Regular Article

In Vitro P-Glycoprotein-Mediated Transport of Tadalafil: A Comparison with Sildenafil

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Tadalafil and sildenafil are selective inhibitors of phosphodiesterase type 5, showing marked pharmacokinetic variability in patients with pulmonary arterial hypertension. It has been reported that sildenafil is a substrate for P-glycoprotein (P-gp), but whether tadalafil is a substrate for P-gp remains to be determined. The objective of the present study was to elucidate whether tadalafil is a substrate for P-gp. Transcellular transport of sildenafil and tadalafil (5 μ M each) was examined using renal epithelial LLC-PK₁ and P-gp-expressing LLC-GA5-COL150 cell monolayers. The efflux ratio of the basal to apical (B to A) transport of sildenafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (1.52) was significantly higher than that in LLC-PK₁ cells (0.711). The efflux ratio of the B to A transport of tadalafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (10.4) was significantly higher than that in LLC-PK₁ cells (1.23). In LLC-GA5-COL150 cell monolayers, the $V_{\max}$ and K_m values of sildenafil transport calculated from a modified Michaelis–Menten equation were 101 ± 64 pmol/min/cm² and 112 ± 47 μ M, respectively. On the other hand, those of tadalafil transport were 13.6 ± 4.8 pmol/min/cm² and 22.7 ± 9.3 μ M, respectively. In the presence of a P-gp inhibitor (PSC833), the B to A transport of tadalafil was decreased by 28.6% in LLC-GA5-COL150 cells, and the A to B transport of tadalafil was 6.59-fold greater than that in its absence. These results indicate that tadalafil is a substrate for P-gp.

Key words tadalafil; sildenafil; P-glycoprotein

Tadalafil and sildenafil are potent and selective inhibitors of cyclic GMP-specific phosphodiesterase type 5 (PDE5) in vascular smooth muscle.¹⁾ These drugs have been used as treatments not only for erectile dysfunction, but also for pulmonary arterial hypertension (PAH).²⁾ Sildenafil has a short half-life of about 3–5 h,³⁾ whereas tadalafil has a long half-life of about 17.5 h.⁴⁾ Due to its long half-life, tadalafil has the advantage of once-a-day dosing compared with 3-times-a-day dosing for sildenafil.⁵⁾ For patient convenience and compliance, the transition from sildenafil to tadalafil has been tried in stable patients with PAH.⁵⁾ However, it is necessary to understand the difference between the pharmacokinetics of the two drugs for their safe use.

Sildenafil is eliminated predominantly by hepatic metabolism and converted to an active metabolite, *N*-desmethyl sildenafil.⁶⁾ In healthy men, metabolites are predominantly excreted into the feces (73–83%) and to a lesser extent urine (6–15%) after an oral dose.⁶⁾ In our previous study, we confirmed that the *N*-demethylation of sildenafil was mediated by both CYP3A4 and CYP3A5 isoforms.⁷⁾ That is, the Michaelis–Menten constant (K_m) values for CYP3A4 and CYP3A5 isoforms were estimated to be 27.7 ± 7.5 and 17.4 ± 3.2 μ M, respectively, and the maximum transport rate ($V_{\max}$) values were 30.7 ± 11.3 and 18.9 ± 6.1 pmol/min/pmol P450, respectively.⁷⁾ In addition, Choi and Song⁸⁾ reported that sildenafil was a substrate for P-glycoprotein (P-gp). That is, the transcellular transport of sildenafil in P-gp-expressing Madin–Darby canine kidney II (MDCKII)-multidrug resistance protein 1 (MDR1) cells in a basal to apical (B to A) direction was 4.87-fold greater than that from A to B. They proposed that P-gp might be the underlying mechanism related to the low and variable oral bioavailability of sildenafil.⁸⁾

