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Title	In vitro and in vivo evaluation of organic anion-transporting polypeptide 2B1-mediated pharmacokinetic interactions by apple polyphenols
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Citation	Xenobiotica, 51(11), 1318-1325 https://doi.org/10.1080/00498254.2021.1969480
Issue Date	2021-08-24
Doc URL	https://hdl.handle.net/2115/87010
Rights	This is an Accepted Manuscript of an article published by Taylor & Francis in Xenobiotica on 24 Aug. 2021, available online: http://www.tandfonline.com/10.1080/00498254.2021.1969480 .
Type	journal article
File Information	Manuscript_Takahashi et al.pdf



In vitro and in vivo evaluation of organic anion-transporting polypeptide 2B1-mediated pharmacokinetic interaction by apple polyphenols

1. Organic anion-transporting polypeptide (OATP) 2B1 plays a critical role in the intestinal absorption of substrate drugs. Apple juice reportedly interacts with OATP2B1 substrate drugs. The purpose of this study was to investigate the effect of two apple polyphenols, phloretin and phloridzin, on OATP2B1-mediated substrate transport *in vitro* and to evaluate the effect of phloretin on rosuvastatin pharmacokinetics in rats.
2. *In vitro* studies revealed that both polyphenols inhibited OATP2B1-mediated uptake of estrone-3-sulfate. Despite preincubation with phloretin and subsequent washing, the inhibitory effect was retained. Phloretin markedly decreased OATP2B1-mediated rosuvastatin uptake, with an IC₅₀ value of 3.6 µM.
3. On coadministering rosuvastatin and phloretin in rats, the plasma concentration of rosuvastatin 10 min after oral administration was significantly lower than that in the vehicle group. The area under the plasma concentration-time curve of rosuvastatin was not significant, showing a tendency to decrease in the phloretin group when compared with the vehicle group. The *in-situ* rat intestinal loop study revealed the inhibitory effect of phloretin on rosuvastatin absorption.

4. Phloretin has potent and long-lasting inhibitory effects on OATP2B1 *in vitro*.

Phloretin may inhibit OATP2B1-mediated intestinal absorption of rosuvastatin; however, it failed to significantly impact the systemic exposure of rosuvastatin in rats.

Keywords: organic anion-transporting polypeptide 2B1; phloretin; phloridzin; rosuvastatin

Introduction

Apples are among the most widely consumed fruits worldwide owing to the general recognition that they can afford several vital nutritional benefits (Boyer and Liu 2004). Phloretin is a flavone aglycone of phloridzin (phloretin 2'-O-glucose) found to occur in apple-derived products (Bai *et al.* 2016, Schieber *et al.* 2001). Phloretin and phloridzin exhibit numerous biological and pharmacological activities, including anti-inflammatory, antioxidant, anti-hyperglycaemic, and antitumor properties (Zheng *et al.* 2018, Vasantha *et al.* 2010, Han *et al.* 2017, Ma *et al.* 2016, Yang *et al.* 2009).

Organic anion-transporting polypeptide 2B1 (OATP2B1) is broadly expressed in various tissues, including the luminal membrane of enterocytes and the sinusoidal membrane of hepatocytes (Drozdzik *et al.* 2019). Furthermore, accumulated evidence suggests that OATP2B1 is localised at the abluminal membrane in human enterocytes (Keiser *et al.* 2017). In addition, several therapeutic agents, such as HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) inhibitors and fexofenadine, have been identified as OATP2B1 substrates (Ho *et al.* 2006, Imanaga *et al.* 2011). Thus, OATP2B1 is thought to play a critical role in the intestinal absorption and hepatic clearance of these drugs. Several studies have revealed potential pharmacokinetic interaction between food and drugs, indicating that OATP2B1 can act as a target for drug

interactions with natural ingredients, including polyphenols. A representative example is fruit juice-drug interactions. Dresser *et al.* (2002) have reported that plasma fexofenadine levels were markedly reduced in humans after oral administration when administered with fruit juices, especially apple juice. Similarly, Tapaninen *et al.* (2011) have reported that apple juice dramatically reduces systemic exposure to aliskiren, an OATP2B1 substrate. Moreover, Shirasaka *et al.* (2013a) have revealed that several components in fruit juice inhibit OATP2B1-mediated transport of estrone-3-sulfate (E3S), a prototypical substrate of OATP, in *Xenopus* oocyte expression systems. Additional studies from the same research group have demonstrated that apple juice has a long-lasting inhibitory effect on OATP2B1 functions (Shirasaka *et al.* 2013b). Based on these reports, we hypothesised that the underlying mechanisms through which apple juice affects systemic exposure to substrate drugs partially depend on the inhibition of intestinal absorption via OATP2B1 mediated by apple polyphenols. In order to test this hypothesis, we evaluated the competition between OATP2B1 substrates and apple polyphenols, phloretin and phloridzin, using OATP2B1-expressing HEK293 cells, and finally examined the potential pharmacokinetic interactions between rosuvastatin and phloretin in rats.

Materials and Methods

Chemicals

[³H]-labelled E3S ([³H]E3S) was purchased from Perkin Elmer (Waltham, MA, USA). Phloretin (purity: 98%), phloridzin (purity: 97%), and rosuvastatin (purity: 98%) were purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). All other reagents were commercially available with guaranteed purity and were used without further purification.

Cell culture and uptake experiment

OATP2B1-expressing HEK293 cells and mock cells were grown in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% foetal bovine serum (FBS) at 37°C under 5% CO₂. The uptake study was performed as described previously, with minor modifications (Kondo *et al.* 2019). The cells were washed and prewarmed with Hank's balanced salt saline (HBSS) buffer at 37°C for 10 min. Uptake was initiated by adding HBSS containing [³H]E3S or rosuvastatin in the presence or absence of apple polyphenols. Then, to evaluate the effect of pretreatment with each apple polyphenol, cells were exposed to phloretin or phloridzin in HBSS buffer for 0-90 min, with uptake initiated in the absence of these polyphenols after subsequent washing out. Furthermore, to evaluate

the long-lasting inhibitory effect of phloretin, cells were exposed to 10 μ M phloretin in HBSS buffer for 15 min; uptake was initiated in the absence of phloretin after subsequent incubation for the indicated times in HBSS buffer without phloretin. The uptake was terminated by removing the substrate solution, and cells were washed twice with ice-cold HBSS buffer. The cellular accumulation of [3 H]E3S and rosuvastatin was measured by liquid scintillation counting and liquid chromatography/electrospray ionisation tandem mass spectrometry (LC-MS/MS), respectively.

Animals

Male Wistar rats were obtained from Hokudo (Sapporo, Japan). The rats (weighing 200-230 g, aged 7 weeks) were housed under standard conditions ($23 \pm 2^\circ\text{C}$) and maintained under a 12:12-h light-dark cycle. During acclimatisation, the rats were housed individually with free access to food (Rodent Lab Diet EQ 5L37; PMI Nutrition International) and water. All animal experiments were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals of Hokkaido University. Ethical approval was granted by the Laboratory Animal Committee of Hokkaido University (Approval No. 17-0005).

