

Full-length article

Difference in oral absorption of ginsenoside Rg₁ between *in vitro* and *in vivo* modelsMin HAN, Xiao-ling FANG¹*Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 200032, China***Key words**ginsenoside Rg₁; *Panax*; Caco-2; bioavailability; pharmacokineticsCorrespondence to Dr Xiao-ling FANG.
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Accepted 2005-12-21

doi: 10.1111/j.1745-7254.2006.00303.x

Abstract

Aim: To clarify the cause of poor oral absorption of ginsenoside Rg₁ (Rg₁), the active ingredient in *Panax notoginseng saponins* (PNS) used for treating hemorrhage. **Methods:** Caco-2 cell monolayers were used as an *in vitro* model to study the transport mechanism of Rg₁ across the intestinal mucosa. Moreover, the serum concentration-time profiles after peroral (*po*), intraduodenal (*id*), portal venous (*pv*) and tail venous (*iv*) administration of Rg₁ in rats were compared to evaluate the first-pass effects in the stomach, intestine, and liver. **Results:** Uptake of Rg₁ by Caco-2 cell monolayers was temperature-dependent, but was not influenced by cyclosporin A. The change in the apical pH produced no obvious effect on the uptake of Rg₁. The uptake and transport of Rg₁ was non-saturable; whereas the flux from the apical compartment to the basolateral compartment (A–B) increased in a linear manner with the increase in concentration, indicating passive transport. An apparent permeability coefficient of $(2.59 \pm 0.17) \times 10^{-7}$ cm/s ($C_0 = 1$ mg/mL) predicted incomplete absorption. A significant difference was observed between the *po* (F_{po} was 3.29% at a dose of 1500 mg/kg), *id* (F_{id} was 6.60% at a dose of 1200 mg/kg) and *pv* (F_{pv} was 50.56%) administration methods, and the barrier function of the intestine was more significant than those of the stomach and liver in the absorption process. **Conclusion:** Elimination in the stomach, large intestine and liver contributed to the low oral bioavailability of Rg₁, but low membrane permeability might be a more important factor in determining the extent of absorption.

Introduction

Ginsenoside Rg₁ (Rg₁), the main ingredient of *Panax notoginseng saponins* (PNS; an important traditional Chinese medicine), has been proven in recent years to possess a number of pharmacological effects, including partial protection against the excitotoxic effect of glutamate in primary hippocampal cultures^[1], a partial neurotrophic and neuroprotective role in dopaminergic cell culture^[2], an ameliorating effect on performance impairment induced by scopolamine^[3], and a protective effect on experimental myocardial injury induced by ischemia and reperfusion in rats^[4].

Rg₁ is poorly absorbed when administered orally, but in addition to factors such as solubility and dissolution related to its high aqueous solubility, it is possible that other fac-

tors contribute to its poor absorption. It has been reported in recent years that Rg₁ is decomposed in gastric juice^[5] and transformed into 20(*S*)-protopanaxatriol (20*S*-Ppt) via ginsenoside Rh₁ by enteric bacteria in rats and humans^[5–9]. This suggests the instability of Rg₁ in the stomach and large intestine. The pharmacokinetics of Rg₁ in rats have been studied through TLC and the results show that little Rg₁ is absorbed from the digestive tract^[5,10]. Rg₁ has been reported to be efficiently taken up by the rat liver and secreted into bile, and 57.2%±1.7% of the dose was found to be excreted into bile within 4 h after *iv* administration at a dose of 5 mg/kg^[10]. However, what remains unknown is the transport mechanism across the intestinal mucosa and the first-pass effects of the stomach, intestine and liver which are involved in the absorption process. In order to improve the

oral absorption of Rg_1 , it is necessary to clarify the absorption mechanism systematically.

The first-pass effects of the stomach, intestine and liver can be evaluated by comparing the absolute bioavailability (F_{po} , F_{id} and F_{pv} respectively), which are calculated by dividing the area under the serum concentration *versus* time curve (AUC) after *po*, *id*, and *pv* administration by the AUC after *iv* administration^[12,13]. Because PNS is used more widely in practice than Rg_1 alone, both Rg_1 and PNS (Rg_1 content of 27.36%) are investigated in the present study.

