Drug Development and Industrial Pharmacy, 2011; 37(12): 1415–1421

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.582874

informa
healthcare

RESEARCH ARTICLE

Enhancing effect of Labrasol on the intestinal absorption of ganciclovir in rats

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Abstract

Ganciclovir (GCV), like other nucleoside analogs such as trifluridine and acyclovir (ACV), is hydrophilic, poorly permeable across membranes and orally low-bioavailable. In the present studies, Labrasol was evaluated for improving intestinal absorption of GCV through *in vitro* and *in vivo* experiments. The effect of Labrasol on absorption of GCV in rat small intestine was investigated using an *in situ* single-pass perfusion technique. The apparent absorptive clearance (PeA) of GCV with Labrasol in the duodenum, jejunum and ileum was 1.01, 1.28, and 1.49 mL/min/cm ($n = 6$), respectively, and significant regional differences of GCV absorption among the three segments were observed (p jejunum (p duodenum ($p > 0.05$). The effects of EDTA, verapamil on the permeability of GCV were conducted. The permeability of GCV was increased by EDTA, verapamil, respectively. The results indicated that paracellular absorption and efflux played important roles in GCV absorption. *In vivo* absorption GCV in rats was conducted. When GCV at 1 mg/kg dose was administered with Labrasol (10%, v/v), the mean AUC of was determined as 14.45 ± 3.88 μg*h/mL, compared to 8.05 ± 1.52 μg*h/mL without Labrasol. Based on the results, we could conclude that the absorption of GCV through GI lumen would be enhanced by Labrasol. The effect of Labrasol maybe ascribed to both (i) inhibit efflux of GCV from the enterocytes to the GI lumen; and (ii) enhance GCV absorption from the GI lumen through paracellular pathway.

Keywords: Ganciclovir, Labrasol, paracellular absorption, P-glycoprotein inhibition, apparent absorptive clearance

Introduction

Ganciclovir (GCV) is a selective antiviral agent which has shown activity against herpes simplex viruses I and II, human cytomegalovirus (HCMV). GCV is widely used in the treatment of cytomegalovirus (CMV) retinitis[1,2]. In fact, GCV has been reported to be 26 times more potent than acyclovir (ACV) against HCMV *in vitro*[3]. However, GCV is a BCS type III drug, which is poorly absorbed by oral administration. The bioavailability of orally administered GCV in humans was reported, ranging from 2.6 to 7.3%[4]. The poor bioavailability of GCV is due to its low lipophilicity and membrane partitioning, as evidenced by an octanol-to-water partition coefficient of 0.022[5–7]. Therefore, the absolute oral bioavailability having been determined to be in the range of 15% is considerably poor[8]. As a consequence, oral GCV has been used in long-term maintenance therapy in the treatment of CMV retinitis in immuno-compromised patients with a dose of 3000 mg/d[9]. Such a high dose would cause severe side effects, e.g., life-threatening blood problems-neutropenia and thrombocytopenia. Thus, there have been many attempts to improve the bioavailability of ACV[10] such as the development of the amino acid prodrug valacyclovir, which leads to improved bioavailability[11].

P-glycoprotein (P-gp) represents an important membrane transporter pumping a wide range of structurally unrelated drugs and xenobiotics out of cells[12]. On the intestinal level, it is located in the apical membrane of the epithelial cells and it transports drugs back into the gut lumen. Studies in animals and human have indicated that P-gp plays a major role in limiting drug absorption

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(Received 10 February 2011; revised 12 April 2011; accepted 13 April 2011)

and consequently oral bioavailability[13,14]. These effects have restricted the clinical use of drugs as well as the development of new chemical entities (NCEs), which are substrates of P-gp. Thus, there is considerable interest in trying to enhance their absorption and oral bioavailability by inhibiting the P-gp-mediated drug efflux. Recently, ACV has been suggested to be a substrate of P-gp (MDR1) which is an efflux transporter of the ABC-binding cassette (ABC) family[15]. It has been demonstrated that *in vitro* ACV absorption could be increased due to the use of P-gp-specific inhibitors[16,17].

