JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 381–386

Received July 25, 2008
Accepted November 17, 2008
DOI 10.1211/jpp/61.03.0014
ISSN 0022-3573

Research Paper

Active absorption of ginsenoside Rg1 *in vitro* and *in vivo*: the role of sodium-dependent glucose co-transporter 1

Jing Xiong[a], Minjie Sun[a], Jianxin Guo[b], Luosheng Huang[c], Shujing Wang[a], Boyu Meng[a] and Qineng Ping[a]

[a]Department of Pharmaceutics, China Pharmaceutical University, Nanjing, P.R. China; [b]Technology Department, Talecris Biotherapeutics, Clayton, North Corolina, USA and [c]Department of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing, P.R. China

Abstract

Objectives Our previous study suggested that adrenaline (epinephrine) could be an effective absorption enhancer for ginsenoside Rg1 (Rg1). This study focused on the transport mechanism of Rg1 and the role of sodium-dependent glucose co-transporter 1 in the regulation of Rg1 uptake after exposure to adrenaline.

Methods Caco-2 cells were used as an in-vitro model to assess the absorption mechanism of Rg1. Also the effect of D-glucose on adrenaline-induced absorption of Rg1 was investigated *in vivo* in rats.

Key findings Results showed that the uptake of Rg1 was temperature-dependent. The transport from the basolateral side to the apical side was significantly lower compared with that from the apical to the basolateral side ($P < 0.01$). The transport of Rg1 was concentration dependent (Km was 41.60 mM, Vmax was 353.75 mol/cm^2/min). Cells incubated with D-glucose-free medium exhibited significantly greater Rg1 uptake (+ 62.6%) compared with cells in D-glucose-containing medium. The data indicated that sodium-dependent glucose co-transporter 1 was involved in the transport of Rg1. Adrenaline-induced uptake of Rg1 was significantly inhibited in the presence of phlorizin and the absence of Na^+. In the in-vivo study in rats, it was found that after co-administration with D-glucose, the adrenaline-induced absorption of Rg1 was inhibited. The area under the concentration–time curve ($AUC_{0\rightarrow\infty}$) value was significantly decreased from 64.57 ± 27.08 to 1.37 ± 0.42 μg/ml h ($P < 0.001$).

Conclusions The data suggested that adrenaline enhanced the absorption of Rg1 by regulating sodium-dependent glucose co-transporter 1.

Keywords active transport; adrenaline (epinephrine); Caco-2 cells; ginsenoside Rg1 (Rg1); sodium-dependent glucose co-transporter 1 (SGLT1)

Introduction

As one of the major ingredients of *Panax ginseng*, ginsenoside Rg1 (Rg1) has been proven in recent years to possess a number of pharmacological effects such as immune-modulating activity,[1,2] a preventive effect against memory deficit,[3] a stimulatory effect on the central nervous system[4] and a suppressive effect on hepatoma cell growth.[5]

The pharmacokinetics of Rg1 in rats has been investigated in detail and its poor oral bioavailability has been reported.[6] In-vivo and in-vitro models have been applied to clarify the cause of poor oral absorption of Rg1. The absolute bioavailability of Rg1 after intragastric gavage is about 1.33%. In-vivo studies revealed that the low bioavailability of Rg1 could result from its low membrane permeability,[7] instability in the gastrointestinal tract,[8] elimination in the liver[7] and tendency to form self-micelles.[9] Han and Fang[7] studied the transport mechanism of Rg1 by using Caco-2 cells as an in-vitro model. They reported that the uptake and transport of Rg1 was non-saturable and the flux from the apical compartment (AP) to the basolateral compartment (BL) increased in a linear manner with an increase in concentration. These studies led to the conclusion that Rg1 was passively transported. However, it was also noted that the uptake of Rg1 by Caco-2 cells was temperature dependent and that the apparent permeability coefficient (Papp) for BL-AP transport was significantly lower than that of AP-BL transport ($P < 0.05$).[7] These results imply that some active transporter might be involved in the transport of Rg1, which is not

Correspondence: Q.N. Ping, College of Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing, 210009 China.
E-mail: pingqn2008@yahoo.com.cn

consistent with the passive diffusion conclusion. Therefore, it is imperative that such a controversy be clarified.

