic cation transporter; P-gp, P-glycoprotein; SVG, Steviol glucuronide.

Conflict of interests:

There are no conflict of interests to be disclosed during the conduct of experiments and preparation of the manuscript.

Abstract

Steviol glucuronide (SVG) is the major metabolite derived from steviol, the aglycone of stevioside and rebaudioside A. After the ingestion of stevioside and rebaudioside A, SVG is formed and excreted into the urine in humans. In the present study, transporter mediated efflux and uptake of SVG was investigated in order to understand molecular mechanisms underlying its renal clearance. Results showed that SVG was not a substrate of efflux transporters BCRP, MRP2, MATE1 or P-gp. In contrast, OAT3 played a predominant role in the uptake of SVG in comparison to OATP1B1, OATP1B3, or OATP2B1. Quercetin, telmisartan, diclofenac, and mulberrin displayed a relatively strong inhibition against OAT3 mediated uptake of SVG with IC_{50} values of 1.8, 2.9, 8.0, and 10.0 μM, respectively. Because OAT3 is a major uptake transporter in the kidney, inhibition of OAT3 activity may alter SVG's renal clearance by drugs and natural compounds that are used concomitantly with stevia leaf extracts.

Key words: Steviol glucuronide; OAT3; uptake transport; food-drug interaction

1. Introduction

Because of popularity and easy access, consumers use a variety of dietary supplements and functional food components to promote health and/or manage certain disease conditions (Low Dog, 2009; Roy et al., 2010). Consumption of those products, particularly when used concurrently with therapeutic drugs, would inevitably increase the likelihood of pharmacokinetic and/or pharmacodynamic interactions. Such interactions, often described in the literature as food-drug or botanical-drug interactions, have generated a considerable amount of interests in academics, food and drug industries, and regulatory agencies due to safety concerns (Bailey, 2010; de Lima Toccafondo Vieira and Huang, 2012; Zhou et al., 2007). A well-known example of those interactions is the grapefruit juice-drug interaction, where its components (e.g., furanocoumarins) are potent inhibitors of CYP3A4 (Paine et al., 2005; Schmiedlin-Ren et al., 1997). Recently, fruit juices are also found to inhibit OATP-mediated drug uptake. In particular, grapefruit juice decreases aliskiren plasma concentrations via OATP1A2 inhibition (Rebello et al., 2012), while grapefruit, orange and apple juices are capable of inhibiting OATP2B1 activity (Shirasaka et al., 2013). Since functional components in food products and small molecule drugs share similarities in disposition processes, the above cases demonstrate that drug-metabolizing enzymes and transporters are key molecular mechanisms involved in food-drug interactions.

As major components of stevia leaf extracts, steviol glycosides (such as stevioside and rebaudioside A) are gaining popularity as natural sweeteners in beverages and some food products due to their intense sweetness and minimum caloric intakes (Crammer and Ikan, 1986). Representative products containing stevia leaf extracts include Coca-Cola Life (The

Coca-Cola Company, Atlanta, Georgia) and Pepsi True (PepsiCo Inc, Purchase, New York). In addition, biological activities of those natural occurring compounds have been reported (Chatsudthipong and Muanprasat, 2009). Studies have shown that components of stevia leaf extracts may promote health benefits related to their anti-hyperglycemic, anti-hypertensive, anti-diabetic, anti-inflammatory, and anti-tumor activities (Chen et al., 2005; Nakamura et al., 1995; Yasukawa et al., 2002). However, the lack of pharmacological effects was also reported with respect to their cardiovascular benefits (such as blood glucose levels) in both type II diabetics and non-diabetic hypotensive/normotensive individuals (Barriocanal et al., 2008; Maki et al., 2008). Recently, components of stevia leaf extracts have been shown to modulate glucose transport (Rizzo et al., 2013) and inhibit renal cyst growth (Yuajit et al., 2014).

Stevioside and rebaudioside A are subjected to hydrolysis in the gastrointestinal tract after oral ingestion, leading to the formation of steviol (Gardana et al., 2003; Renwick and Tarka, 2008; Roberts and Renwick, 2008; Wheeler et al., 2008). Upon its formation, steviol is absorbed into the circulation and eliminated from the body mainly as steviol glucuronide via excretion into the urine in humans (Koyama et al., 2003; Wheeler et al., 2008), suggesting that glucuronidation and subsequent excretion are key elimination pathways for steviol and steviol glucuronide. While our previous study has identified that uridine diphosphate glucuronosyltransferase 2B7 is a major enzyme for steviol glucuronidation in the small intestines and liver (Wang et al., 2014), mechanisms of the transmembrane transport of steviol glucuronide remains unclear with respect to its hepatic uptake and renal excretion (Figure 1).

Biological membranes are physical barriers that maintain polar and nonpolar substances

inside the cells for proper functions against concentration gradients. Compounds that cannot freely diffuse across membranes have to rely on membrane transport proteins (i.e., transporters) to achieve certain intracellular concentrations. Based on the direction of the mass transfer, transporters can be loosely categorized into efflux and uptake transporters, and a number of transporters play important roles in nutrients uptake, drug disposition and food-drug interactions (de Lima Toccafondo Vieira and Huang, 2012). In particular, OAT3 has been shown to participate in the renal clearance of many drugs and endogenous substances (Soars et al., 2014).

The formation of steviol glucuronide via the glucuronidation of steviol represents a common disposition scheme shared by many naturally occurring glycosides and their corresponding aglycones (Ma et al., 2014). As part of our continued efforts on delineating mechanisms of steviol and steviol glucuronide disposition, the present study aimed to investigate the role of various human drug transporters in the efflux and uptake of steviol glucuronide and examine if the transmembrane transport of steviol glucuronide (as an interaction victim) could be interfered by a number of drugs and natural compounds. In addition, the interaction between steviol glucuronide (as an interaction perpetrator) and rosuvastatin was evaluated, since the hepatic transport of rosuvastatin is mediated by multiple transporters and the drug has been frequently used as a probe substrate for OATPs (Kitamura et al., 2008). With the availability of mechanistic insights on the overall disposition processes of steviol glycosides, steviol and steviol glucuronide, it is anticipated that safe and effective consumption of functional products containing steviol glycosides can be achieved even when certain drugs and/or natural products are used concurrently.

2. Materials and Methods

2.1 Materials

Steviol glucuronide was synthesized as described previously (Wang et al., 2014). Atorvastatin, diclofenac, isoquercetin, telmisartan and rosuvastatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Scutellarin (scutellarein-7-O-glucuronide, S7G) was purchased from Shanghai Standard Biotech Co., Ltd. (Shanghai, China). Dioscin, glycyrrhizic acid, liquiritin, rutin, paeoniflorin, quercetin and vitexin were purchased from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, Jiangsu, China). Hesperitin, mulberrin and corticosterone were generous gifts from the Laboratory of Natural Medicinal Chemistry of Soochow University (Suzhou, Jiangsu, China). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM) and trypsin were from Hyclone (Logan, UT, USA). Twenty four-well plates biocoated with poly-D-lysine were purchased from BD Biosciences (San Jose, CA, USA). The BCA protein assay kit was purchased from Alpha Pharmaceutical Co. (Jiangsu, China). All other reagents and chemicals were of analytical grade or of the highest quality available commercially.

Samples were analyzed by a LC-MS/MS system consisting of an API4000 Qtrap mass spectrometer equipped with a turbo-V ionization source (Applied Biosystems, Foster City, CA, USA), two LC-20AD pumps with a CBM-20A controller, DGU-20A solvent degasser and a SIL-20A autosampler (Shimadzu, Columbia, MD, USA).

2.2 Assays and methods

2.2.1 Cell cultures

Human embryonic kidney 293 (HEK293) cells overexpressing human OAT1, OAT3, OCT2, OATP1B1, OATP1B3 or OATP2B1 as well as vectors were generously provided by Professor Dafang Zhong, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). Transfected cell lines were maintained as previously described (Li et al., 2015). Human efflux transporters MDR1-, BCRP-, MRP2-, MATE1 or vector-transfected MDCKII (Madin-Darby canine kidney epithelial cells) cell lines were established at PharmaResources (Shanghai) Co. (Shanghai, China). Each of the above cell lines was established to express a single transporter gene and was used to differentiate roles of various transporters individually. Respective control cells were also prepared in similar fashion with vectors that did not contain individual target genes. All cells were grown in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37^{o}C with 5% CO_2 and 95% humidity.