Surfactants with a medium length alkyl chain surfactant may penetrate the lipid bi-layer easily. Thus, Labrasol (caprylocaproyl macrogol-8 glyceride) is comprised of a well-defined mixture of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethyleneglycol, with the predominant fatty acids being caprylic and capric acids[18]. While Labrasol has been reported to increase the solubility of water-insoluble drugs by emulsification[19], it has also been demonstrated to facilitate the oral bioavailability of water-soluble drugs, such as gentamicin sulfate[20], and amphiphilic drugs, such as diclofenac diethylamine[21]. The absorption-enhancing mechanism of Labrasol is responsible for increasing membrane permeability for water-soluble drugs remains unclear and requires clarification. Recently, it has been reported that Labrasol of 0.1 and 1% concentrations has a tight junction opening action. Further, as one of the biological actions of Labrasol in low concentration (0.075–0.1%) on the absorption of water-soluble drugs from intestinal tract, Labrasol inhibits the function of efflux pump in intestinal epithelial cells[22]. While Labrasol affected the transportation of Rodamine123-a specific P-gp substrate, but did not change the transportation of Lucifer yellow, a non-P-gp substrate suggested that the effect of Labrasol on the transport of drugs was specific for rhodamine 123[23].

The aims of the present work are as follows: (i) to investigate the effects and absorption-enhancing mechanism of Labrasol on the intestinal absorption of GCV *in vitro* and *in situ*; and (ii) to test the potential of Labrasol to improve the rat oral bioavailability.

Materials and methods

Materials

Ganciclovier (purity > 99.5%) was purchased from Huayuan Pharmaceutical Co. Ltd (Shanghai, China); Labrasol was a gift from Gattefossé Canada Inc. (Toronto, Canada); All other chemicals used were analytical grade.

Male Sprague-Dawley rats (190–210 g) were purchased from Qinglongshan Experimental Animal Center (Nanjing, China). All animals were housed in stainless steel cage, and could freely access food and water. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation in Science and Technology Government Department in Jiangsu Province.

In situ single-pass perfusion studies

Under sodium pentobarbital anesthesia (25 mg/kg i.p.), rats were placed on an operating plate. Anesthesia was maintained by i.p. injection of additional dose of pentobarbital when rats showed signs of recovery.

Following anesthesia, the animal was laid on a heating pad placed below to maintain the body temperature throughout the experiment. The abdominal area was shaved and cleaned; an incision (2–4 cm) was made slightly below the midline. The intestinal segment of interest was identified. For the duodenal segment, from 1 cm distal to the pyloric end of the stomach, a segment approximately 5 cm in length was perfused. A transverse incision was made on the antimesenteric side of the bowel at both ends of the defined 5 cm segment. Silicone tubing (2.2 mm I.D., 3.0 mm O.D.) was inserted into the lumen at one end and attached to the perfusion pump (Shanghai Qingpu Huxi Instrument Factory, Shanghai, China) and reservoir at the other. To remove the contents of the segment, saline (37°C) was slowly perfused through it until the effluent was clear. The remaining saline in the segment was cleared carefully by infusing air so as not to cause excessive intraluminal pressure. The same procedure was used to prepare the jejunal and ileum segment. Intestinal segments were then returned to the abdominal cavity. Finally, the abdomen was covered with saline-soaked gauze (37°C) to keep it warm and moist throughout the experiment.