Tadalafil is eliminated predominantly by hepatic metabolism and converted to an inactive metabolite, desmethylene tadalafil.⁴⁾ In healthy men, metabolites are predominantly excreted into the feces (61%) and to a lesser extent urine (36%).⁹⁾ In our previous study, we confirmed that the demethylation of tadalafil was mediated by both CYP3A4 and CYP3A5 isoforms.⁷⁾ That is, the K_m values for CYP3A4 and CYP3A5 isoforms were estimated to be 5.29 ± 0.47 and 7.76 ± 1.05 μ M, respectively, and the $V_{\max}$ values were 0.658 ± 0.048 and 0.467 ± 0.069 pmol/min/pmol P450, respectively.⁷⁾ However, to our knowledge, whether tadalafil can be transported by P-gp is still unclear. Recently, we also found the marked variability in the pharmacokinetics of tadalafil in 23 children with PAH.¹⁰⁾ All children received a mean dose of 0.97 ± 0.41 mg/kg tadalafil, and plasma concentrations of the drug at 3.8–4.8 h after the dose showed a 6-fold interindividual difference.¹⁰⁾ Although the mechanisms responsible for the marked variability in the pharmacokinetics of tadalafil have not been documented, we considered that the involvement of both CYP3A and P-gp in the pharmacokinetics of tadalafil was possible as well as those of sildenafil.

The aim of the present study was to elucidate whether tadalafil is a substrate for P-gp. In the present study, we evaluated the transcellular transport properties of tadalafil in renal epithelial LLC-PK₁ and P-gp-expressing LLC-GA5-COL150 cells. In addition, kinetic parameters of tadalafil transport in LLC-GA5-COL150 cells were compared with those of sildenafil transport.

MATERIALS AND METHODS

Reagents Sildenafil and tadalafil were purchased from To-

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ronto Research Chemicals (North York, ON, Canada). PSC833 was purchased from AdooQ BioScience (Irvine, CA, U.S.A.). [^{14}C]-Mannitol (0.1mCi/mL) was purchased from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). All other chemicals and solvents were of the highest purity available.

Cell Lines and Cell Culture LLC-PK₁ cells were purchased from the American Type Culture Collection (Manassas, VA, U.S.A.). LLC-GA5-COL150 cells were purchased from the Riken Bioresource Center (Tsukuba, Japan).

LLC-PK₁ cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% MEM nonessential amino acids, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin in a humidified atmosphere with 5% $\text{CO}_2/95\%$ air at 37°C. LLC-GA5-COL150 cells were cultured under the same conditions with the exception of the addition of 150 ng/mL of colchicine, which was added to the medium to maintain P-gp expression.¹¹⁾ The medium was changed every 2 or 3 d and, when the cells reached 70–80% confluence, they were subcultured using a 0.02% ethylenediaminetetraacetic acid (EDTA)/0.05% trypsin solution. The cells were seeded at a density of 5×10^5 cells/cm² on a 0.9 cm² porous membrane (0.4- μm pore size) in a cell culture insert (BD Bioscience, Bedford, MA, U.S.A.). The seeded LLC-PK₁ and LLC-GA5-COL150 cells were grown for 6 or 7 d.¹¹⁾

The maturity of cell monolayers was evaluated prior to transport experiments by measuring transepithelial electrical resistance (TEER). TEER was measured using a Millicell-ERS resistance system (Millipore, Bedford, MA, U.S.A.). LLC-PK₁ cell monolayers whose TEER was above 40 $\Omega \cdot \text{cm}^2$ were used to assess the transcellular transport. LLC-GA5-COL150 cell monolayers whose TEER was above 100 $\Omega \cdot \text{cm}^2$ were used.

Transcellular Transport of Sildenafil and Tadalafil The transport of sildenafil or tadalafil (5 μM each) across LLC-PK₁ and LLC-GA5-COL150 cell monolayers was examined. Also, the transport of sildenafil (3–100 μM) or tadalafil (1–100 μM) across LLC-GA5-COL150 cell monolayers used for kinetic analysis was examined. The composition of the incubation medium was as follows: 123 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 1 mM sodium pyruvate, and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.4). Cells were pre-incubated with the incubation medium for 30 min at 37°C. The apical or basal side was replaced with the incubation medium containing sildenafil or tadalafil after pre-incubation. An 80- μL (apical side) or a 150- μL (basal side) aliquot of the medium was collected after 30, 60, 90, or 120 min from the compartment opposite to that in which sildenafil or tadalafil was added. To compensate for the volume sampled, an 80- μL (apical side) or a 150- μL (basal side) aliquot of incubation medium pre-warmed at 37°C was added immediately after each sampling. The concentrations of sildenafil and tadalafil in each sample were determined by LC-MS/MS^{8,12)} and HPLC methods,⁷⁾ respectively.