Pharmacokinetic study in rats

An aqueous solution of rosuvastatin (3 mg/mL/kg) dissolved in hydroxypropyl cellulose (HPC) 0.5% in the absence or presence of phloretin (30 µg/mL/kg) was gavage fed as a single oral dose to rats after overnight fasting. The dose setting was based on a dose conversion method between animals and humans. Considering that rosuvastatin is generally prescribed at a daily dose of 5 to 40 mg for adult patients and the average human body weight is 70 kg, the dose of rosuvastatin used in rats is within the daily dose range of rosuvastatin in humans.

Blood samples (300 µL at each time point) were collected from the tail vein at 10, 30, 60, 120, 240, and 360 min after administration. Blood samples were drawn into heparinised tubes and centrifuged immediately at $1,200 \times g$ for 20 min at 4°C to separate the plasma. All samples were stored at -30°C until further analysis of rosuvastatin. Plasma rosuvastatin concentrations were measured using LC-MS/MS, as described below.

In-situ rat intestinal loop study

Intestinal absorption of rosuvastatin was evaluated by the *in-situ* intestinal closed-loop method. During maintenance anesthesia of sevoflurane, the abdominal cavity was opened and an intestinal loop 10 cm in length was made at the lower ileum. An

aqueous 0.5% HPC solution of rosuvastatin (3 mg/mL) in the absence or presence of phloretin (30 µg/mL) was introduced into the intestinal loop. Blood samples were collected from the portal vein at 3, 9, 15, and 30 min after administration and then treated, as described above.

LC-MS/MS analysis

Quantitation of rosuvastatin was performed as previously reported (Kondo *et al.* 2019). In brief, plasma fractions were extracted with ethyl acetate, and then samples were injected into the chromatographic system for analysis using an ACQUITY UPLC® H-Class System coupled with a Xevo® TQ-S mass spectrometer (Waters, Milford, MA).

Statistical analysis

Data obtained from *in vitro* studies are expressed as the mean ± standard error of the mean (SEM), and data of the *in vivo* studies are presented as the mean ± standard deviation (SD). When appropriate, the mean values were compared using Student's t-test (for two means) or analysis of variance followed by Dunnett's test (for >2 means). Statistical significance was set at $p < 0.05$.

Results

Effect of coincubation with apple polyphenols on OATP2B1-mediated E3S uptake

We examined the inhibitory effects of apple polyphenols, phloretin and phloridzin, on OATP2B1-mediated E3S uptake by HEK293 cells. Herein, we observed that phloretin and phloridzin inhibited OATP2B1-mediated E3S uptake in a concentration-dependent manner (Figure 1). IC₅₀ values of phloretin and phloridzin were 4.3 μ M and 110 μ M, respectively. Next, we examined the effect of these two polyphenols on the kinetic parameters of OATP2B1-mediated E3S uptake. Figure 2 and Table 1 present the concentration-dependent uptake of E3S by OATP2B1 and kinetic parameters, respectively. The results revealed that coincubation with phloretin or phloridzin affects K_m rather than V_{max} of E3S transport by OATP2B1.

[Insert Figure 1 here]

[Insert Figure 2 about here]

[Insert Table 1 about here]

Effects of preincubation with apple polyphenols on OATP2B1-mediated E3S uptake

We examined the effect of preincubation with these two apple polyphenols without coincubation. Phloretin inhibited E3S uptake by OATP2B1-expressing HEK293

cells as a result of preincubation for 1-90 min despite subsequent washing (Figure 3B). This inhibitory effect was enhanced with increasing preincubation time for up to 15 min and was maintained until 90 min of preincubation. In contrast to phloretin, preincubation with phloridzin (100 μ M) did not inhibit E3S uptake by OATP2B1-expressing HEK293 cells. In mock cells, these polyphenols demonstrated no inhibitory effects (Figure 3A). We further examined whether preincubation with phloretin induced long-lasting OATP2B1 inhibition. After preincubation with phloretin (10 μ M) for 15 min, the cells were washed and incubated for 0-60 min in the growth medium without phloretin. Subsequently, OATP2B1-mediated E3S uptake was determined in the absence of phloretin. E3S uptake mediated by OATP2B1 was significantly inhibited despite preincubation with phloretin followed by a 30 min incubation period without phloretin, although this inhibitory effect tended to be weaker (Figure 4).

[Insert Figure 3 here]

[Insert Figure 4 here]

Effect of phloretin on OATP2B1-mediated rosuvastatin uptake

We verified the inhibitory effect of phloretin on OATP2B1 using rosuvastatin, a known substrate of this carrier. Rosuvastatin uptake by OATP2B1 was linear for up to 30

s (Figure 5A); therefore, uptake studies using rosuvastatin were performed with a 30-s incubation period. As shown in Figure 5B, phloretin inhibited OATP2B1-mediated rosuvastatin uptake in a concentration-dependent manner, with an IC_{50} value of 3.6 μ M. Furthermore, despite preincubation with phloretin for 1-60 min followed by washing, the inhibitory effect on rosuvastatin uptake was maintained in OATP2B1-expressing HEK293 cells (Figure 5C).

[Insert Figure 5 here]

Impact of concomitant oral phloretin administration on plasma concentration-time profiles of rosuvastatin in rats

Next, to evaluate whether phloretin could affect the pharmacokinetics of rosuvastatin, changes in the plasma concentration of rosuvastatin were assessed when rosuvastatin and phloretin were orally coadministered. Ten minutes after administration, plasma concentrations of rosuvastatin decreased significantly when concomitantly administered with phloretin (Figure 6). The area under the plasma concentration-time curve (AUC) of rosuvastatin showed a tendency to decrease in the phloretin group when compared with the vehicle group, but this difference was not significant (Table 2). Furthermore, the effect of phloretin on the intestinal absorption of rosuvastatin was

evaluated by *in-situ* rat intestinal loops. The concentrations of rosuvastatin in the portal vein at 15 min after intrainestinal administration was significantly reduced by phloretin coadministration, suggesting that a decreased plasma concentration of rosuvastatin at early time points after oral administration may be related to the decrease of intestinal absorption by coadministration with phloretin (Figure 7).

[Insert Figure 6 here]

[Insert Table 2 about here]

[Insert Figure 7 here]

Discussion

Several studies have reported that apple juice can induce drug interactions via intestinal OATP2B1 (Dresser *et al.* 2002, Tapaninen *et al.* 2011, Akamine *et al.* 2014). Although ingredients specifically and abundantly present in apple juice could strongly contribute to these drug interactions, the component of apple juice responsible for this interaction remains unknown. Phloridzin and its aglycone phloretin were reportedly found almost exclusively in apple and apple juice (Oleszek *et al.* 1988, Shirasaka *et al.* 2013a). In the present study, we therefore focused on major apple polyphenols, phloretin and phloridzin, and demonstrated the inhibitory effects of these polyphenols on OATP2B1 *in vitro*. In addition, our *in vivo* pharmacokinetic studies indicated that the plasma concentrations of rosuvastatin following oral administration might be affected by concomitant phloretin.

As shown in Figure 1, both phloretin and phloridzin exhibited inhibitory effects on OATP2B1-mediated E3S uptake. The IC_{50} value of phloretin was 4.3 μ M, 25-fold lower than that of phloridzin, almost consistent with previous reports observed in *Xenopus* oocytes (Shirasaka *et al.* 2013a). To further clarify the interaction of these polyphenols with OATP2B1, kinetic analyses were performed. Kinetic analyses of E3S uptake revealed that phloretin and phloridzin increased K_m values, whereas V_{max} was unaltered by coincubation, suggesting that these polyphenols inhibit OATP2B1-mediated

E3S uptake in a competitive manner (Figure 2 and Table 1).