Rg1 has the structure of a glucoside. Since glucose is actively absorbed by sodium-dependent glucose cotransporter 1 (SGLT1) in the brush-border membrane (BBM) of intestinal epithelial cells,[10–12] SGLT1 may also be involved in the transport of some sugar analogues. In addition, our results obtained in another study[13] suggested that adrenaline (epinephrine) could be an effective absorption enhancer for Rg1 both *in vivo* and *in vitro* and that the enhancement is mediated by the interaction of adrenaline with β_2-adrenoceptors. Adrenaline has been reported to have a stimulatory effect on glucose transport in the rat small intestine by regulating SGLT1.[14] Thus we propose that the transport of Rg1 is mediated by SGLT1 and that adrenaline-induced absorption of Rg1 is related to SGLT1 trafficking.

To demonstrate our hypothesis, this study was designed to investigate the absorption characteristics of Rg1 and the role of SGLT1 in the regulation of Rg1 uptake after exposure to adrenaline. To substantiate Caco-2 cell results, we further examined the effect of D-glucose on adrenaline-induced oral absorption of Rg1 *in vivo* in rats.

Materials and Methods

Materials

Ginsenoside Rg1 was purchased from Kunming Phytopharmaceutical Co. Ltd (Yunnan, China); the purity was 98.1%, verified by HPLC. The Caco-2 cell line was obtained from the American Type Culture Collection (MD, US). Cell culture media and reagents, including fetal bovine serum, were purchased from Gibco (NY, US). Millicell cell culture Insert and Millicell-ERS were purchased from Millipore (MA, US). Adrenaline (epinephrine) was purchased from Sigma Chemical Co. (MO, US). Acetonitrile (Merck, Darmstadt, Germany) and methanol (Shandong Yuwang Industrial & Commercial Co. Ltd, Yucheng City, China) were of HPLC grade. All the other chemicals were of reagent grade.

Caco-2 cell culture

Caco-2 cells at passages 35–50 were used in the experiments and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamate, 1% nonessential amino acids and 1% penicillin streptomycin solution at 37°C in 75-cm^2 flasks in a humidified air–5% CO_2 atmosphere. After being harvested with trypsin–ethylenediamine tetraacetic acid (EDTA), cells in suspension (5×10^4 cells/ml) were seeded onto 24-well plates for Rg1 uptake studies (400 μl/well) and on permeable polycarbonate inserts (0.6 cm^2, 0.40 μm pore size) in 24-well plates for transport studies (400 μl cell suspension in the apical compartment and 600 μl culture medium in the basolateral compartment). Medium was replaced every 2 days thereafter. Cells were cultured for 14 days for uptake studies and 20–22 days for transepithelial transport studies after seeding. The transepithelial electrical resistance (TEER) of the Caco-2 cells was examined routinely before and after the experiment using the Millicell-ERS apparatus (Millipore, MA, US) to assess the integrity of monolayers grown on the permeable membrane in the transepithelial transport studies. The monolayers were used when TEER exceeded 600 Ω cm^2.

Uptake studies

Rg1 uptake was measured using monolayers grown in 24-well plates. The uptake medium consisted of Hanks' balanced salt solution (HBSS) with calcium and magnesium or *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; adjusted to pH 7.4 with 1 M HCl). For Rg1 uptake measurements, cells were washed with HBSS (3×2 ml) and pre-incubated for 15 min in 2 ml HBSS at controlled temperatures (4 or 37°C). Uptake was initiated by the addition of either the control (Rg1 1.25 mM) or test solution. All experiments were conducted in at least quadruplicate. When a sodium-free buffer was required, sodium chloride and sodium phosphate were replaced with equimolar amounts of choline chloride and potassium phosphate, respectively.[15] The reaction was terminated after 2 h by aspiration of the uptake buffer followed by the addition of ice-cold phosphate-buffered saline (PBS). Cells were washed more than twice in ice-cold PBS. Each Caco-2 cell monolayer was collected in 0.5 ml HBSS and then frozen (at −70°C) and thawed (at room temperature) three times. The cells were then lysed ultrasonically. The samples were mixed with isometric methanol by vortexing for 30 s, and centrifuged at 12 000 rev/min for 10 min. The supernatants were collected and 50 μl was used to determine the Rg1 concentration by HPLC. Results were expressed in nM of Rg1 per mg of protein. The protein content of the cells was determined by the Lowry protein assay using bovine serum albumin as the standard.[16]