When kinetic parameters of the membrane permeation of sildenafil or tadalafil in LLC-GA5-COL150 cells were estimated, mannitol was used as a paracellular marker. That is, mannitol (1–100 μM) contained a fixed amount of [^{14}C]-mannitol, and the radioactivity of [^{14}C]-mannitol was measured by a scintillation counter for 3 min. The amount of transcellular transport of sildenafil or tadalafil in the B to A direction was calculated by subtracting the amount of mannitol.

Effect of the P-gp Inhibitor on the Transport of Sildenafil and Tadalafil in LLC-GA5-COL150 Cells To determine the involvement of P-gp on the transport of sildenafil and tadalafil across LLC-GA5-COL150 cell monolayers, the potential inhibition of transport was examined in the presence or absence of P-gp inhibitor (PSC833). In brief, cells were pre-incubated with the incubation medium in the presence or absence of 2 μM PSC833 for 30 min at 37°C. The apical or basal side was replaced with the incubation medium containing 2 μM PSC833 and sildenafil or tadalafil (5 μM each) after pre-incubation. An 80- μL (apical side) or a 150- μL (basal side) aliquot of the medium was collected after 30 min from the compartment opposite to that in which sildenafil or tadalafil was added. To compensate for the volume sampled, an 80- μL (apical side) or a 150- μL (basal side) aliquot of incubation medium was added immediately after each sampling. The concentrations of sildenafil and tadalafil in each sample were determined by LC-MS/MS^{8,12)} and HPLC methods,⁷⁾ respectively.

HPLC Analysis Concentrations of tadalafil were determined by a reversed-phase HPLC method. The total aliquot of the sample was mixed with three times the amount of methanol to remove proteins and centrifuged at 2000 $\times g$ for 10 min at 4°C, and 50 μL of its supernatant was injected into the HPLC system. The mobile phase was 0.1% trifluoroacetic acid (TFA) and acetonitrile (60:40, v/v). The flow rate was 1.0 mL/min, and column temperature was 40°C. The HPLC system consisted of a Shimadzu LC-10AT_{VP} (Kyoto, Japan), a COSMOSIL[®]C18 reversed-phase column (4.6 $\times$ 150 mm), and a model RF-20A fluorescence detector (Shimadzu). The detection wavelengths were 275 nm (λ_{ex}) and 335 nm (λ_{em}).⁷⁾ The limit of quantification for tadalafil was 1 nM.

LC-MS/MS Analysis Concentrations of sildenafil were determined by the LC-MS/MS method. The total aliquot of the sample was mixed with three times the amount of methanol containing diazepam as an internal standard¹²⁾ and centrifuged at 2000 $\times g$ for 10 min at 4°C, and 20 μL of its supernatant was injected into the LC-MS/MS system. The mobile phase was 10 mM ammonium formate (with 0.1% formic acid) and acetonitrile (20:80, v/v). The flow rate was 100 $\mu\text{L}/\text{min}$, and the column temperature was 40°C. LC-MS/MS analysis was carried out in a Thermo Fisher Accela LC system with a COSMOSIL[®]PACKED column of 2.5C18-MS-II (2.0 $\times$ 50 mm) (Thermo Fisher Scientific) coupled to an LTQ-Orbitrap XL ETD system (Thermo Fisher Scientific). The cone voltage was 35 V and collision energy was 30 eV. Mass spectra were recorded by electrospray ionization in the positive mode. The detector was operated in selected reaction monitoring (SRM) mode using the transitions of sildenafil at m/z 475.4 $\rightarrow$ 283.0⁸⁾ and diazepam at m/z 285.0 $\rightarrow$ 193.0,¹²⁾ respectively. The peak areas were calculated using Qualbrowser software (Thermo Fisher Scientific). The limit of quantification for sildenafil was 10 nM.