The long-lasting inhibition of OATP2B1-mediated transport by apple juice using an oocyte expression system has been previously reported (Shirasaka *et al.* 2013b). However, the precise apple juice component that induces a long-lasting inhibitory effect against OATP2B1 remains elusive. Preincubation with phloretin, but not phloridzin, potently inhibited E3S uptake by OATP2B1-expressing HEK293 cells despite subsequent washing out (Figure 3B). In addition, this inhibitory effect was maintained even after preincubation with phloretin followed by a 30 min incubation period without phloretin (Figure 4). These results suggest that phloretin competitively inhibits OATP2B1-mediated transport and, interestingly, also causes long-lasting irreversible inhibition. Moreover, this finding supports a previous report revealing that apple juice inhibits OATP2B1 through both competitive and irreversible inhibitory mechanisms (Shirasaka *et al.* 2013b).

Similar to the results obtained using E3S as an OATP2B1 substrate, phloretin inhibited OATP2B1-mediated rosuvastatin uptake, with an IC_{50} value of 3.6 μ M (Figure 5B). Rosuvastatin uptake into OATP2B1-expressing HEK293 cells was inhibited despite preincubation with phloretin, followed by a washout; this was not observed in mock cells (Figure 5C). Thus, phloretin exerted a similar inhibitory effect on OATP2B1-mediated

rosuvastatin transport, as observed with E3S. Reportedly, phloretin is mainly present as a glycoside (phloridzin) in apple juice (Kahle *et al.* 2007, Shirasaka *et al.* 2013a). Phloridzin was detected as several tens of micromoles in apple juice (Hrubá *et al.* 2021). Using an *in-situ* rat model, Crespy *et al.* (2001) have reported that 80% of phloridzin undergoes hydrolysis in the intestinal wall into the aglycon. Moreover, in humans, phloretin and its conjugated forms reach the intestine at micromolar concentrations after apple juice consumption (Kahle *et al.* 2007). Therefore, as phloretin inhibited OATP2B1-mediated transport, with IC₅₀ values in the low micromolar range, phloretin might be responsible for OATP2B1-mediated drug interactions induced by apple juice during intestinal absorption. To confirm this possibility, *in vivo* experiments were performed using rats. At early time points after administration, plasma rosuvastatin concentrations decreased with concomitant phloretin administration, but the effect on its systemic exposure was limited (Figure 6 and Table 2). This finding is consistent with a previous report, which reported that coadministration of fexofenadine with apple juice decreases C_{max} (Kamath *et al.* 2005). Moreover, the authors revealed that the AUC of fexofenadine in rats was moderately decreased when compared with that reported in humans (Dresser *et al.* 2002, Akamine *et al.* 2014). In the present study, the effect of phloretin on rosuvastatin pharmacokinetics was limited in rats; however, systemic exposure to

OATP2B1 substrate drugs may be significantly influenced by phloretin coadministration in humans. The limited effect of phloretin in rats could be attributed to species differences in transport activity or the participation of other transporters mediating rosuvastatin absorption in the gastrointestinal tract. Although human OATP2B1 and rat Oatp2b1 have a high amino acid identity (77%), the K_m value of rosuvastatin for human OATP2B1 is reportedly 6.4 μ M, while the affinity of rat Oatp2b1 remains unknown (Hagenbuch and Meier 2003, Kitamura *et al.* 2008). Moreover, other members, such as Oatp1a5, are also expressed in the rat intestinal epithelium and could mediate rosuvastatin transport (MacLean *et al.* 2010, Klaassen and Aleksunes 2010). Therefore, Oatp1a5 might predominantly contribute to the intestinal absorption of rosuvastatin in rats. The *in-situ* rat intestinal loop study showed a partly inhibitory effect of phloretin on rosuvastatin absorption; however, to the best of our knowledge, no currently available report has examined the inhibitory effect of phloretin on the rat Oatps (Figure 7). To explain the decrease of intestinal rosuvastatin absorption by coadministration with phloretin, further studies regarding the inhibitory effect of phloretin on rat Oatps are warranted. Like OATPs, breast cancer resistance protein (BCRP), an ATP-driven efflux pump localised at the luminal side, is expressed in the rat small intestine (Tanaka *et al.* 2015). Considering that BCRP also mediates rosuvastatin transport, the limited effect of phloretin on

rosuvastatin pharmacokinetics *in vivo* might be explained via BCRP inhibition in rats. Following oral administration, the AUC of rosuvastatin can be affected by the ABCG2 genotype associated with decreased BCRP activity (Keskitalo *et al.* 2009). In addition, Karibe *et al.* (2015) reported that BCRP is a determinant of the oral bioavailability of rosuvastatin in mice. Zhao *et al.* (2020) recently reported that phloretin interacts with intestinal efflux transporters, including BCRP, using an *in situ* rat intestinal perfusion technique. Accordingly, we cannot exclude the contribution of inhibited BCRP-mediated intestinal rosuvastatin efflux on the limited phloretin effect observed in rats (Zhang *et al.* 2004). After apple juice consumption, several polyphenols, including their conjugated forms, are present in the gastrointestinal tract (Kahle *et al.* 2007). However, the pharmacokinetic interactions between these compounds and intestinal transporters, such as OATP2B1 and BCRP, need to be comprehensively elucidated. Further studies, including not only intestinal transporter-mediated mechanisms but also other mechanisms (e.g., osmolarity change in the lumen), are warranted to identify the contributing factor(s) responsible for drug interactions mediated by apple juice (Funai *et al.* 2019).

The present study focused on the underlying mechanisms through which apple juice affects systemic exposure to OATP2B1 substrate drugs, demonstrating that phloretin, a flavonoid present in apples, has both competitive and long-lasting inhibitory effects on

OATP2B1. Moreover, when rosuvastatin was orally co-administered with phloretin in rats, peak plasma concentrations of rosuvastatin decreased, although the effect of phloretin on systemic exposure was limited. Thus, the mechanisms underlying the impact of apple juice on the oral availability of OATP2B1 substrate drugs can be partially explained by the inhibitory effect of phloretin on OATP2B1-mediated intestinal absorption.

Acknowledgements

This work was supported by JSPS KAKENHI (grant number JP 18K14416 to KN). We would like to thank Editage (www.editage.com) for English language editing.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

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Tables

Table 1

Kinetics of OATP2B1-mediated E3S transport in the absence or presence of phloretin or phloridzin.

Substrate	Treatment	$V_{\max}$ (pmol/mg protein/15 s)	K_m (μ M)
E3S	None (Control)	589 $\pm$ 12	26.2 $\pm$ 1.6
	phloretin	543 $\pm$ 14	54.5 $\pm$ 3.2
	phloridzin	694 $\pm$ 118	53.9 $\pm$ 20

The kinetic parameters for the uptake of E3S were calculated using the Michaelis-

Menten equation using data in Figure 2 and calculated using the following equation: $V =$

$V_{\max} [S]/(K_m + [S])$, where V represents the uptake rate, $V_{\max}$ is the maximum uptake

rate, $[S]$ is the substrate concentration, and K_m is the Michaelis-Menten constant. Data

represent the mean $\pm$ standard error of the mean (SEM) of three independent

experiments.