Transport studies

Caco-2 cells were washed twice with pre-warmed (37°C) HBSS before the experiments. The monolayers were pre-incubated at 37°C for 15 min in a CO_2 incubator with warm (37°C) HBSS. The TEER was measured to ensure that the epithelium was tight. The HBSS solution on both sides of the cells was then removed by aspiration. Rg1 was added to either the apical (0.4 ml) or basolateral (0.6 ml) compartment. Experiments were performed for 2 h. At a predetermined time (1, 1.5 or 2 h), a sample (200 μl) was taken from the basolateral compartment. A volume of 50 μl was used to determine the Rg1 concentration by HPLC. All the experiments were conducted in at least quadruplicate.

The apparent permeability coefficient (*P*app) was determined according to the equation:

$$P\text{app} = (\mathrm{d}Q/\mathrm{d}t)/(A \times C_0) \quad (1)$$

where $\mathrm{d}Q/\mathrm{d}t$ is the drug permeation rate (μmol/s), A is the surface area of the epithelium (cm^2) and C_0 is the initial concentration in the donor compartment at time 0 (μM).

A non-linear regressions analysis was performed in fitting the plot to the following equation:

$$V = V\text{max}\ S/(K\text{m} + S) + K \times S \quad (2)$$

where V is the apparent linear initial rate (μmol/cm^2/min) and S is the initial concentration (μmol/ml) of Rg1 in the donor compartment. *V*max and *K*m are the maximum rate and the Michalis–Menten constant, respectively, and K represents the linear clearance.

Oral bioavailability study of Rg1 in rats

Sprague–Dawley rats, about 220–250 g, were obtained from the Jiangsu animal breeding centre, Nanjing. The animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University. The rats were fasted for 12 h before the experiment.

Aqueous solutions of Rg1 containing adrenaline prepared with or without D-glucose (200 mg/kg) were administered orally at a dose of 200 mg/kg to fasted rats and free access to water was available. Each group contained six rats. Blood samples were collected at 5, 15, 30, 45 and 60 min and 1.5, 2, 4, 8, 12 and 24 h after administration under light ether anaesthesia and pretreated with solid phase extraction (SPE) cartridges (OASIS; Waters, MA, US).

Pharmacokinetic data analysis

The area under the concentration–time curve ($AUC_{0\to\infty}$) for Rg1 was obtained using the linear trapezoidal rule from time zero to the last measured time point, followed by the addition of the extrapolated tail area, calculated by dividing the last measured plasma concentration by the terminal rate constant.

Determination of Rg1 by HPLC

HPLC was conducted with an Agilent 1100 liquid chromatography system consisting of a binary solvent delivery system, an autosampling device and a UV detector. A Diamonsil C18 reverse phase column (5 μm, 4.6 i.d. × 250 mm; Dikma, Beijing, China) was applied and maintained at 40°C. UV absorption was measured at 203 nm. The mobile phase was composed of acetonitrile–water (25 : 75, v/v) and the flow rate was 1 ml/min.

For in-vitro experiments, the lower limit of concentration was 0.395 mM. The intra- and inter-day precision, determined as relative standard deviation, was less than 3.0%. For in-vivo experiments, the lower limit of concentration was 526 ng/ml. The mean recovery was 91.88 ± 5.27%. The intra- and inter-day precision, determined as relative standard deviation, was less than 5.0%. The method has been demonstrated to be sensitive and accurate for the determination of Rg1 in-vitro and in-vivo experiments.

Statistical analysis

The statistical value of differences between means was determined by Student's test or the analysis of variance test with $P = 0.05$ as the minimal level of significance.

Results

Temperature dependency

Rg1 (1.25 mM) uptake by Caco-2 cells was studied at 37°C and 4°C. The intracellular level of Rg1 increased remarkably during the first 30 min and reached a plateau afterwards (Figure 1) at 37°C. In contrast, the uptake rate at 4°C was slow and reached a plateau in 20 min. The amount of Rg1 taken up at 37°C was approximately twice that at 4°C.