2.2.2 Transporter-mediated efflux of steviol glucuronide

To determine if efflux transporters were involved in the transmembrane transport of steviol glucuronide, MDCKII cells were seeded on transwell membrane inserts (6.5-mm diameter, 0.4-μm pore size; Corning Costar, catalog #3401) at a density of 200,000 cells per well. Cells were cultured in the growth medium for approximately 5 days to allow the formation of cell monolayer and full maturation. Culture medium was replaced every 48 hours

and the day before the experiment. On the experimental day, cell monolayers were washed three times with pre-warmed Hank's balanced salt solution (HBSS) and then pre-incubated in the assay buffer for 30 min at 37^{o}C prior to the addition of test compounds. The HBSS (pH 7.4) contained 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 5.4 mM KCl, 0.4 mM KH_2PO_4, 137 mM NaCl, 4.2 mM $NaHCO_3$, 0.3 mM Na_2HPO_4, 10 mM HEPES and 5 mM D-glucose. The integrity of the monolayer was checked by measuring transmembrane resistance and a value of $>$200Ω was considered adequate. In addition, positive control compounds were included during the incubation to ensure proper transporter functions. The transmembrane transport assay was initiated by replacing the HBSS on the apical side or basal side with the HBSS containing test compounds at defined concentrations. Incubations were carried out at 37^{o}C and aliquots of the HBSS were taken out from each compartment at specified time points (2 or 3 hrs post incubations). Samples were processed and analyzed by the LC-MS/MS method described below.

For protein assay, the total protein contents were measured using the Pierce^{TM} BCA Protein Assay Reagent Kit (Thermo Scientific, Rockford, USA) with albumin as the standard.

2.2.3 Transporter-mediated uptake of steviol glucuronide

Cellular uptake of steviol glucuronide was investigated using HEK293 cells overexpressing individual human uptake transporters. Cells were seeded in 24-well plates at a density of 200,000 cells per well and cultured in high glucose DMEM with phenol red for 48 hrs. On the third day and prior to the start of the experiment, cells were washed twice with pre-warmed HBSS and pre-incubated with HBSS for 30 min at 37^{o}C. Uptake experiments

were carried out by the addition of 500 µL HBSS containing defined concentrations of substrates. Incubations were terminated at 5 min by washing with ice-cold HBSS. Cells were immediately lysed with 250 µL of 0.2M NaOH solution for 1 hr at 4^{o}C, and the mixtures were then neutralized with 250 µL of 0.2M HCl solution. Intracellular drug accumulations were determined by the LC-MS/MS method.

2.2.4 Inhibition of steviol glucuronide uptake

The above efflux and uptake studies revealed that transmembrane transport of steviol glucuronide was mainly mediated by OAT3 and to a lesser extent by OATP1B1, OATP1B3 or OATP2B1. Therefore, the interaction potential of SVG with other compounds was examined using OAT3-, OATP1B1-, OATP1B3- or OATP2B1-transfected HEK293 cells under the same conditions with the substrate concentration set to 10 µM (below K_m). The inhibition potency was first estimated in the presence of 100 µM inhibitors for a series of compounds after 30-minute incubations. Based on the observed potency, IC_{50} values of quercetin, diclofenac, telmisartan and mulberrin against OAT3-mediated uptake of SVG were determined using inhibitor concentrations in the range of 0.1 to 300 µM. Uptake inhibition was calculated using 10 µM SVG in the absence of inhibitor compounds. In all experiments, active uptake of steviol glucuronide was obtained by subtracting cellular concentrations in control cells from the total uptake in transfected cells to correct for the contribution of passive diffusion (and/or background transport activities).

2.2.5 Inhibition of OATP1B1 and OATP1B3 mediated uptake of rosuvastatin by SVG

Inhibition of OATP-mediated uptake of rosuvastatin by SVG was investigated in OATP1B1-, OATP1B3 or control-HEK293 cells. Prior to the experiment, cells were washed twice with pre-warmed HBSS (pH7.4) and pre-incubated with the HBSS containing different concentration of SVG (0 to 300 μM) for 30 minutes at 37^{o}C. The uptake was initiated by the addition of HBSS containing rosuvastatin (1 μM, at or below K_m). The incubation was terminated by washing cells with ice-cold HBSS. The cells were then lysed with 250 μL of 0.2M NaOH solution for 1hr at 4^{o}C and then neutralized with 250 μL of 0.2M HCl solution. The intracellular rosuvastatin concentrations were quantified by LC-MS/MS.

2.2.6 LC-MS/MS analysis

For sample processing and analysis, 100 μL acetonitrile containing internal standard (IS) were added to 50 μL of cell homogenate and mixed for 2min. Samples were centrifuged and aliquots of 10 μL of the supernatants were injected for analysis.

All samples were analyzed using LC-MS/MS as described previously (Wang et al., 2014). For chromatographic separation, a gradient elution at a flow rate of 0.3 mL/min was employed using the following mobile phases: A, 0.1% formic acid water solution and B, 0.1% formic acid in methanol. The total run time was 6 min. For MS/MS quantitation of steviol glucuronide, the API 4000 Qtrap mass spectrometer was operated in the ESI negative mode with multiple reaction-monitoring (MRM). The ion transitions and collision energy (CE) were as follows: m/z 493.2 (M-H) $\rightarrow$ 317.3 and CE -25 V for steviol-glucuronide; 461.2 (M-H) $\rightarrow$ 284.9 and -25 V for scutellarein-7-glucuronide (IS), respectively. For the quantitation of rosuvastatin (ESI positive mode), the ion transitions and CE were as follows: m/z 482.2(M+H) $\rightarrow$ 258.1 and CE

46 V for rosuvastatin and m/z 559.4(M+H) $\rightarrow$ 440.0 and CE 32 V for atorvastatin (IS), respectively. Data were collected and processed using AB Sciex Analyst 1.5.2 data collection and integration software. Steviol glucuronide or rosuvastatin was quantified by the standard calibration curves, which were linear from 2 to 2000 nM and 1 to 1000 nM, respectively. The quantitative method displayed good sensitivity and reproducibility.

2.2.7 Data analysis

For transporter-mediated efflux of steviol glucuronide, the rate of passage of the test compound across the cell monolayer was used to determine the permeability coefficient (Papp). Values of A$\rightarrow$B or B$\rightarrow$A apparent permeability were calculated using the following equation:

$$Papp(A\rightarrow B) \text{ or } Papp(B\rightarrow A) = (dQ/dt)/(A*C_0) = (C2h*V)/(t*\pi r^2*C_0)$$

where dQ/dt was the rate of permeation, C_0 was the initial concentration of the test compound, and A ($A = \pi r^2$) was the area of the monolayer. After obtaining values of Papp(A$\rightarrow$B) and Papp(B$\rightarrow$A), the efflux ratio (ER) was calculated by dividing Papp(B$\rightarrow$A) with Papp(A$\rightarrow$B), and a ratio of ≥2 was considered to be positive for efflux activity.

Kinetic parameters of transporter-mediated uptake were estimated by nonlinear regression analysis using Prism 5.01 (Graphpad Software Inc., San Diego, CA, USA). Equations used were as follows:

$$v = (V_{\max} \times [S]) / (K_m + [S])$$

Where v was the rate of uptake, V_{max} was the maximum velocity, K_m was the Michaelis constant (substrate concentration at half of the V_{max}), and [S] is the substrate concentration.

For the inhibition study, the IC_{50} values were obtained by fitting the data to the following equation by nonlinear regression analysis using Prism 5.01:

$$\% \text{ Control} = 100/(1 + I / IC_{50})$$

where I is the inhibitor concentration.