Table 2

Mean pharmacokinetic parameters of rosuvastatin after administration alone or co-administrated with phloretin.

	AUC (ng · h / mL)	t _{1/2} (h)
Vehicle	27.9 ± 5.1	3.07 ± 1.2
Phloretin	21.5 ± 5.8	2.66 ± 1.9

In order to analyse the pharmacokinetics of rosuvastatin, the area under the curve (AUC) was calculated using the trapezoidal rule. The elimination rate constant (K_{el}) was determined by log-linear regression, considering at least three final data points. The apparent elimination half-life ($t_{1/2}$) was calculated using the formula $0.693/K_{el}$.

Figure captions

Figure 1

Inhibitory effects of apple polyphenols on OATP2B1-mediated E3S uptake by HEK293 cells.

The uptake of [³H]E3S (5 nM) by OATP2B1-expressing HEK293 cells and mock cells was performed at 37°C, pH 7.4, for 15 s in the absence or presence of various concentrations of phloretin (A) and phloridzin (B). The specific uptake of E3S by OATP2B1 was calculated by subtracting the uptake by mock cells from that by OATP2B1-expressing HEK293 cells. Data are presented as uptake values relative to the uptake in the absence of an inhibitor (%). To determine the IC₅₀ value of each inhibitor, the data points were analysed using nonlinear regression analysis, fitting a 4-parameter logistic equation using SigmaPlot 12.5. Data represent the mean ± standard error of the mean (SEM) of three independent experiments. OATP2B1, organic anion-transporting polypeptide 2B1; E3S, estrone-3-sulfate.

Figure 2

Concentration-dependent uptake of E3S by OATP2B1 in the absence (open circles) and presence (filled circles) of apple polyphenols.

Uptake assays were initiated by adding either [^3H]E3S or a mixture of [^3H]E3S and non-radiolabelled E3S to each well. Cells were incubated at 37°C and pH 7.4 for 15 s, with varying concentrations of E3S in the absence or presence of 10 μM phloretin (A) and 100 μM phloridzin (B). The specific uptake of E3S by OATP2B1 was calculated by subtracting the uptake by mock cells from that by OATP2B1-expressing HEK293 cells. Each point represents the mean $\pm$ standard error of the mean (SEM) of three independent experiments. OATP2B1, organic anion-transporting polypeptide 2B1; E3S, estrone-3-sulfate.

Figure 3

Time-dependence of effects of preincubation with phloretin and phloridzin on E3S uptake by OATP2B1-expressing HEK293 cells.

After preincubation with HBSS buffer (open circles), 10 μM phloretin (filled circles), or 100 μM phloridzin (filled triangles) for 0, 1, 15, 30, 60, and 90 min, E3S uptake by mock cells (A) and OATP2B1-expressing HEK293 cells (B) was measured in the absence of apple polyphenols for 15 s at 37°C and pH 7.4. The effect of preincubation with apple polyphenols on E3S uptake by HEK293 cells was evaluated by comparing with the uptake in the control group (preincubation with HBSS). Asterisks indicate significance compared

with the control ($*p < 0.05$). Data represent the mean $\pm$ standard error of the mean (SEM) of three independent experiments. OATP2B1, organic anion-transporting polypeptide 2B1; E3S, estrone-3-sulfate; HBSS, Hank's balanced salt saline.

Figure 4

Long-lasting inhibitory effect of phloretin on OATP2B1-mediated E3S uptake by HEK293 cells.

After preincubation in the absence or presence of phloretin (10 μ M) for 15 min, OATP2B1-expressing HEK293 cells and mock cells were incubated for 0, 30, and 60 min in DMEM supplemented with 10% FBS without phloretin. E3S uptake (5 nM) was measured in the absence of phloretin for 15 s at 37°C and pH 7.4. The specific uptake of E3S by OATP2B1 was calculated by subtracting the uptake by mock cells from that by OATP2B1-expressing HEK293 cells. Data are presented as uptake values relative to uptake when preincubation without phloretin was performed (%). $*p < 0.05$, significantly different from control (preincubation without phloretin). Data represent the mean with standard error of the mean (SEM) of three independent experiments. OATP2B1, organic anion-transporting polypeptide 2B1; E3S, estrone-3-sulfate; DMEM, Dulbecco's modified Eagle medium; FBS, foetal bovine serum.

Figure 5

Inhibitory effects of phloretin on OATP2B1-mediated rosuvastatin uptake by HEK293 cells.

(A) Time-dependence of OATP2B1-mediated rosuvastatin uptake. OATP2B1-expressing HEK293 cells (filled circles) and mock cells (open circles) were incubated for the indicated times at 37°C and pH 7.4 with rosuvastatin (0.01 μ M). OATP2B1-mediated rosuvastatin uptake (filled triangles) was calculated by subtracting rosuvastatin uptake by mock cells (open circles) from that by OATP2B1-expressing HEK293 cells (filled circles).

(B) Concentration-dependent inhibition of OATP2B1-mediated rosuvastatin uptake by phloretin. The uptake of rosuvastatin (0.01 μ M) was evaluated at 37 °C and pH 7.4 for 30 s, in the presence or absence of phloretin at specified concentrations. The specific uptake of rosuvastatin by OATP2B1 was calculated by subtracting its uptake by mock cells from that by OATP2B1-expressing HEK293 cells. Data are presented as the uptake values relative to the uptake in the absence of phloretin (%). To determine the IC₅₀ value of phloretin, the data points were analysed using nonlinear regression analysis by fitting a 4-parameter logistic equation using SigmaPlot 12.5. (C) Time-dependence of effects of phloretin preincubation on rosuvastatin uptake by OATP2B1-expressing HEK293 cells.

After preincubation with HBSS buffer (open circles) or 10 μ M phloretin (filled circles) for 0, 1, 15, 30, 60, and 90 min, rosuvastatin uptake by mock cells (left) and OATP2B1-expressing HEK293 cells (right) was measured in the absence of phloretin for 30 s at 37°C and pH 7.4. The effect of phloretin preincubation on rosuvastatin uptake by HEK293 cells was evaluated by comparing the uptake in the control group (preincubation with HBSS). Asterisk indicates significance from control (* p < 0.05; ** p < 0.01). Data represent the mean $\pm$ standard error of the mean (SEM) of three independent experiments. OATP2B1, organic anion-transporting polypeptide 2B1; HBSS, Hank's balanced salt saline.

Figure 6

Mean plasma concentration-time curve of rosuvastatin coadministered with phloretin in Wistar rats.

Rats were orally administered 3 mg/mL/kg rosuvastatin in the absence (open circles) or presence (filled circles) of 30 μ g/mL/kg phloretin. Blood samples were collected by serial sampling at 10, 30, 60, 120, 240, and 360 min after administration. Asterisks indicate significance compared with the vehicle group (** p < 0.01). Data values are presented as the mean $\pm$ standard deviation (SD) of four rats.