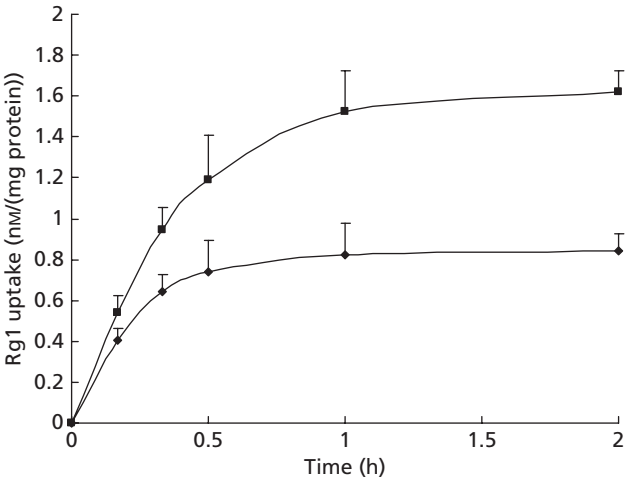

Figure 1 Uptake of Rg1 into Caco-2 cells. Measurement of Rg1 (1.25 mM) uptake into Caco-2 cells was performed at 4°C (◆) and 37°C (■). Each point represents the mean ± SD, $n \geq 4$.

Transport studies of Rg1 in Caco-2 cells

The effect of Rg1 concentration on its transport across the Caco-2 cells was investigated. The cumulative transepithelial flux of Rg1 through Caco-2 cell monolayers over 2 h increased linearly with increasing concentration in the apical compartment (Figure 2). As Rg1 concentration increased over the range of 0.625–31.25 mM, the absorptive flux increased (Figure 3a), but the *P*app (AP-BL) decreased (Figure 3b). The results suggested that the AP-BL transport was mediated by some active transporter. The results were analysed by Lineweaver–Burk transformation of the transport kinetics data. The kinetic analysis of Rg1 transport according to the Michaelis–Menten equation yielded an apparent *K*m value of 41.60 mM and *V*max value of 353.75 μmol/cm^2/min.

On the other hand, the flux (BL-AP) increased linearly with an increase in Rg1 concentration (0.625–31.25 mM).

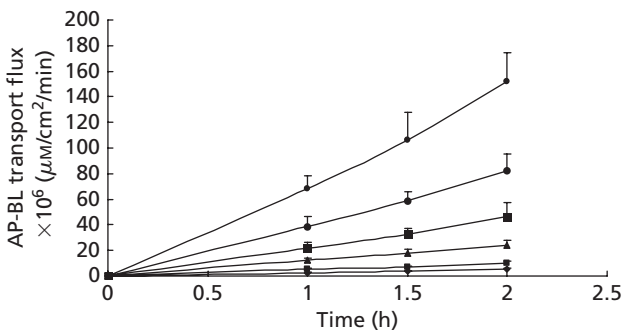

Figure 2 Cumulative transport of Rg1 across Caco-2 cells in the AP-BL direction. Cumulative transport of Rg1 was measured as a function of time at different final concentrations (⬥, 0.625 mM; ■, 1.25 mM; ▲, 3.125 mM; ■, 6.25 mM; ●, 12.5 mM, •, 31.25 mM) of Rg1. Each point represents the mean ± SD, $n \geq 4$.