All experiments were conducted in three separate incubations unless indicated otherwise. Statistical analysis was performed using a two-way ANOVA (Prism 5.01).

3. Results

3.1 Transporter-mediated efflux of steviol glucuronide

The MDCKII cell generally has low expression of transporter proteins and low metabolic activity, and it is thus an ideal polarized epithelia cell line to study bi-directional transport of nutrients and drug molecules (Horio et al., 1989). In the present study, MDCKII cells stably transfected with human efflux transporters MDR1 (P-gp), BCRP, MRP2 or MATE1 were used to determine if those transporters were involved in the transmembrane transport of steviol glucuronide. Table 1 summarized the apparent permeability (Papp values) of SVG in those cell lines. Based on the calculated efflux ratios, it appeared that SVG was not a substrate of these human efflux transporters.

3.2 Transporter-mediated uptake of steviol glucuronide

Intracellular uptake of steviol glucuronide could be achieved by passive diffusion and transporter-mediated processes. Using HEK293 cells stably transfected with human uptake transporters such as OAT1, OAT3, OCT2, OAPT1B1, OATP1B3 or OATP2B1, the active uptake of SVG was investigated. Compared with the intracellular concentrations in the control cells (passive diffusion and/or background transport activities), the uptake of SVG was predominantly mediated by OAT3 at both low (5 μM) and high (30 μM) concentrations (Figure 2A). In comparison, OATP1B1, OATP1B3 or OATP2B1 were also involved in the SVG uptake albeit to a lesser extent.

Kinetic parameters of SVG uptake were estimated by subtracting intracellular concentrations in the control cells from the total intracellular concentrations of a given transfected cell line (Figure 2B). As summarized in Table 2, OAT3 mediated SVG uptake had a calculated Km of 143.3 μM, demonstrating a relatively higher affinity to OAT3 among uptake transporters. Collectively, Km values of SVG to the tested uptake transporters were all in the high micromolar range, suggesting that the binding of SVG was not in the high affinity category. Judging from the Vmax/Km ratios (the intrinsic uptake clearance), the role of transporters in SVG uptake followed the order of OAT3 > OATP1B3 > OATP1B1 > OATP2B1. The above data suggested that OAT3 might play a predominant role in the uptake of SVG at lower concentrations (≤5 μM).

3.3 Inhibition of steviol glucuronide uptake by selected drugs and natural compounds

Because OAT3, OATP1B1, OATP1B3 or OATP2B1 were involved in the uptake transport

of SVG, the interaction potential of these transporters with multiple drugs and natural product extracts were subsequently examined. As illustrated in Figure 3A and 3C, multiple compounds showed strong inhibitory effects (>50%) on the OAT3- or OATP1B3-mediated SVG uptake. For OTA3, telmisartan, quercetin, diclofenac and mulberrin exhibited the highest inhibition potency (>85%). In contrast, rutin and atorvastatin were compounds that showed obvious inhibition (>50%) against OATP1B1-mediated uptake of SVG (Figure 3B); whereas minimal or no inhibition was observed for OATP2B1-mediated uptake (Figure 3D). The above data suggested that transporter-mediated SVG uptake was subject to compound and transporter dependent inhibition.

Further inhibition studies revealed that telmisartan, quercetin, diclofenac and mulberrin inhibited the OAT3-mediated SVG uptake in a concentration-dependent manner, displaying IC_{50} values of 2.9, 1.8, 8.0, and 10.0 μM, respectively (Figure 4), indicating the likelihood of interactions between those compounds and OAT3 mediated SVG uptake.

3.4 Stimulation of steviol glucuronide uptake by dioscin

Among the tested compounds, it was interesting to note that dioscin displayed a weak inhibitory effect against OAT3 and OATP1B3 but a stimulatory effect toward OATP1B1 and OATP2B1 (Figure 3A-3D). Further experiments revealed that dioscin stimulated OATP1B1- and OATP2B1-mediated uptake of SVG in a concentration-dependent manner (Figure 5A-C), with apparent EC_{50} values of 376.8 μM and 1460 μM, respectively.

3.5 Inhibition of rosuvastatin uptake by steviol glucuronide

OATP1B1 and OATP1B3 play important roles in the hepatic uptake of numerous drugs such as statins. To investigate if SVG might affect the function of OATP1B1 and OATP1B3, rosuvastatin was used as a surrogate substrate and the inhibition of its uptake was evaluated. As displayed in Figure 5D-F, SVG exhibited an inhibitory effect on the uptake of rosuvastatin, with IC_{50} values of 26.4 μM and 39.8 μM for OATP1B1 and OATP1B3, respectively. In contrast, SVG exhibited a weak inhibitory effect against OAT3 mediated rosuvastatin uptake.

4. Discussion

Naturally occurring glycosides are important phytochemicals that are frequently used as nutritional supplements and functional food components. As key ingredients in stevia leaf extracts, the health benefits of steviol glycosides (such as stevioside and rebaudioside A) have been increasingly recognized, in addition to their usage as natural sweeteners (Chatsudthipong and Muanprasat, 2009). Biological effects of glycosides and their corresponding aglycones can be affected by numerous factors, particularly their disposition mechanisms (Ma et al., 2014; Xiao, 2015). Due to similarities in chemical linkages, glycosides in general follow similar steps in their disposition after oral ingestion. For example, stevioside and rebaudioside A are hydrolyzed by intestinal microflora in human to steviol, which is then absorbed into the circulation (Gardana et al., 2003; Koyama et al., 2003; Wingard et al., 1980). Steviol can undergo oxidative metabolism in the presence of NADPH, forming monohydroxy- and dihydroxy-metabolites (Koyama et al., 2003), as well as glucuronidation to form steviol glucuronide (Wang et al., 2014). Steviol glucuronide is the major metabolite observed in human urine, suggesting that renal clearance is an important

elimination pathway for steviol related substance (Geuns et al., 2007; Geuns et al., 2006). The present study investigated transporter-mediated efflux and uptake of steviol glucuronide and provided evidence that OAT3 might play a predominant role in the renal clearance of steviol glucuronide. The current findings thus filled the important knowledge gap in the grand scheme of glycoside disposition, thus enabling an overall assessment on pre-systemic hydrolysis of glycosides, absorption and metabolism of glycosides and aglycones, and excretion of aglycone metabolites (Figure 1). Consequently, investigations into each step of glycoside disposition, as exemplified by research on steviol glycosides, would lead to better understanding in molecular mechanisms that govern the efficacy and safety of glycosides and their aglycones (Ceunen and Geuns, 2013; Kim and Himaya, 2012; Ma et al., 2014; Xiao, 2015).

Glucuronidation and subsequent excretion of glucuronides are important pathways by which the body uses to eliminate endogenous substances and xenobiotics. Glucuronides are generally more polar than their corresponding aglycones and their elimination is often mediated by transporters (Jiang and Hu, 2012). The current data showed that steviol glucuronide was not a substrate of major human efflux transporters. In contrast, cellular uptake of steviol glucuronide could be mediated by OAT3, OATP1B1, OATP1B3 or OATP2B1, and a number of drugs and naturally occurring xenobiotics had either inhibitory or stimulatory effect on those uptake processes. For OAT3-mediated uptake, telmisartan, quercetin, diclofenac and mulberrin displayed the most potent inhibition, while for OATP1B1 and OATP2B1; dioscin exhibited a marked stimulatory effect. The present study provided valuable

evidence for the first time that OAT3 and OATPs (to a less extent) were important mechanisms in the transmembrane transport of SVG and likely play a role in its elimination from the body.