For each intestinal segment ($n=3$), GCV was perfused alone and with Labrasol (0.1%, v/v) at 15 μg/mL in Krebs–Ringer solution (27.4 mM NaCl, 1 mM KCl, 0.2 mM $MgSO_4$, 0.06 mM KH_2PO_4, 2.8 mM Trizma base, 0.56 mM $CaCl_2$, and 1.8 mM glucose PH 7.4) at a flow rate of 0.2 mL/min. Samples were taken from the perfusion syringe before and after the perfusion study. Effluent perfusate samples were collected every 15 min for 120 min in tared 3-mL centrifuge tube. The sample was determined by HPLC. At the end of each experiment, the animal was executed by giving excess pentobarbital anesthesia (i.p). And the intestinal segment was excised. Its length was measured by placing the segment flat over a ruler wetted with saline. This length was used in the calculations of length-normalized absorptive clearance. The inflow perfusion rate (Q_{in}) in each experiment was estimated from a linear regression of the volume remaining in the perfusion syringe vs. time. The perfusion pump was also calibrated before each experiment by weighing the blank perfusion solution collected over selected time periods. The volume of the outflow samples was measured gravimetrically, and the outflow perfusion rate (Q_{out}) was calculated by dividing the volume by the time interval (15 min) between sample collections. The time to reach a steady-state rate of loss from the segment was determined by examining the outflow concentration-time profiles. The average outflow concentration (C_{out}) and average outflow perfusion rate were then determined over the collection intervals at steady state. The inflow concentration (C_{in}) was an average of the concentrations taken from syringes before

and after perfusion. The apparent absorptive clearance (PeA) was then calculated by a newly developed model from Equation (1) and normalized by the length of the perfused intestinal segment[24].

$$PeA = Q_{ave} \cdot \ln\left(\frac{Q_{in} \cdot C_{in}}{Q_{out} \cdot C_{out}}\right) \quad (1)$$

where Q_{ave} was calculated as follows:

$$Q_{ave} = \frac{Q_{in} \cdot Q_{out}}{\ln\left(\frac{Q_{in}}{Q_{out}}\right)} \quad (2)$$

Permeation studies utilizing a Caco-2 cell culture monolayer system and rat intestine in Valia-Chien chambers

Caco-2 cells were maintained in the media described above at 95% humidity and 37°C in an atmosphere of 5% CO_2. The media was changed daily and cells were split twice a week. The following experiments were conducted during passages 80–90. Cells were plated directly after splitting in a density of 1×10^5 cells onto the membrane inserts of 12-well plates. The cells were allowed to grow and differentiate for 24 days, during which time the media was changed every 48 h. Transepithelial electrical resistance (TEER) of the monolayers was measured with the EVOM instrument (World Precision Instruments, Sarasota, FL).

Rats were fasted for at least 16 h before tissue procurement. Under light ether anesthesia, abdominal incisions were made and small intestine (lower jejunum) was removed. The intestinal fat adhered to the mesentery was isolated. Each segment was cut open with a scalpel and the epithelial surface was washed with ice-cold saline. The modified Valia-Chien chambers made of glass were used in this experiment[25]. Pieces of intestinal tissue were mounted as flat sheets between the opposing faces of two halves of chambers, in which a surface area of 0.78 cm^2 was exposed. The volume of each diffusion chamber was 5 mL. The pH of the prepared $NaHCO_3$ buffered with 40 mM HEPES was adjusted to 6.8 and the osmolarity to 310 mosM/kg. All experiments were performed at 37°C and a mixture of 5% CO_2 and 95% O_2 was continuously bubbled through the donor and acceptor compartments. Over 2 h of incubation time, aliquots of 200 μL were taken from the other compartment every 30 min, and the volume was substituted by 200 μL incubation medium pre-equilibrated at 37°C. The amount of permeated GCV was determined by HPLC.