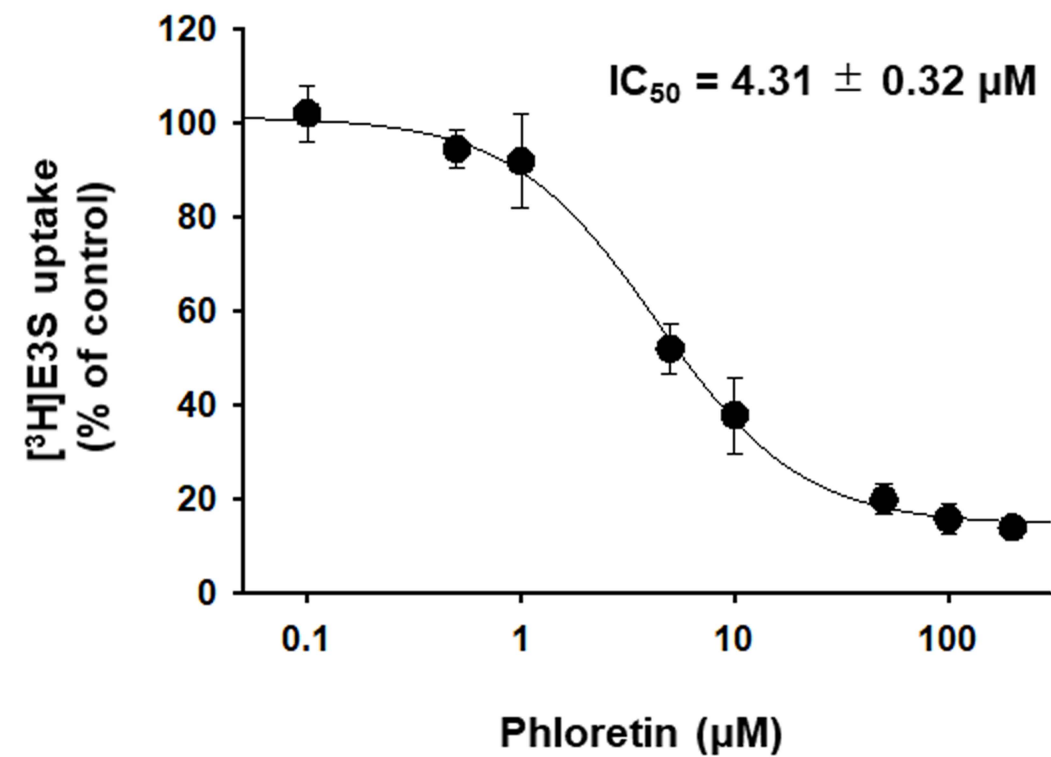
Figure 7

Effect of phloretin coadministration on the rosuvastatin concentration in the portal vein by *in-situ* rat intestinal loops after intrainestinal administration.

Rosuvastatin (3 mg/mL) in the absence (open circles) or presence (filled circles) of phloretin (30 µg/mL) was administered. Blood samples were collected from the portal vein at 3, 9, 15, and 30 min after intraileum administration and the rosuvastatin concentrations were determined. Asterisks indicate significance compared with the vehicle group (* $p < 0.05$). Data values are presented as the mean $\pm$ standard deviation (SD) of four to five rats.

Figure 1

(A)



(B)

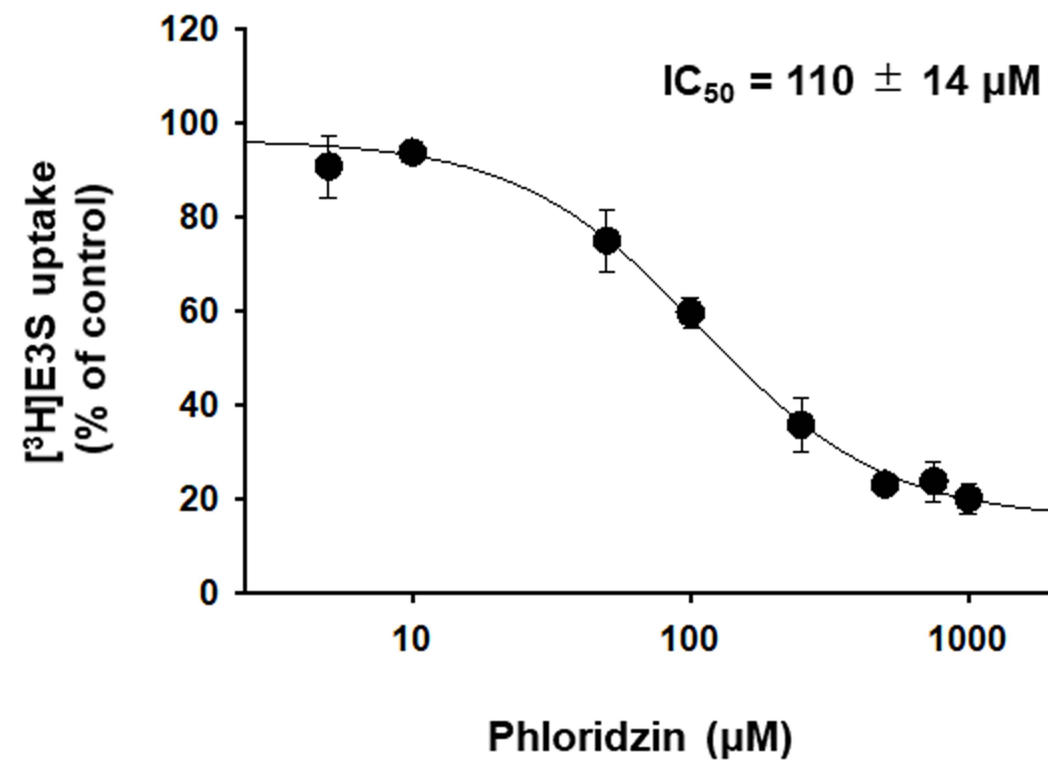
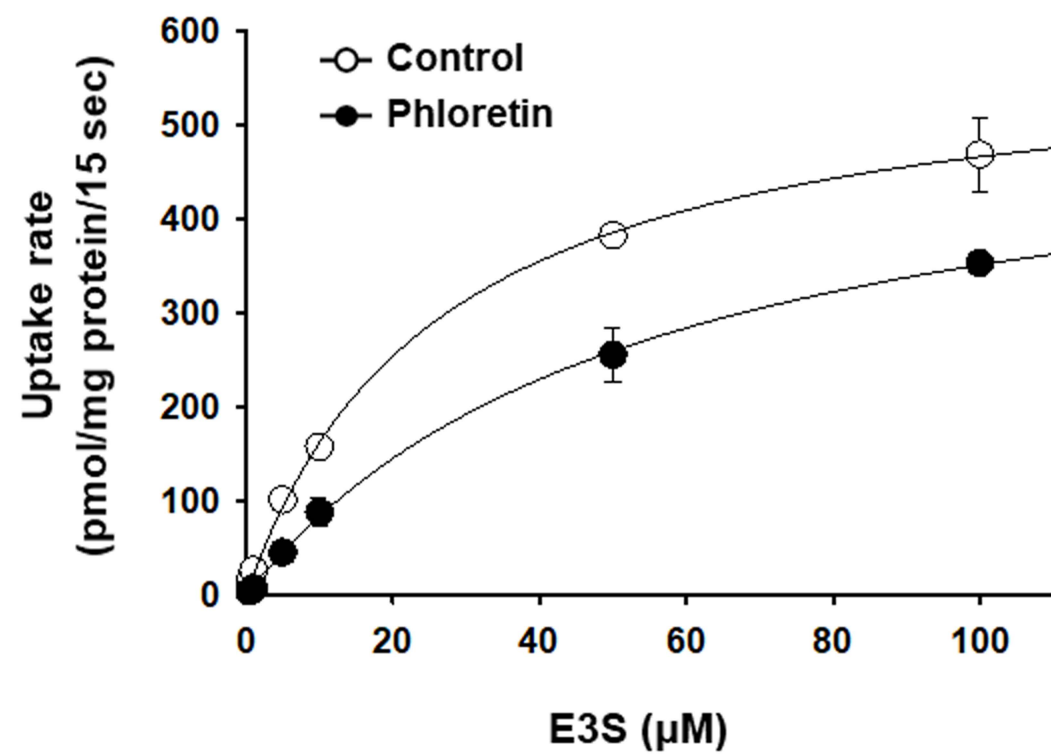