Transporters are functional membrane proteins that govern vital substances in and out of cells or tissues. Factors affect this process would lead to changes in cellular concentrations of transporter substrates and potentially affect biological functions. Cumulative evidence have indicated that a number of transporters play important roles in nutrients uptake, drug disposition and food-drug interactions (de Lima Toccafondo Vieira and Huang, 2012). The significance of the involvement of uptake transporters in SVG transmembrane transport is two folds with respect to transporter-mediated food-drug interactions. As a substrate of OAT3 and OATPs, cellular uptake of SVG can be affected by various compounds as observed in the present study. In this case, SVG is the “victim” of food-drug interactions. Specifically, OAT3-mediated uptake can be inhibited by telmisartan, quercetin, diclofenac and mulberrin. As OAT3 plays a role in the renal clearance of many endogenous substances and xenobiotics (Soars et al., 2014), concurrent use of the above substances would lead to a reduction in SVG renal clearance. Other the other hand, SVG can behave as a “perpetrator” in transporter-mediated food-drug interactions in which it can interfere with the transmembrane transport of OATP- or OAT3 substrates. As shown in the present study, SVG displayed an inhibitory effect on OATP1B1-mediated uptake of rosuvastatin, suggesting that the hepatic uptake of this important lipid-lowering drug can be affected by SVG or when the drug is used concomitantly with stevia leaf extracts. Because rosuvastatin is prescribed widely with Cmax

values up to 39 nM (Martin et al., 2003), further studies are warranted for the above dual roles of transporter-mediated SVG uptake to define its clinical relevance.

For purified stevioside and rebaudioside A, an acceptable daily intake (ADI) of up to 4 mg per kilogram body weight per day as steviol equivalents has been recommended by the Joint FAO/WHO Expert Committee on Food Additives based on safety and tolerability studies in animal species and human (Curry and Roberts, 2008; Hsieh et al., 2003). At this dose level, the use of these glycosides is generally considered safe (Urban et al., 2013; Urban et al., 2015). Human pharmacokinetic studies have found that plasma levels of stevioside and steviol were extremely low or undetectable after an oral administration of the 250 mg capsule of stevioside three times per day for 3 days (Geuns et al., 2007). For steviol glucuronide, however, its plasma concentrations could be as high as 33 μg/mL (or approximately 67 μM), with significant inter-subject variability observed (Geuns et al., 2007). Assuming linear pharmacokinetics for stevioside, an ADI of 4 mg/kg (or 240 mg total dose for a 60 kg person) of stevioside would yield a peak plasma concentration of steviol glucuronide at around 22 μM. Although the inhibitory potency of SVG was not very strong against OATP1B1 (IC_{50}, ~26 μM), the possibility exists for the interaction between SVG and rosuvastatin, particularly in subjects with renal function impairment, where a reduced renal clearance would lead to high SVG plasma concentrations after the ingestion of stevioside and rebaudioside A.

Steviol glucuronide can be chemically categorized as an acyl-O-glucuronide (Geuns et al., 2006). Unlike other types of glucuronides, acyl glucuronides are subject to intramolecular

rearrangement and intermolecular reactions with macromolecules such as proteins. It is this presumed chemical reactivity of acyl glucuronides that has led to the hypothesis of a causal relationship for idiosyncratic toxicity induced by certain xenobiotics (Bailey and Dickinson, 2003; Regan et al., 2010). Because there is a significant structural diversity that exists in aglycones, differences in intrinsic reactivity and pharmacokinetic properties of acyl glucuronides would have distinctive impacts on pharmacological and toxicological outcomes. Given the fact that steviol glucuronide may have a high plasma concentration after oral ingestion of stevioside and rebaudioside A, it would be important to understand its reactivity and biological significance. This is particularly prudent in situations where steviol glycosides are concurrently used with certain drugs or natural products, or in populations whose renal functions are compromised.

In conclusion, the present study investigated the roles of various human drug transporters in the efflux and uptake of steviol glucuronide. Results indicated that SVG was not a substrate of efflux transporters BCRP, MRP2, MATE1 or P-gp. Uptake transporters, such as OAT3, OATP1B1, OATP1B3, or OATP2B1, were involved in the cellular uptake of SVG, with OAT3 playing a more predominant role. Quercetin, telmisartan, diclofenac, and mulberrin displayed a relatively strong inhibition against OAT3 activity, while dioscin exhibited a stimulatory effect toward OATP1B1 and 2B1. Because OAT3 is a major uptake transporter in the kidney, inhibition of OAT3 activity may alter SVG's renal clearance by drugs and natural compounds if those substances are used concomitantly with stevia leaf extracts. The current findings filled the important knowledge gap, which would enable a better assessment of the

disposition mechanisms of steviol glycosides in particular and other naturally occurring glycosides in general. More importantly, the present study provides in vitro evidence for further mechanistic investigations on potential transporter-mediated food-drug interactions. It is anticipated that in vivo studies would define clinical relevance of transporter-mediated uptake of steviol glucuronide and lead to safe and effective use of stevioside and rebaudioside A.

Acknowledgement

The current work was supported in part by a grant from Soochow University, Suzhou, China [Grant No. Q413200711], and a grant from the National Natural Science Foundation of China [Grant No. 81473278].

References

Bailey, D.G., 2010. Fruit juice inhibition of uptake transport: a new type of food-drug interaction. British journal of clinical pharmacology 70, 645-655.

Bailey, M.J., Dickinson, R.G., 2003. Acyl glucuronide reactivity in perspective: biological consequences. Chem Biol Interact 145, 117-137.

Barriocanal, L.A., Palacios, M., Benitez, G., Benitez, S., Jimenez, J.T., Jimenez, N., Rojas, V., 2008. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. Regulatory toxicology and pharmacology : RTP 51, 37-41.

Ceunen, S., Geuns, J.M., 2013. Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod 76, 1201-1228.

Chatsudthipong, V., Muanprasat, C., 2009. Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacology & therapeutics 121, 41-54.

Chen, T.H., Chen, S.C., Chan, P., Chu, Y.L., Yang, H.Y., Cheng, J.T., 2005. Mechanism of the hypoglycemic effect of stevioside, a glycoside of Stevia rebaudiana. Planta medica 71, 108-113.

Crammer, B., Ikan, R., 1986. Sweet Glycosides from the Stevia Plant. Chem Brit 22, 915-&.

Curry, L.L., Roberts, A., 2008. Subchronic toxicity of rebaudioside A. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46 Suppl 7, S11-20.

de Lima Toccafondo Vieira, M., Huang, S.M., 2012. Botanical-drug interactions: a scientific perspective. Planta medica 78, 1400-1415.

Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., Pietta, P., 2003. Metabolism of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. Journal of agricultural and food chemistry 51, 6618-6622.

Geuns, J.M., Buyse, J., Vankeirsbilck, A., Temme, E.H., 2007. Metabolism of stevioside by healthy subjects. Exp Biol Med (Maywood) 232, 164-173.

Geuns, J.M., Buyse, J., Vankeirsbilck, A., Temme, E.H., Compernolle, F., Toppet, S., 2006. Identification of steviol glucuronide in human urine. J Agric Food Chem 54, 2794-2798.

Horio, M., Chin, K.V., Currier, S.J., Goldenberg, S., Williams, C., Pastan, I., Gottesman, M.M., Handler, J., 1989. Transepithelial transport of drugs by the multidrug transporter in cultured Madin-Darby canine kidney cell epithelia. The Journal of biological chemistry 264, 14880-14884.

Hsieh, M.H., Chan, P., Sue, Y.M., Liu, J.C., Liang, T.H., Huang, T.Y., Tomlinson, B., Chow, M.S., Kao, P.F., Chen, Y.J., 2003. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clinical therapeutics 25, 2797-2808.

Jiang, W., Hu, M., 2012. Mutual interactions between flavonoids and enzymatic and transporter elements responsible for flavonoid disposition via phase II metabolic pathways. RSC Adv 2, 7948-7963.

Kim, S.K., Himaya, S.W., 2012. Triterpene glycosides from sea cucumbers and their biological activities. Adv Food Nutr Res 65, 297-319.

Kitamura, S., Maeda, K., Wang, Y., Sugiyama, Y., 2008. Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. Drug metabolism and disposition: the biological fate of chemicals 36, 2014-2023.

Koyama, E., Sakai, N., Ohori, Y., Kitazawa, K., Izawa, O., Kakegawa, K., Fujino, A., Ui, M., 2003. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 41, 875-883.

Li, X.L., Guo, Z.T., Wang, Y.D., Chen, X.Y., Liu, J., Zhong, D.F., 2015. Potential role of organic anion transporting

polypeptide 1B1 (OATP1B1) in the selective hepatic uptake of hematoporphyrin monomethyl ether isomers. Acta pharmacologica Sinica 36, 268-280.