Materials and methods

Materials PNS was purchased from Kunming Pharmaceutical Co (Kunming, China). Standard ginsenoside Rg_1 was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China. Cyclosporin A (CsA) was obtained from the Guangdong Medicine Group Co, Ltd (Guangzhou, China). The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) at passage 35. Cell culture media and reagents including fetal bovine serum were purchased from Gibco (Grand Island, NY, USA). Permeable polycarbonate inserts and Millicell-ERS (electrical resistance system) were purchased from Millipore (Bedford, MA, USA). All other chemicals and reagents were of analytical or high performance liquid chromatography (HPLC) grade as appropriate.

Caco-2 cell culture Caco-2 cells at passages 35–42 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-cm² flasks (Costar) in a humidified air-5% CO₂ atmosphere. After being harvested with trypsin-ethylenediamine tetraacetic acid (EDTA), cells in suspension (2.5×10^5 cells/mL) were seeded onto 6-well plates for Rg_1 uptake studies (2 mL/well) and on permeable polycarbonate inserts (4.2 cm², 0.40 μm pore size) in 6-well plates (Nunc, Roskilde, Denmark) for transepithelial transport studies (1 mL cell suspension in the apical compartment and 2 mL culture medium in the basolateral compartment). Cells were cultured for 14 d for uptake studies and 20–22 d for transepithelial transport studies after seeding. The transepithelial electrical resistance (TEER) of the Caco-2 cell monolayers was examined routinely before and after the experiment using the Millicell-ERS system to assess the integrity of monolayers grown on the permeable membrane in the transepithelial transport studies. The monolayers were used when TEER exceeded 500 Ω·cm².

Uptake studies Rg_1 uptake was measured using mono-

layers grown in 6-well plates. The uptake medium consisted of Hanks' balanced salt solution (HBSS) with calcium and magnesium, 25 mmol/L *D*-glucose, 20 mmol/L *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; adjusted to pH 7.0 or 7.4 with 1 mol/L HCl) or 2-*N*-morpholine-ethanesulfonic acid (MES; adjusted to pH 5.5, 6.0 or 6.5 with 1 mol/L NaOH). The medium pH was measured and adjusted after addition of all compounds tested in uptake studies. For Rg_1 uptake measurements, cells were washed with HBSS (3×2 mL) at the appropriate pH and preincubated for 15 min in 2 mL HBSS at controlled temperatures (4 or 37 °C). The medium was discarded, and Rg_1 uptake was studied after various incubation times at different final concentrations. Cyclosporin A was added to the incubation medium on the cell monolayers to investigate its effect on Rg_1 uptake. At the end of the incubation period, the incubation solution was aspirated and the monolayers were carefully rinsed with ice-cold HBSS. Each Caco-2 cell monolayer was collected with 1 mL HBSS and frozen (at -80 °C) then thawed (at room temperature) 3 times, and then the cells were lysed ultrasonically. After the solubilized monolayers were centrifuged at 9000×*g* for 10 min, the supernatants were collected and 10 μL was used to determine the Rg_1 concentration using HPLC. Results were expressed as μg of Rg_1 per mg of protein. The protein contents of the cell monolayers were determined by using Coomassie staining.

Transepithelial transport study For Rg_1 transepithelial flux measurement, Caco-2 cell monolayers were washed 3 times with prewarmed (37 °C) HBSS prior to the experiments. Then the monolayers were preincubated at 37 °C for 15 min in a CO₂ incubator with warm (37 °C) HBSS (pH 6.5 and 7.4 for the apical and basolateral compartments, respectively). The TEER was measured to ensure that the epithelium was tight, and the HBSS solution on both sides of the cell monolayers was then removed by aspiration. Rg_1 or PNS was added to either the apical (1.5 mL; pH 6.5) or basolateral (2 mL; pH 7.4) compartments. At various times, samples (200 μL) were taken from the opposite compartment followed by immediate replacement with fresh HBSS of the same volume (200 μL). Ten microliters was used to determine the Rg_1 concentration using HPLC. All experiments were conducted in triplicate. Results were expressed as μg of Rg_1 transported per cm² of epithelium.

The drug transport capacity of the epithelium was quantified by calculating the apparent permeability coefficient (P_{app}) that reflects the passage velocity of the drug through the epithelium. P_{app} was calculated as follows:

$$P_{app} = (dQ/dt) / (A \times C_0)$$

where dQ/dt is the drug permeation rate (μg/s), A is the sur-

face area of epithelium (cm²), and C₀ is the initial concentration in the donor compartment (μg/cm³).