Figure 2

(A)



(B)

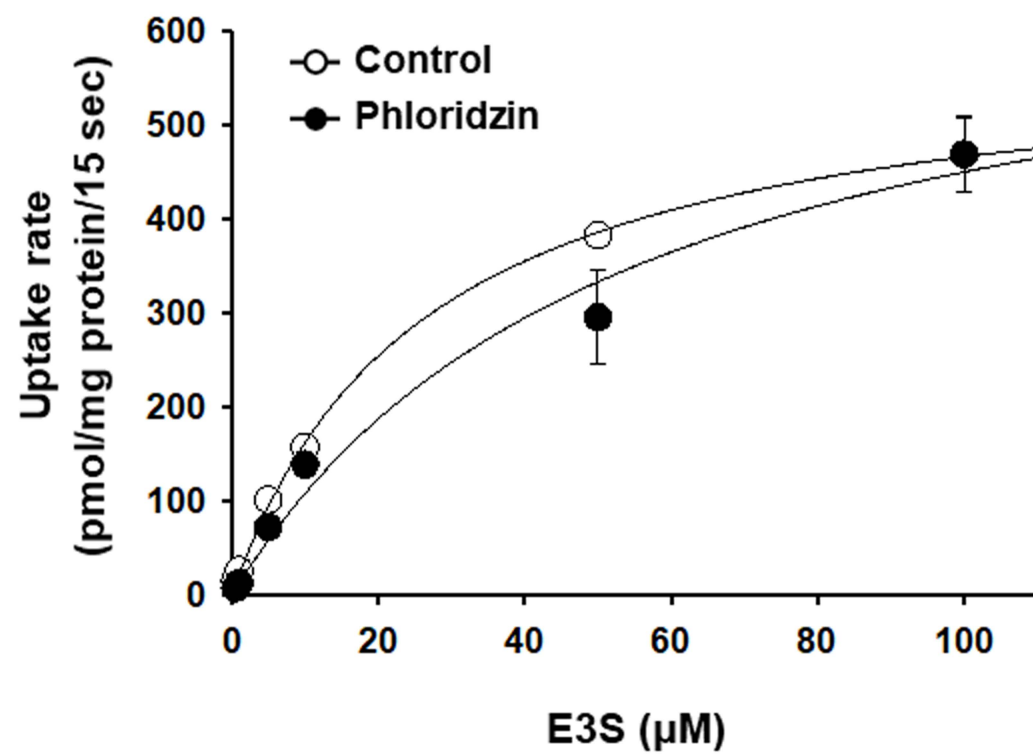
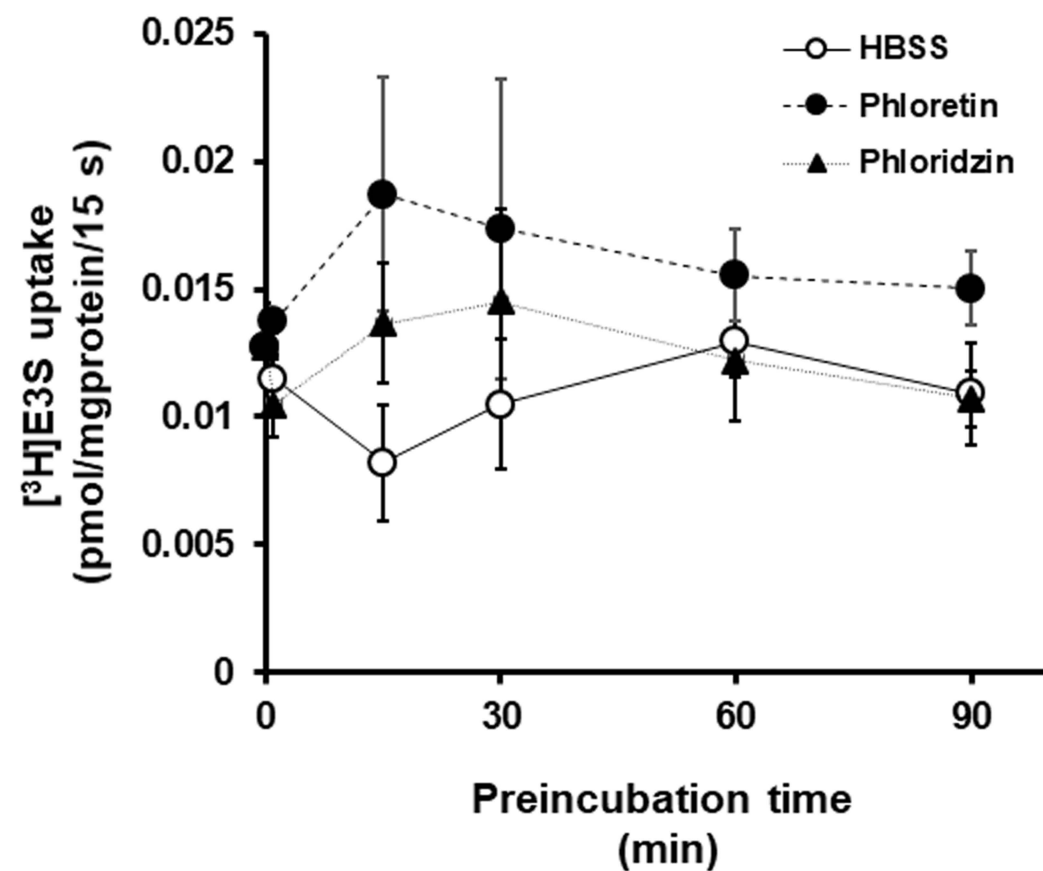