Low Dog, T., 2009. The use of botanicals during pregnancy and lactation. Alternative therapies in health and medicine 15, 54-58.

Ma, Y., Zeng, M., Sun, R., Hu, M., 2014. Disposition of flavonoids impacts their efficacy and safety. Curr Drug Metab 15, 841-864.

Maki, K.C., Curry, L.L., Reeves, M.S., Toth, P.D., McKenney, J.M., Farmer, M.V., Schwartz, S.L., Lubin, B.C., Boileau, A.C., Dicklin, M.R., Carakostas, M.C., Tarka, S.M., 2008. Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46 Suppl 7, S47-53.

Martin, P.D., Warwick, M.J., Dane, A.L., Brindley, C., Short, T., 2003. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. Clinical therapeutics 25, 2553-2563.

Nakamura, Y., Sakiyama, S., Takenaga, K., 1995. Suppression of syntheses of high molecular weight nonmuscle tropomyosins in macrophages. Cell motility and the cytoskeleton 31, 273-282.

Paine, M.F., Criss, A.B., Watkins, P.B., 2005. Two major grapefruit juice components differ in time to onset of intestinal CYP3A4 inhibition. The Journal of pharmacology and experimental therapeutics 312, 1151-1160.

Rebello, S., Zhao, S., Hariry, S., Dahlke, M., Alexander, N., Vapurcuyan, A., Hanna, I., Jarugula, V., 2012. Intestinal OATP1A2 inhibition as a potential mechanism for the effect of grapefruit juice on aliskiren pharmacokinetics in healthy subjects. European journal of clinical pharmacology 68, 697-708.

Regan, S.L., Maggs, J.L., Hammond, T.G., Lambert, C., Williams, D.P., Park, B.K., 2010. Acyl glucuronides: the good, the bad and the ugly. Biopharm Drug Dispos 31, 367-395.

Renwick, A.G., Tarka, S.M., 2008. Microbial hydrolysis of steviol glycosides. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46 Suppl 7, S70-74.

Rizzo, B., Zambonin, L., Angeloni, C., Leoncini, E., Dalla Sega, F.V., Prata, C., Fiorentini, D., Hrelia, S., 2013. Steviol glycosides modulate glucose transport in different cell types. Oxidative medicine and cellular longevity 2013, 348169.

Roberts, A., Renwick, A.G., 2008. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46 Suppl 7, S31-39.

Roy, A., Lurslurchachai, L., Halm, E.A., Li, X.M., Leventhal, H., Wisnivesky, J.P., 2010. Use of herbal remedies and adherence to inhaled corticosteroids among inner-city asthmatic patients. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 104, 132-138.

Schmiedlin-Ren, P., Edwards, D.J., Fitzsimmons, M.E., He, K., Lown, K.S., Woster, P.M., Rahman, A., Thummel, K.E., Fisher, J.M., Hollenberg, P.F., Watkins, P.B., 1997. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. Drug metabolism and disposition: the biological fate of chemicals 25, 1228-1233.

Shirasaka, Y., Shichiri, M., Mori, T., Nakanishi, T., Tamai, I., 2013. Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions. Journal of pharmaceutical sciences 102, 3418-3426.

Soars, M.G., Barton, P., Elkin, L.L., Mosure, K.W., Sproston, J.L., Riley, R.J., 2014. Application of an in vitro OAT assay in drug design and optimization of renal clearance. Xenobiotica 44, 657-665.

Urban, J.D., Carakostas, M.C., Brusick, D.J., 2013. Steviol glycoside safety: is the genotoxicity database sufficient? Food Chem Toxicol 51, 386-390.

Urban, J.D., Carakostas, M.C., Taylor, S.L., 2015. Steviol glycoside safety: are highly purified steviol glycoside sweeteners food allergens? Food Chem Toxicol 75, 71-78.

Wang, M., Lu, J., Li, J., Qi, H., Wang, Y., Zhang, H., 2014. Steviol glucuronidation and its potential interaction with UDP-glucuronosyltransferase 2B7 substrates. Food Chem Toxicol 64, 135-143.

Wheeler, A., Boileau, A.C., Winkler, P.C., Compton, J.C., Prakash, I., Jiang, X., Mandarino, D.A., 2008. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46 Suppl 7, S54-60.

Wingard, R.E., Jr., Brown, J.P., Enderlin, F.E., Dale, J.A., Hale, R.L., Seitz, C.T., 1980. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36, 519-520.

Xiao, J., 2015. Dietary Flavonoid Aglycones and Their Glycosides: Which Show Better Biological Significance? Crit Rev Food Sci Nutr, 0.

Yasukawa, K., Kitanaka, S., Seo, S., 2002. Inhibitory effect of stevioside on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. Biological & pharmaceutical bulletin 25, 1488-1490.

Yuajit, C., Muanprasat, C., Gallagher, A.R., Fedeles, S.V., Kittayaruksakul, S., Homvisasevongsa, S., Somlo, S., Chatsudthipong, V., 2014. Steviol retards renal cyst growth through reduction of CFTR expression and inhibition of epithelial cell proliferation in a mouse model of polycystic kidney disease. Biochemical pharmacology 88, 412-421.

Zhou, S.F., Zhou, Z.W., Li, C.G., Chen, X., Yu, X., Xue, C.C., Herington, A., 2007. Identification of drugs that interact with herbs in drug development. Drug discovery today 12, 664-673.

Figure Legends

Figure 1 Proposed disposition pathways of stevioside and rebaudioside A after oral ingestion

Figure 2 Uptake of steviol glucuronide in OAT1-, OAT3-, OCT2-, OATP1B1-, OATP1B3-, and OATP2B1-transfected HEK293 cells (A); Uptake kinetics of steviol glucuronide by OAT3 (B), OATP1B1 (C), OATP1B3 (D), and OATP2B1 (E).
**P < 0.01; *** P < 0.001. Data were presented as mean± sd.

Figure 3 Inhibition of steviol glucuronide uptake by selected drugs and natural compounds in transporters-transfected HEK293 cells. OAT3 (A), OATP1B1 (B), OATP1B3 (C), and OATP2B1 (D). Grey solid bar, Control; Blank solid bars, Strong inhibitors; Blank bars, other inhibitors; Dashed bar, Dioscin. *Representing P values ranging from < 0.0001 to < 0.05 for clarity. Data were presented as mean± sd.

Figure 4 Inhibitory effects of quercetin (A), telmisartan (B), diclofenac (C) and mulberrin (D) on the uptake of steviol glucuronide mediated by OAT3.

Figure 5 Interaction of transporter mediated uptake.
Selective effect of dioscin on the uptake of steviol glucuronide in transfected cell lines, OATP1B3 (A), OATP2B1 (B), and OATP1B1 (C);
Inhibition of rosuvastatin uptake by steviol glucuronide, OATP1B1 (D), OATP1B3 (E), and OAT3 (F).

Table 1. Bidirectional transport of steviol glucuronide in MDCKII cells stably transfected with human MDR1, BCRP, MRP2, or MATE1 gene

Transporter	Papp(A→B) (x 10^{-6} cm/s)	Papp(B→A) (x 10^{-6} cm/s)	Efflux Ratio (Papp(B→A)/Papp(A→B))
MDR1	0.92 ± 0.17	0.84 ± 0.06	0.91
BCRP	1.49 ± 0.21	1.49 ± 0.12	1.00
MRP2	0.50 ± 0.17	0.40 ± 0.08	0.80
MATE1	0.37 ± 0.17	0.24 ± 0.15	0.65

Transporters tested were MDR1 (multi-drug resistance protein), BCRP (breast cancer resistant protein), MRP2 (multidrug resistant protein 2) and MATE1 (multidrug and toxin extrusion protein 1). The concentration of SVG was set to 10 μM; and the incubation times were 120 min for MDR1, BCRP and MATE1 and 180 min for MRP2. Data were the average of three separate incubations. Data were presented as mean± sd.