First-pass effects of Rg₁ The first-pass effects of Rg₁ were evaluated after *po*, *id*, *pv* and *iv* administration of PNS to rats. Male Sprague-Dawley rats weighing 250–300 g were used. They were fasted with free access to water for 20 h prior to *po* and *id* administration. For *iv* administration, PNS dissolved in 0.9% NaCl solution (50 mg/mL) was given via the tail vein at a dose of 50 mg/kg. For *pv* administration, the rats were anesthetized lightly with diethyl ether, the portal vein was surgically exposed, and PNS dissolved in 0.9% NaCl solution (50 mg/mL) was delivered into the portal vein through the mesenteric vein over 15 s at a dose of 50 mg/kg. For *po* and *id* administration, PNS dissolved in water was administered perorally at doses of 600 mg/kg or 1500 mg/kg (dose volume 6 mL/kg), and intraduodenally at doses of 600, 900 or 1200 mg/kg (dose volume 4 mL/kg). Blood samples were collected from the eye socket at 0.08, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after *iv* and *pv* administration of Rg₁, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, and 72 h after *po* administration of Rg₁, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24, 72 h after *id* administration of Rg₁. Serum samples were obtained by centrifugation at 4000×g for 10 min after the blood collection, and the serum concentration of Rg₁ was determined by HPLC.

Model-independent methods based on the theory of statistical moments were used to characterize the pharmacokinetics of Rg₁ after *iv*, *id*, *pv* and *po* administration of Rg₁. AUC and mean residence time (MRT) were calculated by numerical integration under the trapezoidal rule as described by Yamaoka *et al*^[14]. Because this was observed over a limited period of time (72 h), extrapolation to time=infinity was carried out using a monoexponential equation. The absolute bioavailability (*F*) of Rg₁ was calculated by dividing AUC after *po*, *id*, and *pv* administration by the AUC after *iv* administration.

Determination of Rg₁ by HPLC Solid phase extraction (SPE) cartridges (Oasis, Waters, USA) were conditioned under vacuum with 1 mL of methanol and 1 mL of water. Then, 200 μL of serum samples were mixed with 4 μL of 85% (*v/v*) phosphoric acid, and centrifuged at 9000×g for 10 min. A volume of 100 μL of supernatant was applied to the SPE cartridges and drawn through under low vacuum (10–15 in Hg). Cartridges were washed with 1 mL of 5% (*v/v*) methanol aqueous solution, eluted with 1 mL of methanol and evaporated to dryness under a nitrogen stream at 40 °C. Finally, the residues were reconstituted in 100 μL of the mobile phase and 10 μL was used for HPLC.

HPLC was carried out using a YMC ODS C₁₈ column (5 μm, 4.2 ID×150 mm, Japan) with a liquid chromatograph (LC-

10AT; Shimadzu, Japan) and a UV-VIS Detector (SPD-101; Shimadzu) at 203 nm, and a data workstation system (HS2000; Hangzhou Empire, Hangzhou, China). A mobile phase at a speed of 1 mL/min consisting of acetonitrile with 20 mmol/L NaH₂PO₄ (32:68, *v/v*) was used for chromatographic resolution. The peak area was linearly related to the amount of Rg₁ over the range of 10.8–432 ng. The within-day precision of determination of Rg₁ in rat serum by using HPLC was 3.45%, 6.36%, and 4.66% for 21.6, 108, and 432 ng Rg₁, respectively. The between-day precision of determination of Rg₁ in rat serum by HPLC was 5.40%, 9.18%, and 7.32% for 21.6, 108, and 432 ng Rg₁ respectively. The recovery of Rg₁ in rat serum were 85.24%±2.96%, 86.11%±5.48%, and 81.61%±3.80% for 21.6, 108, and 432 ng Rg₁, respectively.

Statistical analysis Values are expressed as mean±SD. The difference among mean values was analyzed by using the two-tailed Student's *t*-test and comparisons among more than 3 groups were performed by using one-way ANOVA. *P*<0.05 was considered to be significant.

Results

Uptake of Rg₁ by Caco-2 cell monolayers Rg₁ (1 mg/mL) and PNS (4 mg/mL) uptake by Caco-2 cell monolayers was studied at 37 °C and 4 °C. The intracellular amounts sharply increased during the first 30 min and reached almost a steady state afterwards (Figure 1). For both Rg₁ alone and Rg₁ in PNS, uptake was temperature-dependent, and intracellular Rg₁ amounts measured at 37 °C were approximately 1.5-fold higher than those at 4 °C.