Figure 3

(A) HEK293-mock cells



(B) HEK293-OATP2B1 cells

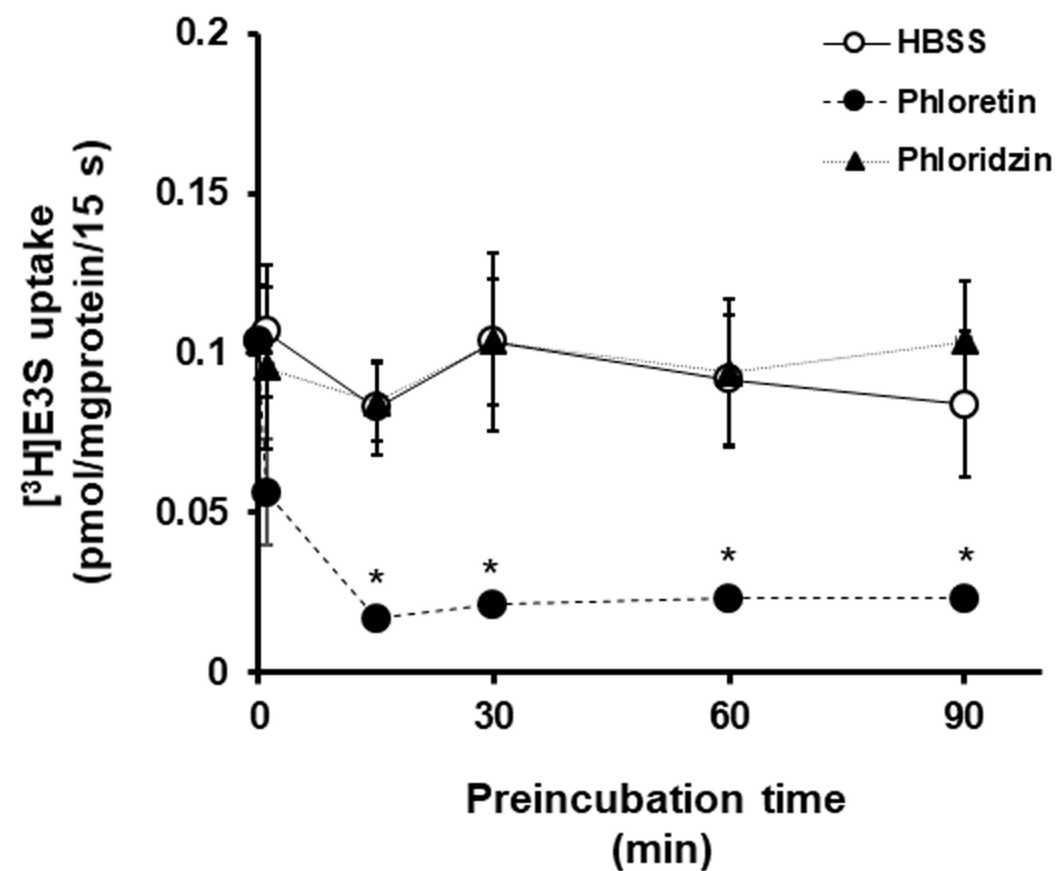


Figure 4

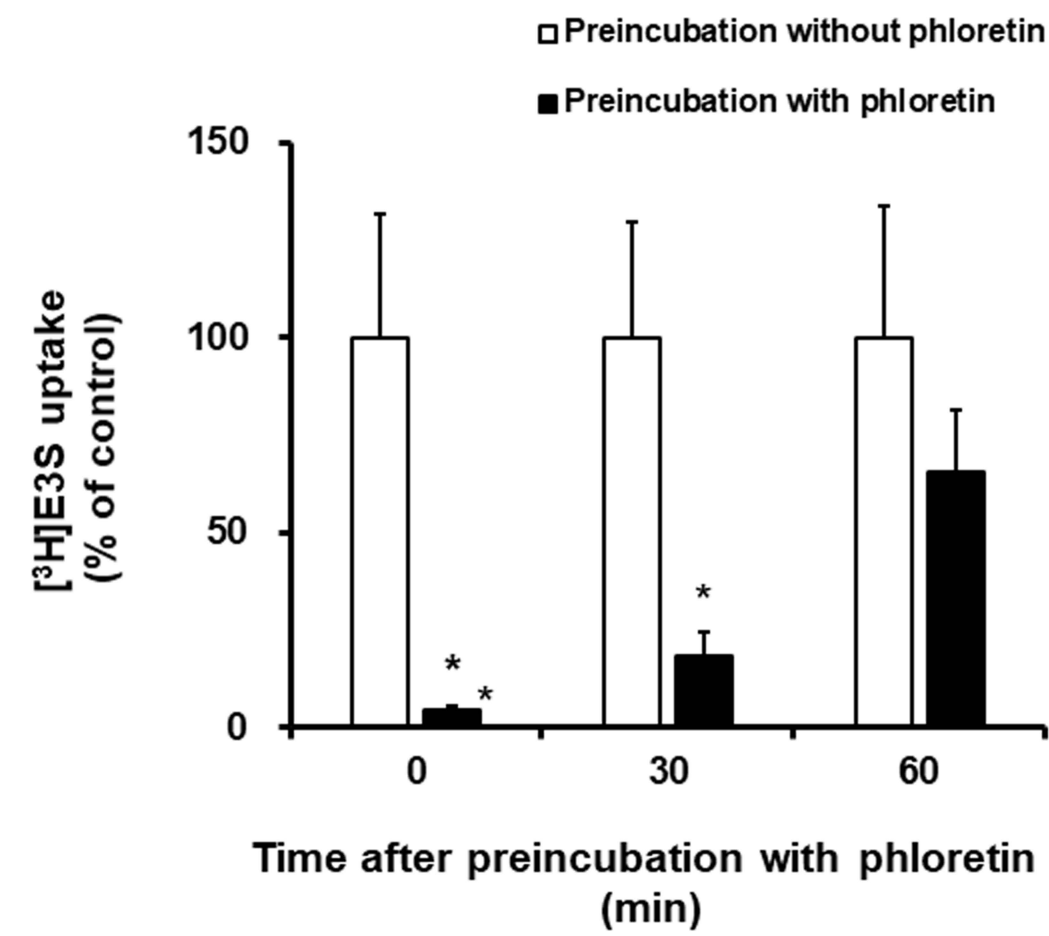
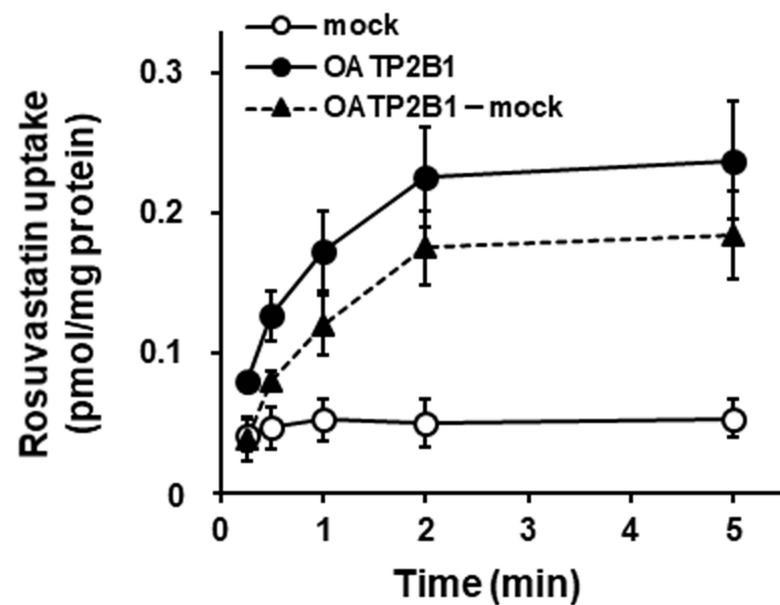
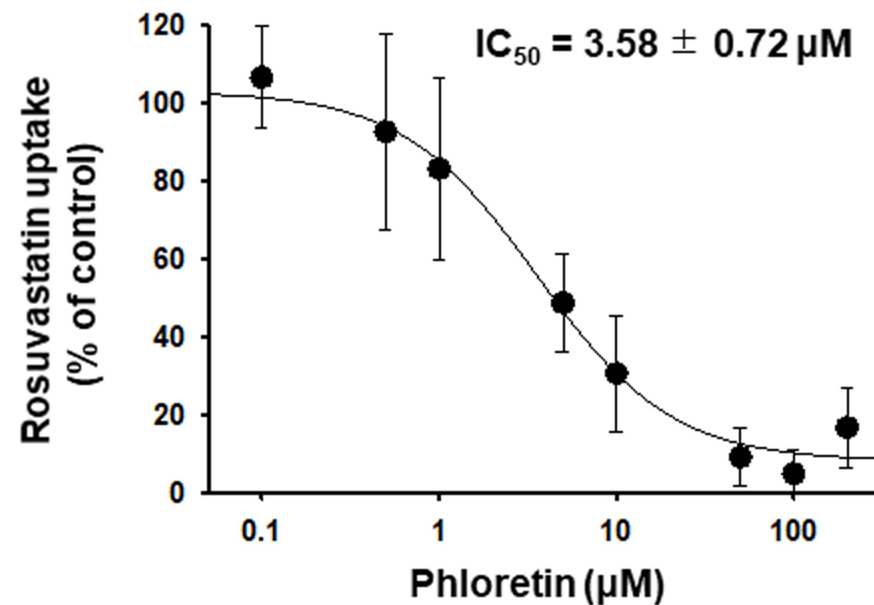