Kinetic Analysis The kinetic parameters for the transport of sildenafil and tadalafil were estimated using a modified Michaelis–Menten equation (Eq. 1):

$$V = \frac{V_{\text{max}} \cdot S}{K_{\text{m}} + S} + K \cdot S \quad (1)$$

where V is the velocity of the reaction, S is the substrate concentration, V_{max} is the maximum transport rate, K_{m} is the Michaelis–Menten constant, respectively, and K represents the

Fig. 1. Transcellular Transport of (a) Sildenafil and (b) Tadalafil in the LLC-PK₁ Cell Monolayers

The concentrations of sildenafil and tadalafil were fixed at 5 μ M. Open and closed symbols represent basal to apical and apical to basal transport, respectively. Each point represents the mean $\pm$ S.E. of 5–6 samples.

Fig. 2. Transcellular Transport of (a) Sildenafil and (b) Tadalafil in the LLC-GA5-COL150 Cell Monolayers

The concentrations of sildenafil and tadalafil were fixed at 5 μ M. Open and closed symbols represent basal to apical and apical to basal transport, respectively. Each point represents the mean $\pm$ S.E. of 5–6 samples.

coefficient of non-specific diffusion.⁸⁾

Statistical Analysis The significance of the differences between the two groups was evaluated using Student's *t*-test if the variance of the group was similar. If this was not the case, the Mann–Whitney *U*-test was applied. $p < 0.05$ was considered significant. Data are expressed as the mean $\pm$ standard error (S.E.)

RESULTS

To elucidate whether tadalafil is a substrate for P-gp, the transcellular transport of sildenafil and tadalafil (5 μ M each) was conducted using LLC-PK₁ and LLC-GA5-COL150 cell monolayers (Figs. 1, 2). Because it has been reported that sildenafil is a substrate for P-gp,⁸⁾ it was used as a positive control in the present study. In LLC-PK₁ cells, the transport of sildenafil and tadalafil in both directions was increased periodically over 120 min (Fig. 1). Similarly, the transport of the two drugs in LLC-GA5-COL150 cells was increased periodically over 120 min, and the direction selectivity of

Table 1. Efflux Ratio of Sildenafil and Tadalafil in LLC-PK₁ and P-gp-Expressing LLC-GA5-COL150 Cells

	Sildenafil	Tadalafil
LLC-PK ₁	0.711	1.23
LLC-GA5-COL150	1.52	10.4

Efflux ratio represents the ratio of B to A transport to that in the opposite direction. ($n=5-6$).

tadalafil transport was clearer than that of sildenafil (Fig. 2). The efflux ratio of the B to A transport of sildenafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (1.52) was significantly higher than that in LLC-PK₁ cells (0.711) (Table 1). The efflux ratio of the B to A transport of tadalafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (10.4) was significantly higher than that in LLC-PK₁ cells (1.23) (Table 1).

To evaluate the transport properties of tadalafil, we conducted kinetic analysis of the transport of sildenafil and tadalafil using P-gp-expressing LLC-GA5-COL150

Fig. 3. Concentration Dependence of the Basal to Apical Transport of (a) Sildenafil and (b) Tadalafil across LLC-GA5-COL150 Cell Monolayers

The transport rate was measured after 30-min incubation. The values were calculated by subtracting the value of mannitol. The Eadie-Hofstee plot (velocity vs. velocity/substrate concentration) of saturable drug transport is shown in the inset. Each point represents the mean $\pm$ S.E. of 6–13 samples.