In addition, the pH-dependence of Rg₁ (or PNS) uptake

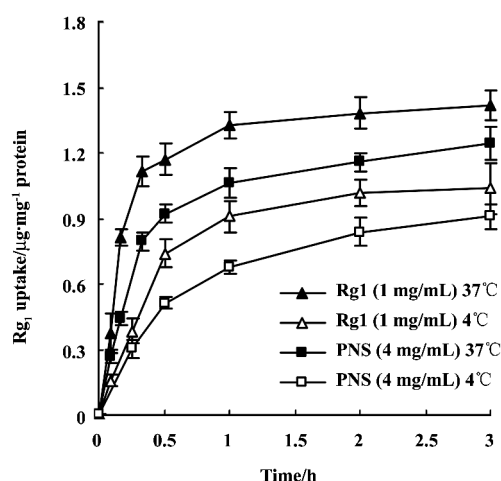


Figure 1. Uptake of Rg₁ in Caco-2 cell monolayers. The kinetics of uptake for Rg₁ (1 mg/mL) and PNS (4 mg/mL) were studied at 37 °C and 4 °C with Caco-2 cells cultured on 6-well plates for various lengths of time. Each point represents mean±SD. *n*=3.

was examined. No significant difference was observed when Caco-2 cell monolayers were incubated with Rg₁ (or PNS) solution at different pH levels (pH 5.5, 6.0, 6.5, 7.0, 7.4; Table 1). Given the weak acid microclimate that exists above the epithelial cell layer in the upper part of the small intestine^[15], the following studies were performed at pH 6.5 on the apical side of the monolayer.

Table 1. Influence of pH on Rg₁ uptake in Caco-2 cell monolayers. Monolayers were incubated in 1 mg/mL Rg₁ (or 4 mg/mL PNS) solution at different pH values, at 37 °C for 20 min. No statistically significant ($P < 0.05$) difference was observed among different pH groups for Rg₁ and PNS uptake. Data are mean \pm SD. $n=3$.

pH	Rg ₁ uptake ($\mu\text{g}/\text{mg}$ protein)	PNS uptake ($\mu\text{g}/\text{mg}$ protein)
5.5	0.989 \pm 0.050	0.789 \pm 0.030
6.0	1.066 \pm 0.081	0.810 \pm 0.030
6.5	1.068 \pm 0.071	0.823 \pm 0.027
7.0	1.123 \pm 0.095	0.795 \pm 0.040
7.4	1.058 \pm 0.053	0.824 \pm 0.036

The concentration-dependence of uptake of Rg₁ by Caco-2 cell monolayers was examined. The intracellular amounts of Rg₁ at 37 °C and 4 °C increased linearly with the increase in concentration over the range of 0.25 mg/mL to 5 mg/mL for Rg₁, and 1 mg/mL to 40 mg/mL for PNS (Figure 2), which indicates passive membrane diffusion.

CsA was used as a P-gp and MRP inhibitor to investigate the role of efflux transporters in Rg₁ uptake. In the absence and presence of CsA (50 $\mu\text{mol}/\text{L}$), Rg₁ uptake was 1.027 \pm 0.056 and 0.986 \pm 0.093 $\mu\text{g}/\text{mg}$ protein, respectively, for 1 mg/mL Rg₁ solution ($n=3$), and 0.826 \pm 0.055 and 0.840 \pm 0.054 $\mu\text{g}/\text{mg}$ protein, respectively, for 4 mg/mL PNS solution ($n=3$). No effect was observed when CsA was present. The results further confirmed that Rg₁ uptake was not mediated by the efflux transporters in Caco-2 cell monolayers.