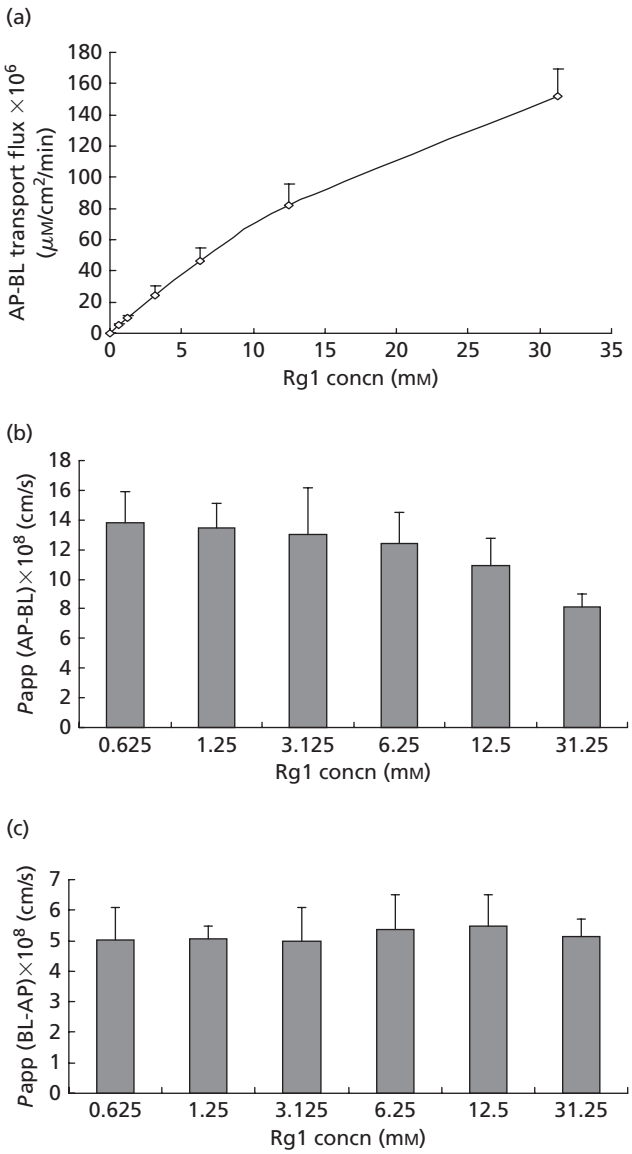

Figure 3 Effect of concentration on the transepithelial transport flux (a), *P*app (AP-BL) of Rg1 (b) and *P*app (BL-AP) (c) by Caco-2 cells. Each data point represents the mean ± SD, $n \geq 4$.

The efflux *P*app (BL-AP) of Rg1 remained almost constant over the concentration range (Figure 3c). The *P*app (BL-AP) was 5.06 ± 0.43 × 10^{-8} cm/s when the concentration of Rg1 was 1.25 mM in the basolateral compartment ($n = 3$), which was significantly lower than that seen in AP-BL transport ($P < 0.01$).

Effect of D-glucose on Rg1 uptake into Caco-2 cell monolayers

To investigate the effect of D-glucose on the uptake of Rg1, Caco-2 cells were incubated with D-glucose-containing medium or D-glucose-free medium for 2 h. D-Mannitol was employed in the D-glucose-free medium to maintain osmolarity.[17] Cells incubated with D-glucose-free medium exhibited significantly greater Rg1 uptake (+ 62.6%) compared with those incubated with D-glucose ($P < 0.01$).

Effect of phloridzin and Na^+ depletion on adrenaline-induced uptake of Rg1 by Caco-2 cells

Adrenaline (10 μM) significantly enhanced cellular uptake of Rg1 (1.96 ± 0.14 with adrenaline vs 1.58 ± 0.16 nM/mg protein/min without adrenaline; $P < 0.05$) (Figure 4). Phloridzin, at a concentration of 100 μM, blocked the effect of adrenaline on the uptake of Rg1 (30.0% reduction) ($P < 0.05$) and a reduction in the adrenaline-induced uptake of Rg1 was observed in the sodium-free medium (52.2% reduction) ($P < 0.05$).

Effect of D-glucose on adrenaline-induced oral absorption of Rg1 *in vivo*

The effect of D-glucose on the adrenaline-enhanced absorption of Rg1 was further investigated in rats. The time course of Rg1 aqueous solutions after oral administration to rats with and without D-glucose was plotted in Figure 5. After

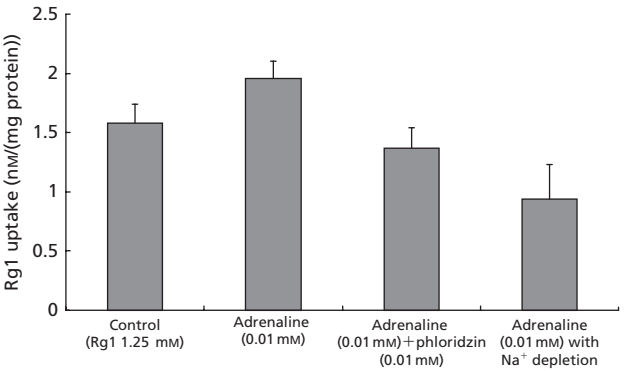

Figure 4 Adrenaline-induced uptake of Rg1 in Caco-2 cells. Adrenaline-induced uptake of Rg1 (1.25 mM) was measured in Caco-2 cells in the presence of phloridzin and Na^+ depletion medium. Results are expressed as means ± SD, $n \geq 4$.