cell monolayers (Fig. 3). The B to A transport of sildenafil and tadalafil was slight, but clearly saturable over the tested concentration range, suggesting the involvement of P-gp-mediated efflux (Fig. 3). The kinetic parameters of sildenafil transport with the $V_{\max}$ and K_m values calculated from the modified Michaelis-Menten equation were 101 ± 64 pmol/min/cm² and 112 ± 47 μ M, respectively, and the K value was negligible (less than 0.01 μ L/min/cm²) (Table 2). The kinetic parameters of tadalafil transport with the $V_{\max}$, K_m , and K values were 13.6 ± 4.8 pmol/min/cm², 22.7 ± 9.3 μ M, and 0.062 ± 0.035 μ L/min/cm², respectively. These results suggest that tadalafil had a higher affinity for P-gp as compared with sildenafil (Table 2). The lower K_m value of tadalafil transport may support the finding that the direction selectivity of tadalafil transport was clearer than that of sildenafil at the concentration of 5 μ M each (Fig. 2). Eadie-Hofstee plot analysis suggested that no efflux transporters other than P-gp were involved (Fig. 3).

It was reported that P-gp-mediated efflux observed during absorptive and secretory transport *via* polar cell membranes, such as Caco-2 cells, was asymmetrical.¹³⁾ Therefore, we could not conclude that tadalafil is a substrate for P-gp based solely on the efflux ratio (Table 2) or direction selectivity of the transcellular transport (Fig. 2). We then conducted the transport experiment using PSC833, a specific inhibitor of P-gp, in LLC-GA5-COL150 cells (Fig. 4). In the presence of PSC833, the B to A transport of sildenafil and tadalafil were decreased by 19.0 and 28.6%, respectively, and the A to B transport was 3.33- and 6.59-fold greater than in its absence, respectively. In the B to A direction, the effect of PSC833 on the transport of the two drugs was not marked, but it was noticeable in the A to B direction (Fig. 4). These results reveal that tadalafil is a substrate for P-gp as well as sildenafil.

DISCUSSION

The objective of the present study was to elucidate whether tadalafil is a substrate for P-gp. Because sildenafil is a known substrate for P-gp, transcellular transport properties of sildenafil and tadalafil were compared using renal epithelial LLC-PK₁ and P-gp-expressing LLC-GA5-COL150 cell monolayers

Table 2. Kinetic Parameters for the Transport of Sildenafil and Tadalafil in LLC-GA5-COL150 Cell Monolayers

	Sildenafil	Tadalafil
$V_{\max}$ (pmol/min/cm ²)	101 ± 64	13.6 ± 4.8
K_m (μ M)	112 ± 47	22.7 ± 9.3
K (μ L/min/cm ²)	<0.01	0.062 ± 0.035

The kinetic parameters of sildenafil and tadalafil were calculated from the modified Michaelis-Menten equation. Values are expressed as the mean $\pm$ S.E. ($n=6-13$).

(Figs. 1, 2). The efflux ratio of the B to A transport of sildenafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (1.52) was significantly higher than that in LLC-PK₁ cells (0.711) (Table 1). The efflux ratio of the B to A transport of tadalafil to the A to B transport after 120-min incubation in LLC-GA5-COL150 cells (10.4) was significantly higher than that in LLC-PK₁ cells (1.23) (Table 1). The kinetic parameters of sildenafil transport with the $V_{\max}$ and K_m values calculated from the modified Michaelis-Menten equation were 101 ± 64 pmol/min/cm² and 112 ± 47 μ M, respectively (Table 2). The kinetic parameters of tadalafil transport with the $V_{\max}$ and K_m values were 13.6 ± 4.8 pmol/min/cm² and 22.7 ± 9.3 μ M, respectively (Table 2). In the presence of PSC833, the B to A transport of tadalafil was decreased by 28.6% in LLC-GA5-COL150 cells, and the A to B transport of tadalafil was 6.59-fold greater than that in its absence (Fig. 4). These results revealed that tadalafil is a substrate for P-gp.