Trans epithelial transport studies Rg₁ trans epithelial transport across Caco-2 cell monolayers was measured to evaluate intestinal permeability. Transport velocity from the apical to the basolateral (A–B) compartment was approximately constant during the whole kinetic study at 37 °C (Figure 3). The amount of Rg₁ cumulative trans epithelial flux through Caco-2 cell monolayers over 180 min increased linearly with increasing concentration in the apical compartment. P_{app} was (2.66 \pm 0.41) $\times 10^{-7}$, (2.44 \pm 0.09) $\times 10^{-7}$, (2.59 \pm 0.17) $\times 10^{-7}$ and (2.19 \pm 0.17) $\times 10^{-7}$ cm/s when the initial Rg₁ concentration in apical compartment was 0.2, 0.5, 1.0, and 3.0 mg/mL, respectively ($n=3$). In the presence of CsA (50 $\mu\text{mol}/\text{L}$), P_{app}

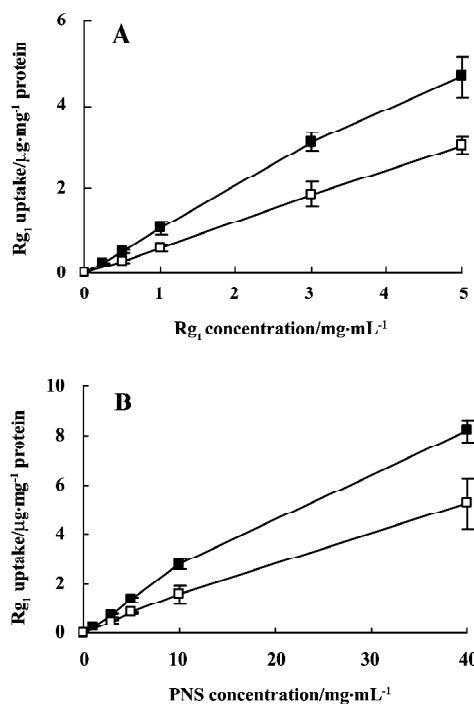


Figure 2. Effect of concentration on the uptake of Rg₁ (A) and PNS (B) by Caco-2 monolayers. Caco-2 monolayers were incubated in Rg₁ or PNS solution at different final concentrations at 37 °C (for 20 min; black markers) or 4 °C (for 30 min; white markers). Cells were lysed and the intracellular concentration of Rg₁ was measured by HPLC. Each point represents mean \pm SD ($n=3$).

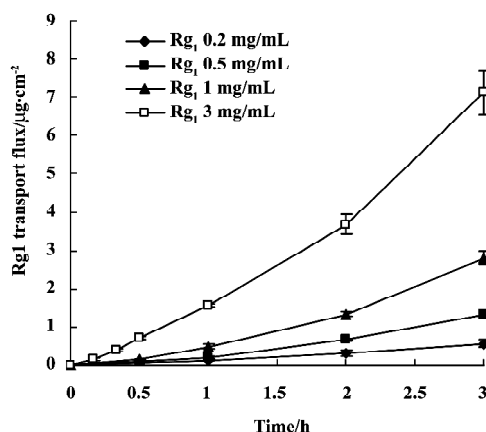


Figure 3. Cumulative transport of Rg₁ across Caco-2 cell monolayers in the apical-basolateral direction as a function of time at different final concentrations (0.2, 0.5, 1.0, 3.0 mg/mL). Each point represents mean \pm SD. $n=3$.

(A–B) was (2.45 \pm 0.20) $\times 10^{-7}$ cm/s when Rg₁ was 1 mg/mL ($n=3$). Thus, the P_{app} (A–B) of Rg₁ was not significantly different at any concentration tested or in the presence of CsA. However,

the $P_{app}(B-A)$ was $(1.13 \pm 0.11) \times 10^{-7}$ cm/s when Rg_1 was 1 mg/mL in the basolateral compartment ($n=3$), which is significantly lower than that of A-B transport ($P < 0.05$).

Gastric, intestinal and hepatic first-pass effects of Rg_1

The gastric, intestinal and hepatic first-pass effects were evaluated after *po*, *id*, *pv* and *iv* administration. Figure 4 shows the serum concentration-time profiles of Rg_1 after *iv* and *pv* administration of PNS. For each route of administration, 2 phases of decline were observed: a rapid decline followed by a slow decline, which could be described by a 2-compartment model. The pharmacokinetic parameters are listed in Table 2. AUC_{0-8} of Rg_1 after *pv* administration of PNS (50 mg/kg) in rats was lower (approximately 49.44%) than that after *iv* administration of PNS (50 mg/kg). This indicates that part of the dose disappeared (may be due to biliary excretion) following the first pass through the liver after *pv* administration. After *pv* administration, the MRT_{0-8} value decreased by approximately 50% compared with *iv*