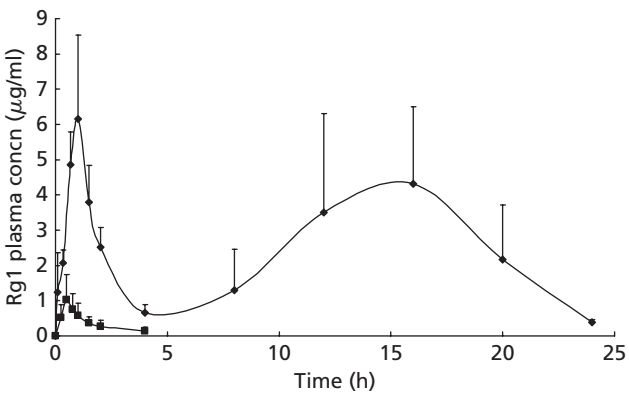

Figure 5 Plasma concentration–time profiles of Rg1 and adrenaline after oral administration to rats with or without D-glucose. The plasma concentration of Rg1 (200 mg/kg) and adrenaline (4 mg/kg) was measured after oral administration to rats with (■) or without (◆) D-glucose (200 mg/kg). Each point represents the mean ± SD of 6 rats.

co-administration with D-glucose at such a high dose (1.11 mM/kg), the adrenaline-induced absorption of Rg1 was inhibited, resulting in lower plasma levels and a shorter residence time. The $AUC_{0\to\infty}$ value was significantly decreased from 64.57 ± 27.08 to 1.37 ± 0.42 μg/ml h ($P < 0.001$). It appeared that D-glucose blocked adrenaline-induced enhancement of Rg1 absorption.

Discussion

Temperature dependency

The Rg1 uptake by Caco-2 cells at 37°C was greater than that at 4°C, which was in agreement with literature reports.[7] One explanation could be that the temperature dependence of uptake might be attributed to the cell membranes being more rigid at low temperatures. This would lead to a lower level of diffusion through the membrane.[18] Even so, the possibility of an active transport cannot be excluded.

Transport studies of Rg1 in Caco-2 cells

The transport characteristics of Rg1 in Caco-2 cells were different from those reported by Han and Fang,[7] who found the uptake and transport of Rg1 to be non-saturable. An explanation for these discrepancies could be the difference in Rg1 concentration. In our experiments, the highest concentration was 31.25 mM as opposed to 6.25 mM in Han and Fang's study. At a dose as large as 31.25 mM, it was possible to saturate the absorption.

The absorptive transport (AP-BL) was 1.6- to 2.7-fold greater than the corresponding efflux transport (BL-AP) of Rg1. The data suggested that the transport of Rg1 in the AP-BL direction was composed of both passive and active transport, while the transport of Rg1 from BL to AP was only passive. According to the structure of Rg1, we inferred that SGLT1 was involved in its absorption. SGLT1 is frequently expected to be located exclusively in the apical BBM of enterocytes,[19] which offered a reasonable explanation for the greater *P*app in the AP to BL direction than that in the BL to AP direction.

Effect of D-glucose on Rg1 uptake into Caco-2 cell monolayers

The substrate specificity of SGLT1 is reported to be in the order of D-glucose > α-methyl-D-glucose > D-galactose > 3-*O*-methylglucoside >> mannitol and L-glucose.[20–22] Since D-glucose exhibits a high affinity for SGLT1, the binding of other monosaccharide substrates would be inevitably inhibited in the presence of D-glucose. The uptake of Rg1 in cells incubated in D-glucose-free medium was significantly enhanced compared with uptake in cells incubated in medium containing D-glucose. These results suggest that the transport of Rg1 is mediated by SGLT1.