During all transport studies, GCV was applied at a concentration of 15 μg/mL. To confirm the P-gp affinity of GCV, it was admitted to the apical side to investigate the transport in the absorptive direction (A→B) and to the basolateral side to investigate the transport in the secretory direction (B→A). Additionally, transport studies were performed in presence of the well-known P-gp inhibitor verapamil (100 mM) and Labrasol (0.1%, v/v) which were added to both sides of the rat intestinal mucosa. To elucidate that GCV could undergo the paracellular route of absorption, transport studies of GCV were performed in presence of EDTA in the apical side to open the paracellular route by chelation with calcium in a concentration of 0.25%[26]. GCV solution was used as a control. The apparent permeability coefficients (Papp, cm/s) for GCV were calculated according to Equation (3):

$$Papp = \frac{dQ}{dt \cdot C_0 \cdot A} \quad (3)$$

where dQ/dt is the change in the transported amount of GCV per minute. A, C_0 are the diffusion area, the initial concentration of GCV in the donor compartment, respectively. Transport enhancement ratio (ER) was calculated from Papp values according to Equation (4):

$$ER = \frac{Papp_{additive}}{Papp_{control}} \quad (4)$$

In vivo absorption experiment

Absorption studies were performed in male Sprague-Dawley rats. Six rats were divided into two groups randomly. The animals were fasted overnight prior to the experiment, but had free access to water. At time zero, the three rats of each group were dosed by oral gavage with dose of GCV of 10 mg/kg dissolved at 2 mg/mL in water and Labrasol solution (0.1%, v/v), respectively. The rats received each of the test preparations in a crossover fashion with a washout period of 1 week. The blood samples (approximately 300–400 μL) were collected from the eye sockets into heparinized centrifuge tube at 0, 0.25, 1, 1.5, 2, 4 and 6 h after oral administration. Methanol (500 μL) was added to precipitate proteins, mixed vigorously for 5 min and centrifuged at 5000*g* for 20 min. The supernatant plasma was collected into another centrifuge tube for HPLC analysis of GCV.

HPLC analysis of GCV

GCV analysis was performed via reversed-phase HPLC using a Waters 515 pump, and a Waters 2487 HPLC detector (Waters, Nanjing, China) set at 254 nm, and a Sanrui Chromatography Workstation (Sanrui Sci-Tech Co., Shanghai, China). GCV was separated on a C18 column (5 μm, 100 Å, 4.6 × 250 mm, Agela technologies Inc.) at 40°C. The isocratic mobile phase was composed of an ion pair solution (10 μM sodium phosphate dibasic and 10 μM 1-octanesulfonic acid sodium) and methanol in a ratio of 95:5. The pH of the mobile phase was adjusted to 2.5 with phosphoric acid and the flow rate was 1 mL/min. Injection volume was 20 μL.

Statistical and pharmacokinetic data analysis

Data were expressed as mean ± SD. For multiple-group comparisons, a one-way analysis of variance followed by

Turkey's HSD test was used. For a two-group comparison, a two-tailed non-paired Student's *t*-test was employed. And $p < 0.05$ was considered as the level of significance. The pharmacokinetic data analysis of the concentration-time profile was performed with the pharmacokinetic software 3P87 (Chinese Pharmacology Association). According to the AIC values and the goodness of fit of the data, the compartment of the model and the weight were selected.

Results

In situ single-pass perfusion studies

Table 1 summarizes the PeA of GCV in different regions of the intestine when perfused alone or co-perfused with Labrasol at 15 μg/mL. When perfused alone, the PeA of GCV in the duodenum, jejunum, and ileum was 0.87, 0.71, and 0.67 mL/min/cm ($n = 6$), respectively. When Labrasol was added, the PeA of GCV significantly increased in intestine segments, with a PeA of 1.01, 1.28, and 1.49 mL/min/cm in duodenum, jejunum, and ileum, respectively (Table 1). In the above studies, Labrasol was founded to increase the permeability in rat small intestine. Under this condition, significant regional differences of GCV absorption among the three segments were observed ($p < 0.05$). Compared to the control group, the absorbability of the Labrasol group was found to be: ileum ($p < 0.05$) > jejunum ($p < 0.05$) > duodenum ($p > 0.05$). The results confirmed that Labrasol is an effective absorption enhancer for GCV in the intestine.