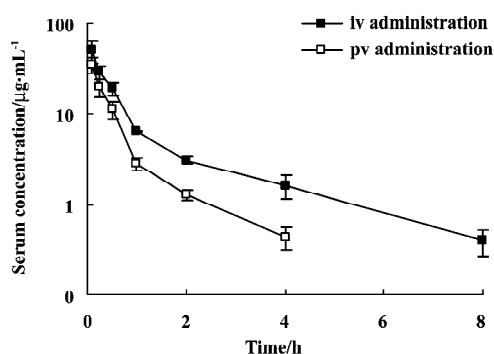


Figure 4. Serum concentration of Rg_1 after *iv* and *pv* administration at doses of 50 mg/kg PNS in rats. Each point represents mean±SD. $n=4$.

administration, which could be due to the hepatic first-pass effect after *pv* administration.

After *po* administration of PNS at a dose of 600 mg/kg, no Rg_1 could be detected in serum samples. However, after *po* administration of 1500 mg/kg PNS in rats, the serum concentration of Rg_1 was low, could be detected 2 h after administration, and reached a peak (5.38 µg/mL) 6 h after administration (Figure 5). Absolute bioavailability (F_{po}) was estimated to be 3.29%. After *id* administration of PNS at

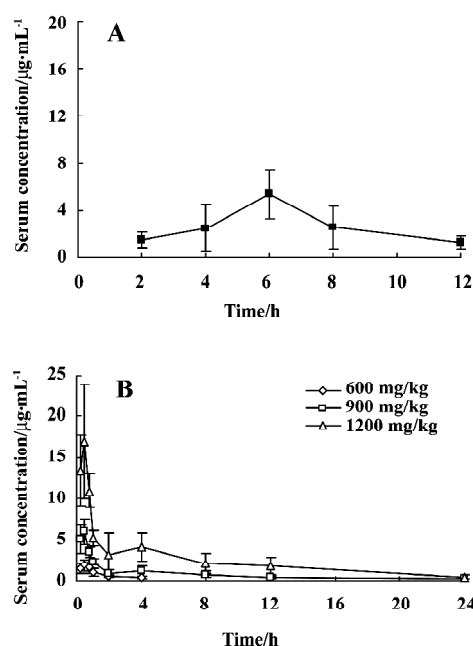


Figure 5. (A) Serum concentration of Rg_1 after *po* administration of PNS (1500 mg/kg) in rats. (B) Serum concentration of Rg_1 after *id* administration of PNS (600, 900 or 1200 mg/kg) in rats. Each point represents mean±SD. $n=3$.

Table 2. Pharmacokinetic parameters of Rg_1 after *iv* (50 mg/kg), *pv* (50 mg/kg), and *po* (A: 600 mg/kg; B: 1500 mg/kg), *id* (A: 600 mg/kg; B: 900 mg/kg; C: 1200 mg/kg) administration of PNS solution in rats. Data are mean±SD. $n=3$ or 4. ^b $P < 0.05$ vs *iv* group. ^c $P < 0.05$ vs *pv* group. ^h $P < 0.05$ vs *id* group.

Parameters	Administration route						
	<i>iv</i>	<i>pv</i>	<i>id</i> (A)	<i>id</i> (B)	<i>id</i> (C)	<i>po</i> (A)	<i>po</i> (B)
AUC ($\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$)	37.975±3.67	19.201±2.87	6.920±0.55	23.340±4.68	60.187±19.23	BQL	37.437±10.54
AUC/ X_0 ($\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$)	0.760±0.073	0.384±0.057 ^b	0.012±0.001 ^{b,e}	0.026±0.005 ^{b,e}	0.050±0.016 ^{b,e}	BQL	0.025±0.007 ^{b,e,h}
MRT (h)	1.55±0.39	0.70±0.14	7.12±3.64	13.29±1.88	10.27±3.13	BQL	8.75±3.04
<i>F</i> (%)	100	50.56	1.52	3.41	6.60	BQL	3.29

BQL, below quantitation limit.

doses of 600 mg/kg, 900 mg/kg and 1200 mg/kg in rats, absolute bioavailability (F_{id}) was, respectively, 1.52%, 3.41%, and 6.60% (Figure 5). For a dose of 600 mg/kg PNS administered via the iv route, the serum concentration was below quantification limits more than 8 h after id administration. Thus, a comparison of F_{po} with F_{id} , and F_{id} with F_{pv} , shows that the gastric and intestinal first-pass effects are both significant.