In addition, many papers[10–12,23] have also confirmed that SGLT1 is the main Na^+–glucose co-transporter among the SGLT family expressed in the small intestine and that D-glucose is mainly actively absorbed by SGLT1 in the BBM of intestinal epithelial cells. It is also suggested that the existence of D-glucose in the luminal phase might inhibit the absorption of Rg1.

Effect of phloridzin and Na^+ depletion on adrenaline-induced uptake of Rg1 by Caco-2 cells

In our previous study, we demonstrated that the incubation of Caco-2 cells with adrenaline resulted in a significant increase in the uptake and amount transported of Rg1.[13] Studies have demonstrated that adrenaline could increase the amount of SGLT1 in the BBM of intestinal epithelial cells by interacting with β-adrenoceptors.[24] It is therefore of interest to investigate whether the adrenaline-induced absorption of Rg1 is related to SGLT1 trafficking.

Phloridzin is widely used to inhibit the active transport of glucose and glycoside conjugates by SGLT1.[25–27] Moreover, the Na^+ gradient is known to provide the driving force for the uptake of most substrates by SGLT1. To validate our speculation, the adrenaline-induced uptake of Rg1 was investigated in the presence of phloridzin or absence of Na^+.

The data from Caco-2 cells (Figure 3) suggested that the transport of Rg1 was composed of both passive and active transport. The active uptake was abolished in the presence of phloridzin and absence of Na^+, while the passive uptake was not blocked. The adrenaline-induced uptake of Rg1 was inhibited to such a great extent that the enhancement was completely abolished. Thus, a reasonable conclusion might be drawn that adrenaline-induced absorption of Rg1 was related to SGLT1 trafficking.

Effect of D-glucose on adrenaline-induced oral absorption of Rg1 *in vivo*

Based on this study, the apparent *K*m value of Rg1 of SGLT1 was 41.60 mM. It is reported that the *K*m value of D-glucose is 10–50 μM,[28] which is much smaller than that of Rg1. After coadministration with D-glucose at such a high dose (200 mg/kg), the adrenaline-induced absorption of Rg1 was completely inhibited by D-glucose and the absorption of Rg1 was even poorer than that of aqueous solution. The result *in vivo* also validated our speculation.

Conclusions

This study has proved that the intestinal efflux of Rg1 is mediated by some active transporters and that SGLT1 is involved in the absorption of Rg1. We also demonstrated that adrenaline enhances the absorption of Rg1 by regulating SGLT1. After co-administration with D-glucose, the adrenaline-induced absorption of Rg1 in rats is significantly inhibited. The D-glucose level in the lumen of the small intestine varies with the nutritional state of the individual; the lower limit is probably 0 mM D-glucose after a fasting period. After a meal, the maximal luminal D-glucose concentrations were calculated to be as high as 200–300 mM.[29] Therefore, pre-prandial administration of Rg1 should be strongly recommended to achieve a greater extent of absorption by the oral route.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The project is supported by the National Natural Science foundation of China (30430790).