Table 2. Kinetic parameters of SVG uptake in HEDK293 cells mediated by Human OAT3, OATP1B1, OATP1B3 and OATP2B1

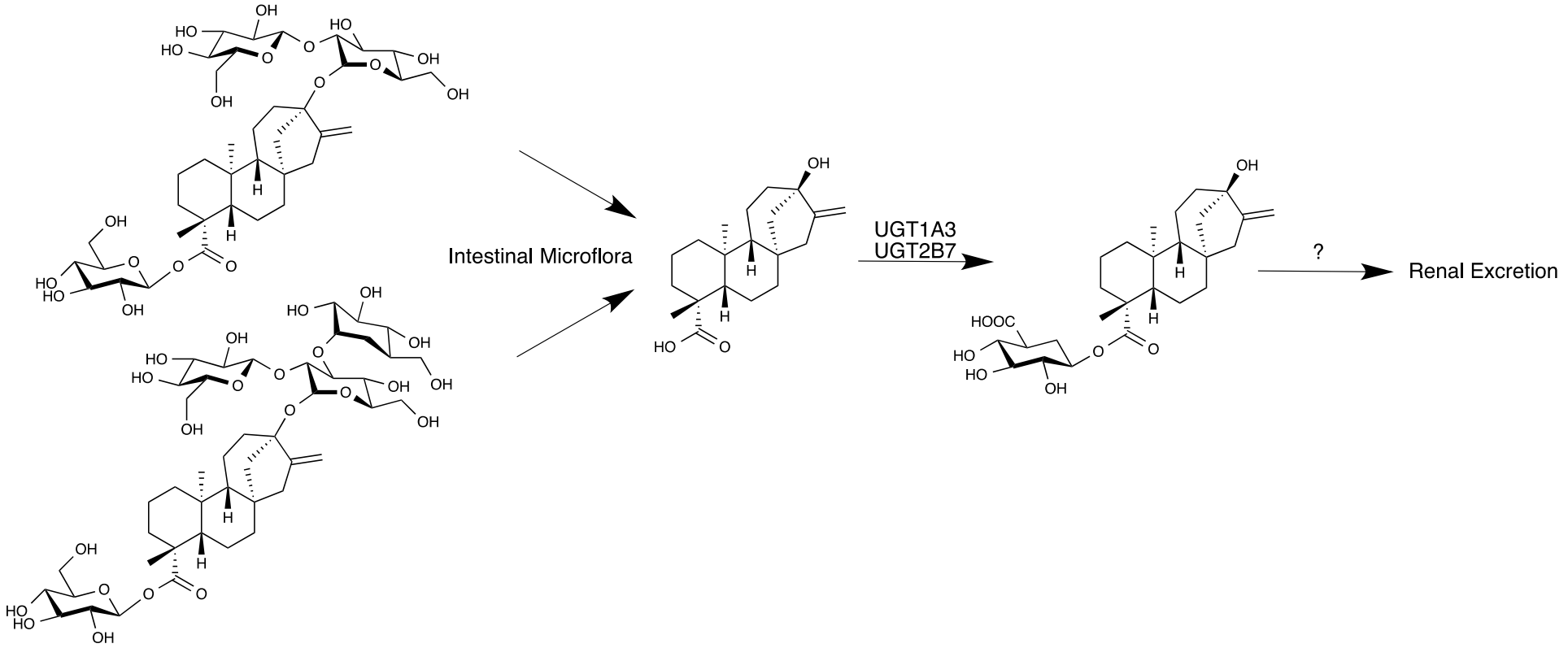
Cell Lines[a]	Kinetic Parameters[b]		
	K_m (μM)	V_{max} (pmol/min/mg protein)	CL_{int}[c] (μl/min/mg protein)
OAT3	143.3 ± 54.5	378.9 ± 77.2	2.64
OATP1B1	363.8 ± 181.5	250.9 ± 88.6	0.69
OATP1B3	396.1 ± 234.5	906.3 ± 299.1	2.29
OATP2B1	297.7 ± 225.2	43.44 ± 22.1	0.15

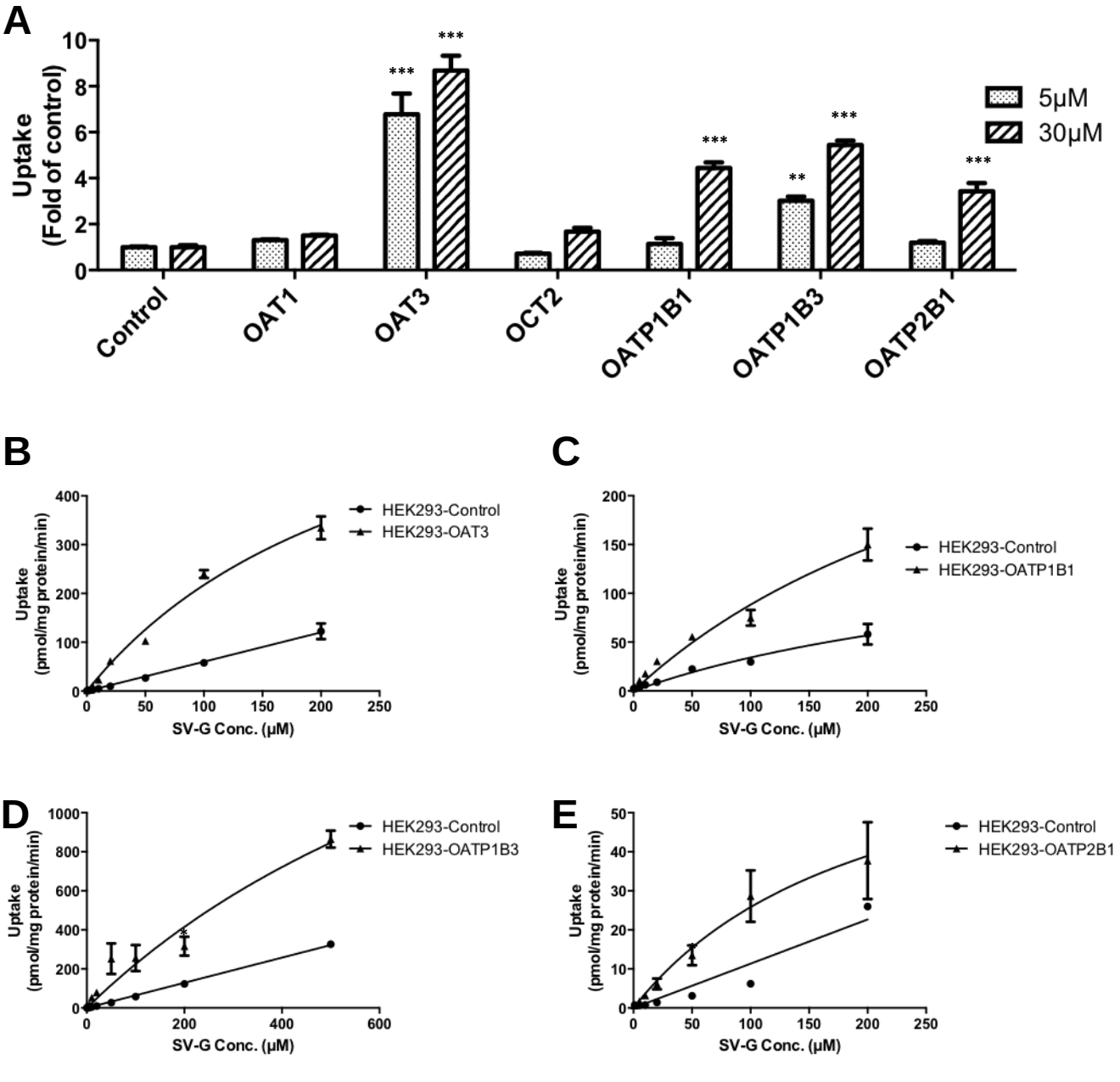
[a]In vitro systems used were HEK293 cells stably transfected with human OAT (organic anion transporter) and OATP (organic anion transport peptide) transporters. Data were the average of three separate incubations.