In vitro permeability experiments

In order to evaluate the mechanism for GCV absorption enhancement by Labrasol, *in vitro* permeability studies of GCV were performed using a modified Valia-Chien chambers method. The profiles of GCV permeation across rat small intestine are shown in Table 2. It was observed that the permeability coefficient (Papp) of GCV in rat small intestine was $2.66 \pm 0.41 \times 10^{-6}$ m/s in the absorptive direction (A→B), whereas it was $4.23 \pm 0.59 \times 10^{-6}$ m/s in the secretory transport (B→A). The ratio of Papp (B→A) to Papp (A→B) was 1.59. That means, the low absorption of GCV from GI gut might due to the secretory transport.

Verapamil, a well-known inhibitor of P-gp, was used to identify the mechanism of secretory transport of GCV. In presence of verapamil, the transport of GCV increased to $5.88 \pm 0.22 \times 10^{-6}$ m/s (ER = 2.98) in the absorptive direction. While the transport of GCV decreased to $3.04 \pm 0.67 \times 10^{-6}$ m/s (ER = 0.71) in the secretory direction. These results indicated that GCV might be a substrate of efflux transporter P-gp. P-gp is a transporter which is located on the apical side of the intestinal membrane, and it is responsible for the efflux of a wide range of xenobiotics. A characteristic feature of P-gp substrates is that they show a higher transport from the basolateral to the apical (B→A) than from the apical to the basolateral (A→B) side of an intestinal membrane.

When Labrasol was added to the donor solution, GCV absorption behavior is similar to that with verapamil. The Papp of GCV significantly increased in absorptive direction($7.93 \pm 0.96 \times 10^{-6}$). Labrasol decreased the GCV permeation in the direction of B→A. The results met well with the assumption that GCV is a substrate of p-gp, and Labrasol is the competitive inhibitor for GCV efflux.

Absorption of large and more hydrophilic drugs is mostly limited to the paracellular pathway. Entry of molecules through the paracellular pathway is primarily restricted by tight junctions. In intact tight junctions, these proteins are strongly associated with the plasma membrane. EDTA depletes Ca^{2+} in the extracellular space including the tight junctional region and activates protein kinase C (PKC), resulting in expansion of the paracellular route[26]. In the present studies, GCV was administered with EDTA to confirm its paracellular absorption. The effect of EDTA on the apparent permeability coefficient (Papp) of GCV is also illustrated in Table 2. The Papp of GCV with EDTA, which could activate PKC by depletion of extracellular calcium resulting in expansion of the paracellular route, was increased to $5.51 \pm 0.54 \times 10^{-6}$ m/s (ER = 2.07). TEER of the Caco-2 monolayers was measured during the transport experiments and was determined to be $372 \pm 35\Omega$ cm^{-2} after the experiment for GCV in buffer and $265 \pm 22\Omega$ cm^{-2} for GCV with EDTA at a concentration of 0.5%. After this experiment, the Caco-2 recovered for 24 h under the culture conditions, TEER value has returned to normal ($358 \pm 27\Omega$ cm^{-2}). This phenomenon indicated that EDTA at a concentration of 0.5% could reversibly open the tight junctions of intestinal epithelium, which increased paracellular transport and drug delivery with less toxicity. The results indicated that the absorption of GCV could be increased by the expansion of the paracellular route.

In vivo absorption experiments

The plasma GCV concentration vs. time profiles following oral administrations of GCV solution or GCV with Labrasol in rats is shown in Figure 1. The blood concentration-time curve of GCV after oral administration was fitted well to the one-compartment model with a weight of $1/c^2$. When Labrasol was added, higher plasma GCV levels were observed after administration. Table 3 summarizes the pharmacokinetic parameters obtained from the plasma concentration-time curves following the oral gavage with GCV dissolved at 2 mg/mL in water and Labrasol solution, respectively. Labrasol showed a

Table 1. Absorptive Clearance (PeA) of GCV in different intestinal regions of rats ($n = 6$).