References

1. Kenaroba B *et al.* Immunomodulating activity of ginsenoside Rg1 from Panax ginseng. *Jpn J Pharmacol* 1990; 54: 447–454.
2. Lee EJ *et al.* Ginsenoside Rg1 enhances CD4(+) T-cell activities and modulates Th1/Th2 differentiation. *Int Immunopharmacol* 2004; 4: 235–244.
3. Yamaguchi Y *et al.* Effects of ginsenosides on impaired performance induced in the rat by scopolamine in a radial-arm maze. *Psychoneuroendocrinology* 1995; 20: 645–653.
4. Tsang D *et al.* Ginseng saponins: influence on neurotransmitter uptake in rat brain synaptosomes. *Planta Med* 1985; 3: 221–224.
5. Lee YJ *et al.* Ginsenoside-Rg1, one of the major active molecules from Panax ginseng, is a functional ligand of glucocorticoid receptor. *Mol Cell Endocrinol* 1997; 133: 135–140.
6. Sun JG *et al.* Simultaneous rapid quantification of ginsenoside Rg1 and its secondary glycoside Rh1 and aglycone protopanaxatriol in rat plasma by liquid chromatography mass spectrometry after solid-phase extraction. *J Pharm Biomed Anal* 2005; 38: 126–132.
7. Han M, Fang XL. Difference in oral absorption of ginsenoside Rg1 between *in vitro* and *in vivo* models. *Acta Pharmacol Sin* 2006; 27(4): 499–505.
8. Hasegawa H *et al.* Main ginseng saponin metabolites formed by intestinal bacteria. *Planta Med* 1996; 62: 453–457.
9. Xiong J *et al.* Self-micelle formation and the incorporation of lipid in the formulation affect the intestinal absorption of Panax notoginseng. *Int J Pharm* 2008; 360(1–2): 191–196.
10. Takata K. Glucose transporters in the transepithelial transport of glucose. *J Electron Microsc* 1996; 45(4): 275–284.
11. Wright EM. Intestinal sugar transport. In: Johnson LR *et al.,* eds. *Physiology of Gastrointestinal Tract*. Philadelphia: Elsevier Press, 1994: 1751–1772.
12. Shirazi-Beechey SP. Molecular biology of intestinal glucose transport. *Nutr Res Rev* 1995; 8: 27–41.
13. Xiong J *et al*. Enhancement by epinephrine of ginsenoside Rg1 transport in Caco-2 cells and oral absorption in rats. *J Pharm Pharmacol* 2009; 61: xx–xx.
14. Aulsebrook KA. Intestinal absorption of glucose and sodium: effects of epinephrine and norepinephrine. *Biochem Biophys Res Commun* 1965; 18: 165–169.
15. Mizuma T *et al.* Intestinal SGLT1-mediated absorption and metabolism of benzyl β-glucoside contained in Prunus mume: carrier-mediated transport increases intestinal availability. *Biochim Biophys Acta* 2005; 1722: 218–223.
16. Lowry OH *et al.* Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
17. Khoursandi S *et al.* Different modes of sodium-D-glucose cotransporter-mediated D-glucose uptake regulation in Caco-2 cells. *Am J Physiol Cell Physiol* 2004; 287: 1041–1047.
18. Brunet JL *et al.* Human intestinal absorption of imidacloprid with Caco-2 cells as enterocyte model. *Toxicol Appl Pharm* 2004; 192: 1–9.
19. Kipp H *et al.* More than apical: distribution of SGLT1 in Caco-2 cells. *Am J Physiol Cell Physiol* 2003; 285: C737–C749.
20. Crane RK. Hypothesis for mechanism of intestinal active transport of sugars. *Fed Proc* 1962; 21: 891–895.
21. Wilson TH, Landau BR. Specificity of sugar transport by the intestine of the hamster. *Am J Physiol* 1960; 198: 99–102.
22. Landau BR *et al.* Hexose transport by hamster intestine *in vitro*. *Am J Physiol* 1962; 203: 237–240.
23. Yoshida A *et al.* Immunohistochemical localization of Na+-dependent glucose transporter in the rat digestive tract. *Histochem J* 1995; 27(5): 420–426.
24. Ishikawa Y *et al*. Mechanism of β-adrenergic agonist induced transmural transport of glucose in rat small intestine: regulation of phosphorylation of SGLT 1 controls the function. *Biochim Biophys Acta* 1997; 1357: 306–318.
25. Mizuma T *et al*. Intestinal active absorption of sugar-conjugated compounds by glucose transport system: implication of improvement of poorly absorbable drugs. *Biochem Pharmacol* 1992; 43: 2037–2039.
26. Mizuma T, Awazu S. Intestinal Na^+/glucose cotransporter-mediated transport of glucose conjugate formed from disaccharide conjugate. *Biochim Biophys Acta* 1998; 1379: 1–6.
27. Uhing MR, Kimura RE. Active transport of 3-O-methylglucose by the small intestine in chronically catheterized rats. *J Clin Invest* 1995; 95: 2799–2805.
28. Khan JM *et al.* Development of the intestinal SGLT1 transporter in rats. *Mol Genet Metab* 2000; 69(3): 233–239.
29. Pappenheimer JR. On the coupling of membrane digestion with intestinal absorption of sugars and amino acids. *Am J Physiol Gastrointest Liver Physiol* 1993; 265: G409–G417.