Figure 5

(A)



(B)



(C)

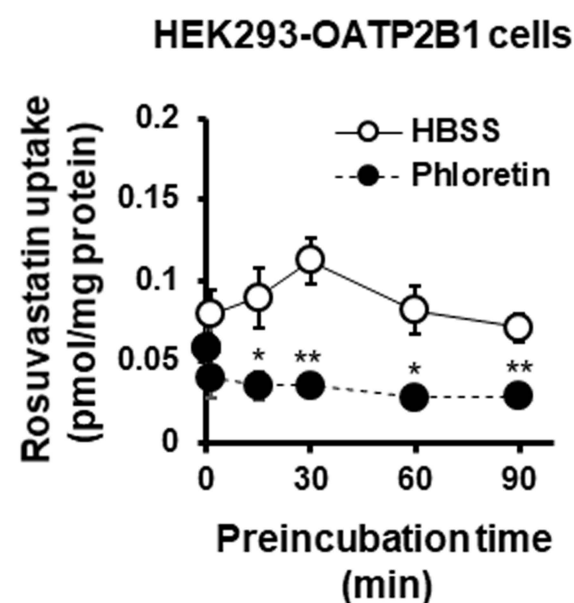
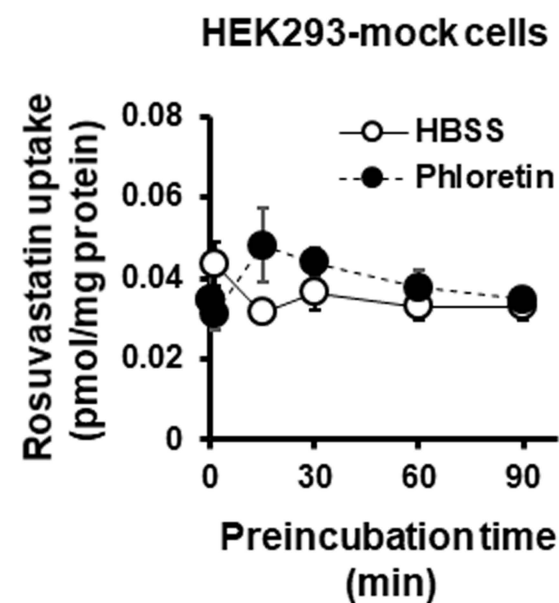


Figure 6

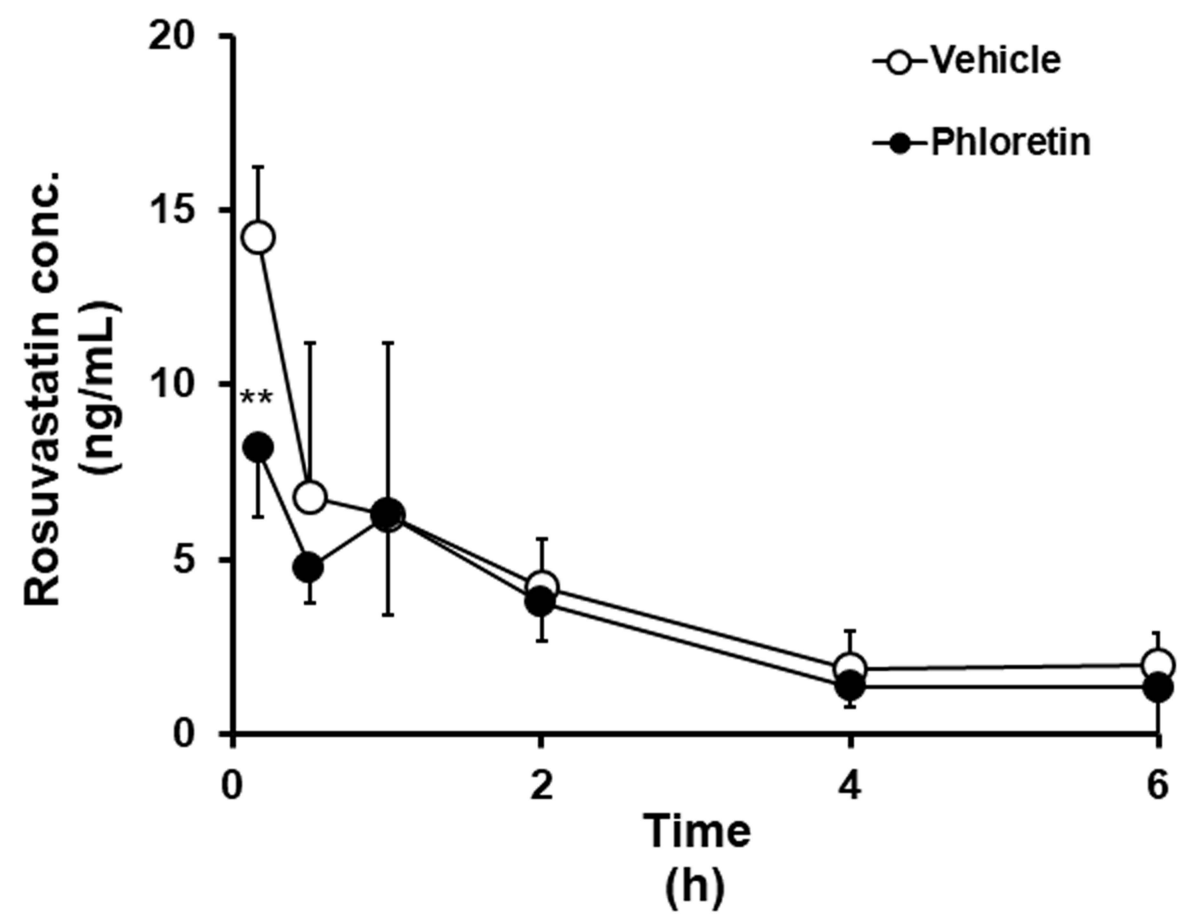


Figure 7