	PeA (mL/min/cm)	
Intestinal segment	GCV solution ($n = 6$)	Co-perfused with Labrasol ($n = 6$)
Duodenum	0.87 ± 0.13	1.01 ± 0.26
Jejunum	0.71 ± 0.15	1.28 ± 0.21*
Ileum	0.67 ± 0.12	1.49 ± 0.33*

*Significantly different from the absorption of control in jejunum and ileum ($p < 0.05$).

Table 2. Effect of surfactant on apparent permeability coefficient (Papp) of GCV in small intestine ($n=6$).

		Papp ($\times 10^{-6}$m/s)			
Intestine cite		Solution	With Labrasol	With verapamil	With EDTA
Small intestine	A→B	2.66 ± 0.41	7.93 ± 0.78*	5.88 ± 0.22*	5.51 ± 0.54*
	ER	1	2.98	2.21	2.07
	B→A	4.23 ± 0.59*	3.56 ± 0.38	3.04 ± 0.67	6.47 ± 1.38
	ER	1	0.84	0.71	1.53

A→B: the transport of GCV in the absorptive direction (apical side→basolateral side); B→A: the transport of GCV in the secretory direction (basolateral side→apical side).
*Significantly different from the absorption in absorptive direction of control ($p<0.05$).

Table 3. Pharmacokinetic parameters of GCV ($n=6$).

	Unit	GCV solution	GCV with Labrasol
A	μg/mL	3.91 ± 0.86	7.00 ± 2.07*
Ke	1/h	0.46 ± 0.09	0.49 ± 0.23
Ka	1/h	7.84 ± 1.19	13.01 ± 9.31*
$t_{1/2}$(Ka)	H	0.090 ± 0.014	0.0090 ± 0.078
$t_{1/2}$(Ke)	H	1.56 ± 0.35	1.73 ± 0.92
T_{max}	H	0.39 ± 0.031	0.35 ± 0.19
C_{max}	μg/mL	3.05 ± 0.23	5.29 ± 1.49**
AUC	μg*h/mL	8.05 ± 1.52	14.45 ± 3.88**

*$p<0.05$, **$p<0.01$; Comparison with control group (GCV solution).

significant absorption enhancement of GCV after oral administration to rats. When Labrasol was added, C_{max} increased from 3.0473 to 5.29 μg/mL and AUC from 8.05 to 14.45 μg*h/mL. The results demonstrate that oral absorption enhancement of GCV can be achieved by co-administrating with Labrasol.

Discussion and conclusion

The small intestine is the most important site of drug absorption following oral administration. Depending largely on their physicochemical properties, drugs can be absorbed through the intestinal mucosa by different mechanisms: passive diffusion via the paracellular space for small polar compounds or via the transcellular route for lipophilic molecules, or via transporters which may function passively or actively (energy dependent). Some of these transporters import their substrates from the intestinal lumen into the epithelial[27]. Other transporters, notably P-gp and the multidrug resistance protein 2 (MRP2), export their substrates out of the enterocytes back into the intestinal lumen[28,29], thus potentially reducing their net absorption.

In this study, Labrasol was found to increase the intestinal absorption of GCV and the effect was prominent (the PeA was about 3-fold higher than control). The order of GCV intestinal absorption was: ileum < jejunum < duodenum, but there were no significant differences among them. When the co-administration of Labrasol, the order was reversed to duodenum < jejunum < ileum (Table 1). Under this condition, significant regional differences of GCV absorption among the three segments were observed ($p<0.05$). Compared to the control group, the absorbability of the Labrasol group was found to

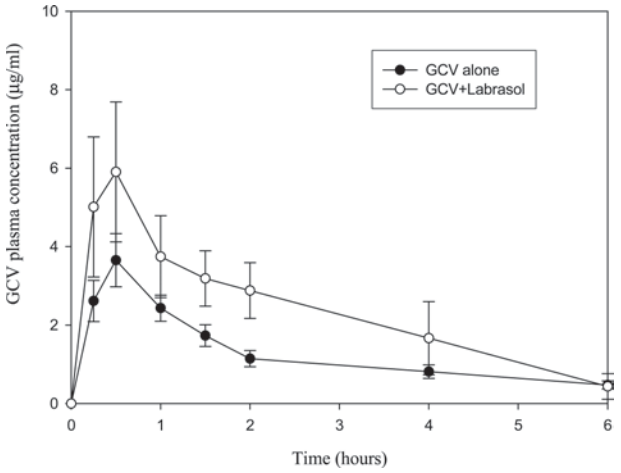

Figure 1. Plasma GCV concentration vs. time profiles following oral administrations of GCV solution alone (•) and with Labrasol (○).