Discussion

Modulation of Rg_1 uptake by a P-glycoprotein (P-gp; or MRP)-related efflux mechanism or enzymatic degradation in Caco-2 monolayers as possible explanations for the low Rg_1 uptake were excluded in the present study because we observed neither concentration-dependence for Rg_1 uptake nor any effect of co-administration of CsA on the Rg_1 uptake.

The Rg_1 uptake by Caco-2 cell monolayers was pH-independent, and greater uptake occurred at 37 °C compared with that at 4 °C. This temperature-dependence of uptake might be attributed to the cell membranes being more rigid at low temperatures, leading to a lower level of diffusion through the membrane^[16].

In the transepithelial transport study, the flux of Rg_1 in the A–B direction increased in a linear fashion with increasing concentration in the apical compartment, and no significant difference was observed after the addition of CsA. These results confirm that Rg_1 is passively transported across Caco-2 cell monolayers. $P_{app}(A-B)$ was approximately 2-fold greater than $P_{app}(B-A)$. This polarized transport occurring in Caco-2 cell monolayers may result from the differences in physiological structure between the apical (A) and basolateral (B) membranes. $P_{app}(A-B)$ was estimated to be $(2.59 \pm 0.17) \times 10^{-7}$ cm/s ($C_0=1$ mg/mL), which is below the suggested threshold value (1×10^{-5} cm/s) for poorly absorbed drugs^[17]. The relatively large molecular weight ($M_r=801$) and low lipophilia value (we found that $\log P$, the log value of the coefficient between water and octanol, was 0.64) of Rg_1 may be partly responsible for its low permeability.

After pv administration of PNS in rats, serum concentration-time profiles of Rg_1 were found to be similar to those observed with iv administration, but the AUC value decreased, which may have been caused by bile excretion following the first pass through the liver. For *po* administration of PNS at a dose of 1500 mg/kg, a delayed peak in serum concentration of Rg_1 can be attributed to the low rate of absorption or the potential specific absorptive sites in the lower part of the intestine instead of biliary excretion, because the amount of biliary excretion is small compared with the *po* dose given in this instance. In contrast, few serum samples could be quantified after *po* administration of PNS

at a dose of 600 mg/kg. With id administration, rapid absorption and a short time to peak serum concentration was observed, which was caused by the high concentration in the local area when PNS was directly delivered to the duodenum. Owing to the saturation of the enzymes in the intestinal tract when a higher dose was administered, absorption of Rg_1 after id administration is dose-dependent, and the absolute bioavailability values were 1.52%, 3.41% and 6.60% for dosages of 600 mg/kg, 900 mg/kg and 1200 mg/kg, respectively.

As a result, the gastric first-pass effect is significant when F_{po} (3.29% at a dose of 1500 mg/kg PNS) is compared with F_{id} (6.60% at a dose of 1200 mg/kg PNS), probably relating to the decomposition of Rg_1 in the acid pH of the stomach^[5]. However, although the gastric first-pass effect is significant, a large amount of Rg_1 remains intact after *po* administration and moves into the intestinal tract, which may also induce saturation of enzymes in the intestinal tract and produce a similar F value to id administration at a relative low dose. Consequently, the primary reason for poor absorption of Rg_1 can be attributed to the intestinal first-pass effect, because F_{id} is still extremely low (6.60% at a dose of 1200 mg/kg PNS), and approximately 7–8 times lower than F_{pv} (50.56%). There are two factors that account for the intestinal first-pass effect. One is metabolism by bacterial β -glucuronidase in the large intestine, but this is not a key factor, because the enzymatic reaction with microflora takes place mainly in the large intestine, but the small intestine is known to be a more important location in the absorption process. Furthermore, it is the low permeability across the intestinal mucosa of Rg_1 that mainly accounts for its poor absorption before reaching the portal vein. This is consistent with the results of our Caco-2 cell studies, in which an extremely low P_{app} indicated a poor membrane permeability for Rg_1 .

Therefore, the low bioavailability of Rg_1 is caused by its decomposition in the stomach, metabolism in the large intestine, low membrane permeability across the intestinal mucosa and elimination in the liver. Of these factors, low membrane permeability is the dominant factor explaining the poor absorption of Rg_1 .

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