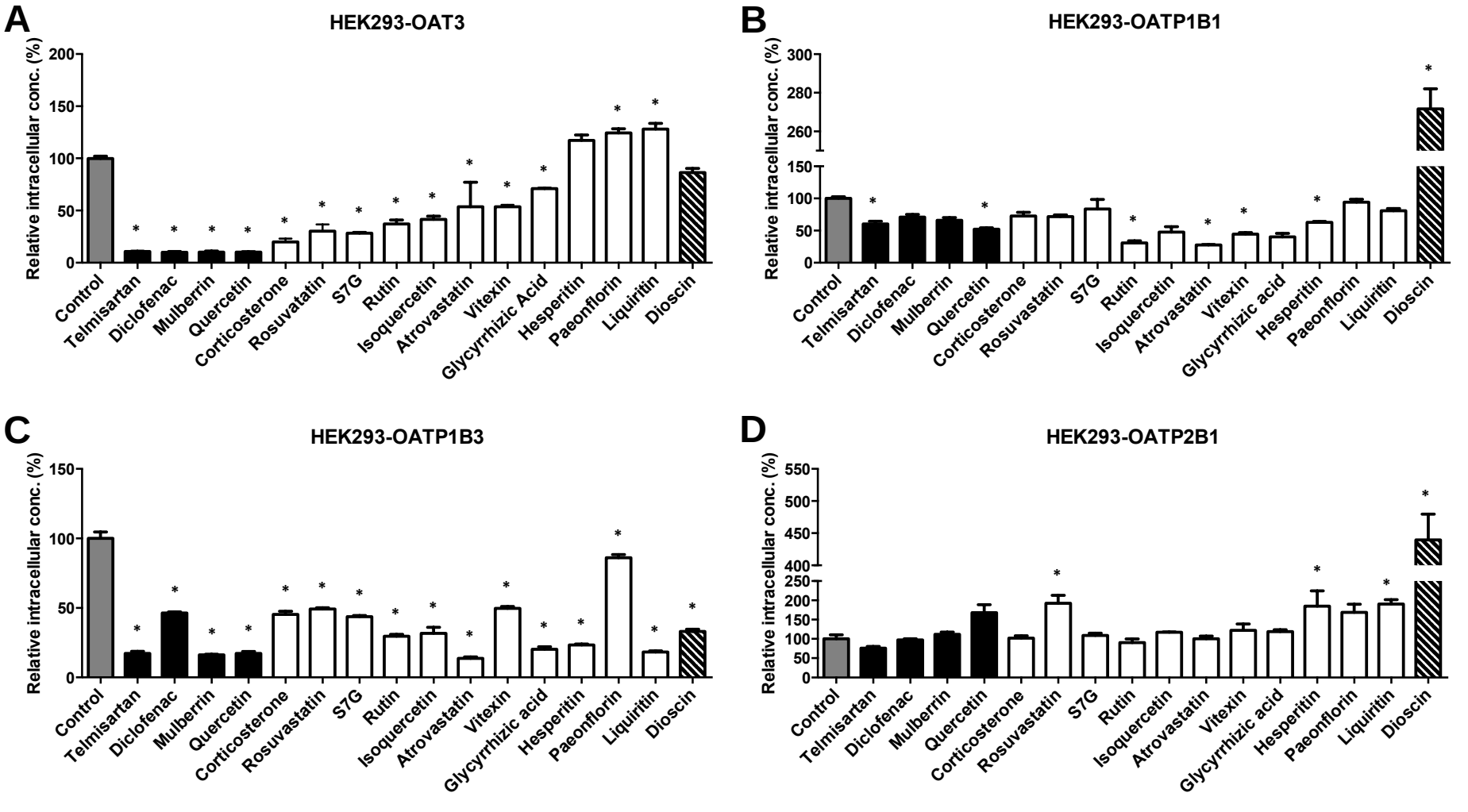
[b]SVG concentrations ranged from 0.2 to 500 μM.

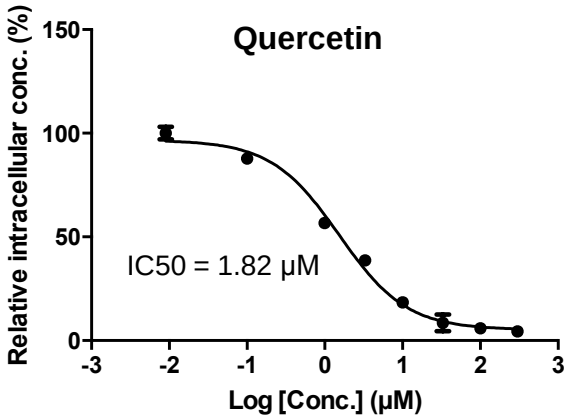
[c]Intrinsic uptake clearance (CL_{int}) was estimated by calculating the ratio of V_{max} over K_m.

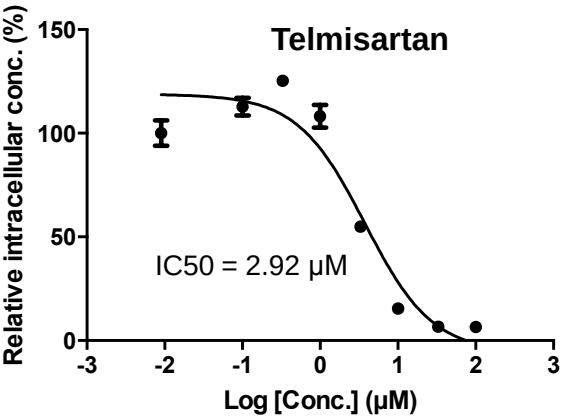
Data were presented as mean± sd.

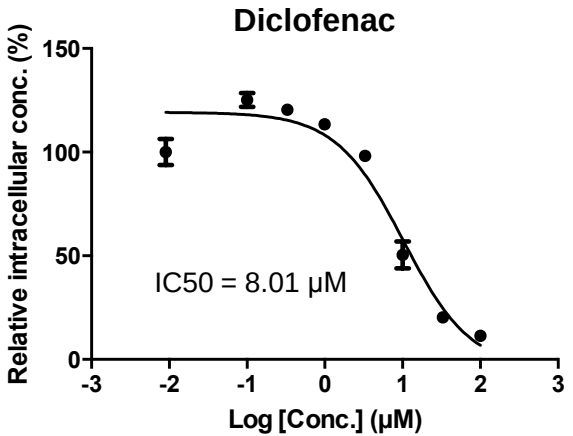

Intestinal Microflora
UGT1A3
UGT2B7
?
Renal Excretion

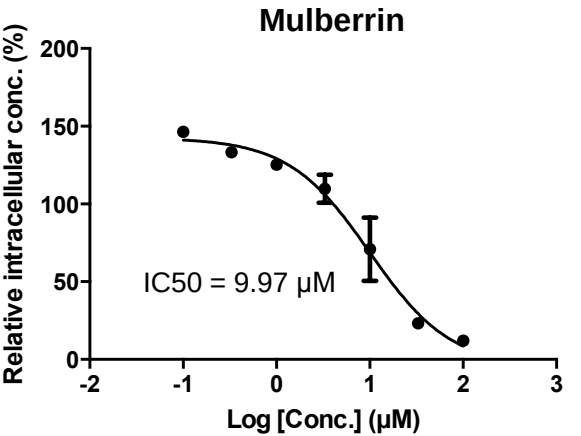

A
Uptake
(Fold of control)
10
8
6
4
2
0
***
***
**
***
***
***
5μM
30μM
Control
OAT1
OAT3
OCT2
OATP1B1
OATP1B3
OATP2B1
B
Uptake
(pmol/mg protein/min)
400
300
200
100
0
0
50
100
150
200
250
SV-G Conc. (μM)
HEK293-Control
HEK293-OAT3
C
Uptake
(pmol/mg protein/min)
200
150
100
50
0
0
50
100
150
200
250
SV-G Conc. (μM)
HEK293-Control
HEK293-OATP1B1
D
Uptake
(pmol/mg protein/min)
1000
800
600
400
200
0
0
200
400
600
SV-G Conc. (μM)
HEK293-Control
HEK293-OATP1B3
E
Uptake
(pmol/mg protein/min)
50
40
30
20
10
0
0
50
100
150
200
250
SV-G Conc. (μM)
HEK293-Control
HEK293-OATP2B1