be: ileum ($p<0.05$) > jejunum ($p<0.05$) > duodenum ($p>0.05$). The increased absorbability, which could be contributed to the inhibited function of P-gp efflux, was consistent with the level of P-gp expressed in rat's small intestine[30].

The intestinal epithelium has specialized transport systems that can secrete drugs in the blood-to-lumen or basolateral-to-apical direction, and these provide a barrier function against drug absorption[31]. A characteristic feature of P-gp substrates is that they show a higher transport from the basolateral to the apical (B→A) than from the apical to the basolateral side (A→B) of an intestinal membrane. From our membrane transport experiments, the transport of GCV from A→B and B→A has been performed across rat intestine, and the determined apparent permeability coefficients (Papp) are listed in Table 2. The secretory transport of GCV was 1.59-fold higher in case of rat small intestine in comparison to the absorptive transport. Due to the inhibitory effect of verapamil, the secretory transport of GCV was 0.45-fold lower in case of rat small intestine in comparison to the absorptive transport. When co-administration of Labrasol was at low concentrations (0.1%), the absorptive directed transport of GCV was significantly increased while its secretory directed transport was decreased. In previous published papers[32], the transport of ACV (with similar chemical structure of GCV) from A→B and B→A has been performed across rat intestine and Caco-2 cell

monolayers, respectively, and the results indicated that ACV is a P-gp substrate. Werle and Hoffer[33] determined for rhodamine 123, a specific P-gp substrate, an efflux ratio of 2.8 which declined to an efflux ratio of 1.0 due to addition of verapamil. Within this study, similar efflux ratios for GCV could be determined which suggest that GCV as P-gp substrate and low concentrations of Labrasol might be the competitive inhibitor for GCV efflux, which inhibiting the function of P-gp in the intestine, thereby increasing intestinal absorption and bioavailability of P-gp substrates.

When EDTA was added, the Papp of GCV increased significantly compared to the control. These data suggested that the compound could undergo the paracellular route for absorption. The results of the recent studies indicated that Labrasol could increase the permeability of mannitol by the opening of tight junctions[34]. So it was thought that Labrasol could enhance the permeability of GCV through the alternation of tight junctions.

In our pre-experiment, TEER of the Caco-2 monolayers was measured during the transport experiments and was determined to be $372 \pm 35\Omega\ cm^{-2}$ after the experiment for GCV in buffer and $265 \pm 22\Omega\ cm^{-2}$ for GCV with EDTA at a concentration of 0.5%. After this experiment, the Caco-2 recovered for 24 h under the culture conditions, TEER value has returned to normal ($358 \pm 27\Omega\ cm^{-2}$). This phenomenon indicated that EDTA at a concentration of 0.5% could reversibly open the tight junctions of intestinal epithelium, which increased paracellular transport and drug delivery with less toxicity.

Administration of GCV in the presence of Labrasol has resulted in increased plasma GCV levels. The values of C_{max} and AUC were increased significantly. In conclusion, the results of the current study indicated that both the poor intestinal membrane permeability and drug efflux systems limit the BA of GCV.

The enhancement of the absorption of GCV with Labrasol was due to an increase in the intestinal permeability (by opening tight junction and influencing the membrane permeability) and the inhibition of secretory transport of GCV from the enterocytes.

Declaration of interest

The authors thank partial financial support from Ministry of Science and Technology of the People's Republic of China under project 2009ZX09310-004.

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