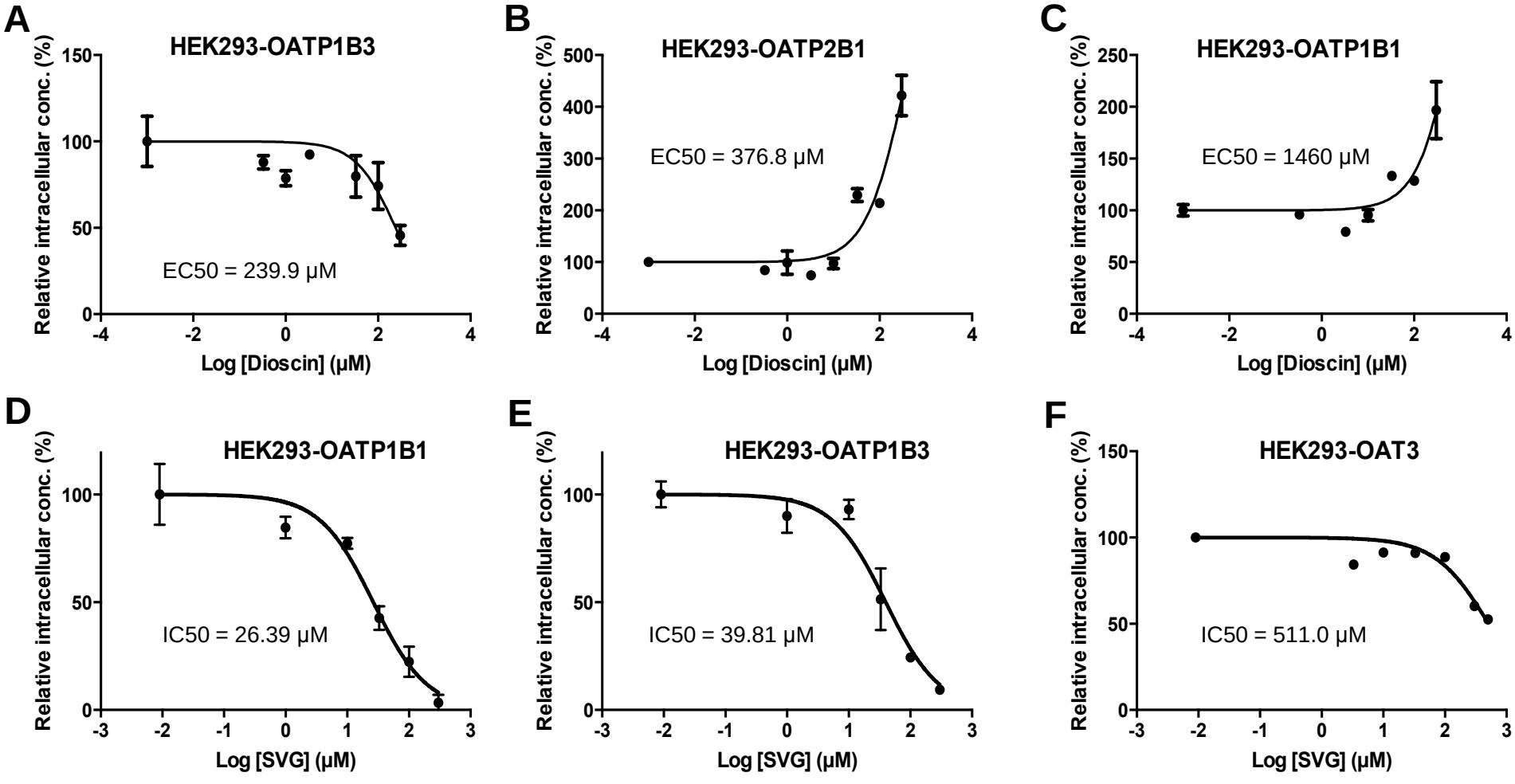

A
HEK293-OAT3
Relative intracellular conc. (%)
200
150
100
50
0
Control
Telmisartan
Diclofenac
Mulberrin
Quercetin
Corticosterone
Rosuvatatin
S7G
Rutin
Isoquercetin
Atrovastatin
Vitexin
Glycyrrhizic Acid
Hesperitin
Paeonflorin
Liquiritin
Dioscin
B
HEK293-OATP1B1
Relative intracellular conc. (%)
300
280
260
150
100
50
0
Control
Telmisartan
Diclofenac
Mulberrin
Quercetin
Corticosterone
Rosuvastatin
S7G
Rutin
Isoquercetin
Atrovastatin
Vitexin
Glycyrrhizic acid
Hesperitin
Paeonflorin
Liquiritin
Dioscin
C
HEK293-OATP1B3
Relative intracellular conc. (%)
150
100
50
0
Control
Telmisartan
Diclofenac
Mulberrin
Quercetin
Corticosterone
Rosuvastatin
S7G
Rutin
Isoquercetin
Atrovastatin
Vitexin
Glycyrrhizic acid
Hesperitin
Paeonflorin
Liquiritin
Dioscin
D
HEK293-OATP2B1
Relative intracellular conc. (%)
550
500
450
400
250
200
150
100
50
0
Control
Telmisartan
Diclofenac
Mulberrin
Quercetin
Corticosterone
Rosuvastatin
S7G
Rutin
Isoquercetin
Atrovastatin
Vitexin
Glycyrrhizic acid
Hesperitin
Paeonflorin
Liquiritin
Dioscin


Quercetin
Relative intracellular conc. (%)
150
100
50
0
-3
-2
-1
0
1
2
3
IC50 = 1.82 μM
Log [Conc.] (μM)


Telmisartan
Relative intracellular conc. (%)
150
100
50
0
-3
-2
-1
0
1
2
3
IC50 = 2.92 μM
Log [Conc.] (μM)


Diclofenac
Relative intracellular conc. (%)
150
100
50
0
-3
-2
-1
0
1
2
3
IC50 = 8.01 μM
Log [Conc.] (μM)


Mulberrin
Relative intracellular conc. (%)
200
150
100
50
0
-2
-1
0
1
2
3
IC50 = 9.97 μM
Log [Conc.] (μM)


A
HEK293-OATP1B3
Relative intracellular conc. (%)
150
100
50
0
-4
-2
0
2
4
Log [Dioscin] (μM)
EC50 = 239.9 μM
B
HEK293-OATP2B1
Relative intracellular conc. (%)
500
400
300
200
100
0
-4
-2
0
2
4
Log [Dioscin] (μM)
EC50 = 376.8 μM
C
HEK293-OATP1B1
Relative intracellular conc. (%)
250
200
150
100
50
0
-4
-2
0
2
4
Log [Dioscin] (μM)
EC50 = 1460 μM
D
HEK293-OATP1B1
Relative intracellular conc. (%)
100
50
0
-3
-2
-1
0
1
2
3
Log [SVG] (μM)
IC50 = 26.39 μM
E
HEK293-OATP1B3
Relative intracellular conc. (%)
100
50
0
-3
-2
-1
0
1
2
3
Log [SVG] (μM)
IC50 = 39.81 μM
F
HEK293-OAT3
Relative intracellular conc. (%)
150
100
50
0
-3
-2
-1
0
1
2
3
Log [SVG] (μM)
IC50 = 511.0 μM

Highlights

- Steviol glucuronide (SVG) was the major metabolite of steviol, the aglycone of steviol glycosides.
- SVG was not a substrate of efflux transporters such as BCRP, MRP2, MATE1 and P-gp.
- Among uptake transporters, OAT3 played a predominant role in the cellular uptake of SVG.
- OTA3-mediated uptake of SVG was inhibited by quercetin, telmisartan, diclofenac, and mulberrin.
- Inhibition of OAT3 activity by drugs and natural compounds may alter SVG's renal clearance.