Choi and Song⁸⁾ demonstrated that efflux transporters were involved in the mechanisms for the transcellular transport of sildenafil across Caco-2 cell monolayers. That is, the B to A transport of sildenafil was 8.81-fold greater than the A to B transport, suggesting limited intestinal absorption due to the efflux transporter-mediated secretion of sildenafil. In the presence of P-gp inhibitor (cyclosporin A), the B to A transport of sildenafil was decreased by 64% compared with that in its absence. They stated that the involvement of P-gp may be a factor contributing to the limited intestinal absorption of sildenafil. Moreover, kinetic analysis of sildenafil transport in P-gp-expressing MDCKII-MDR1 cell monolayers was conducted, and the K_m value calculated from the modified

Fig. 4. Inhibitory Effect of PSC833 on the Transport of (a) Sildenafil and (b) Tadalafil in LLC-GA5-COL150 Cell Monolayers

The concentration of PSC833 was $2\mu\text{M}$ and concentration of sildenafil and tadalafil were $5\mu\text{M}$. The transport rate was measured after 30-min incubation. Open and closed bars represent the transport rate in the absence or presence of PSC833. Each point represents the mean $\pm$ S.E. of 5–9 samples. * $p < 0.05$ vs. control.

Michaelis–Menten equation was $50.4\mu\text{M}$.⁸⁾ This parameter is different from that for sildenafil in the present study (Table 2), but this may be due to the difficulty experienced in the sample solubilization or precipitation control of poorly water-soluble drugs. That is, it is difficult to prepare high ($>100\mu\text{M}$) concentration solutions of tadalafil or sildenafil with water containing less than 1% organic solvent. However, because the direction selectivity of tadalafil transport by P-gp was more significant than that of sildenafil in the present study (Fig. 2), P-gp is also likely to be involved in the mechanisms for the intestinal absorption of tadalafil.

Watkins¹⁴⁾ proposed that P-gp may be a cause of increased drug metabolism in the small intestine. That is, P-gp may function to prolong the duration of absorption and this might increase the duration of exposure of the drug to the enzyme within the enterocytes and, hence, the extent of metabolism. The author suggested that the functions of CYP3A4 and P-gp might not be additive but synergistic in limiting the oral bioavailability of substrates.¹⁴⁾ Several studies have suggested that cyclosporine undergoes significant metabolism in the small intestine after oral administration.^{15,16)} For example, Kolars *et al.*¹⁵⁾ demonstrated the intestinal first-pass metabolism of cyclosporine in two patients during the anhepatic phase of liver transplantation. That is, cyclosporine was administered into the distal duodenum using an enteric feeding tube, and blood samples were drawn from the portal vein periodically until the transplanted liver was reperfused. The authors found that cyclosporine metabolites accounted for 25 and 51%, respectively, of the total identifiable cyclosporine in portal blood from the two patients.¹⁵⁾

Lee *et al.*¹⁷⁾ investigated the pharmacokinetics of oral and intravenous tadalafil in experimental hyperlipidemia rats. After oral administration, the C_{max} of tadalafil in hyperlipidemia rats was 5.91-fold higher than that in control rats, and area under the curve (AUC)_{0– ∞} was 11.9-fold higher in hyperlipidemia rats as compared with control rats. The authors also demonstrated that the absolute oral bioavailability of tadalafil in control rats was 20.5%, whereas that in hyperlipidemia rats was elevated to 115%. These findings suggest that tadalafil was originally well-absorbed from the gut lumen, but some function for influencing first-pass metabolism was impaired in hyperlipidemia rats.¹⁷⁾ Considering the fact that tadalafil is

a drug with low hepatic extraction in humans,⁴⁾ the impaired metabolism of tadalafil in the small intestine may be the cause of the increased oral bioavailability in hyperlipidemia rats.

In the present study, we demonstrated that both sildenafil and tadalafil are substrates for P-gp. On the other hand, it was recently reported that tadalafil but not sildenafil had an effect to induce *ABCB1* (P-gp) mRNA expression in LS180 cells.¹⁸⁾ That is, *ABCB1* mRNA expression after 4-d tadalafil exposure was approximately 4-fold higher than that of the control, whereas sildenafil had no effect on *ABCB1* mRNA expression.¹⁸⁾ Therefore, sildenafil and tadalafil may have different drug interaction profiles, although the two drugs are similar in that both are substrates for P-gp. It has also been suggested that dose linearities of the two drugs are different in healthy subjects.^{3,4)} That is, statistical evidence of non-proportionality in C_{max} and AUC of sildenafil was found over the dose range of 25–200 mg.³⁾ On the other hand, the dose-normalized AUC values of tadalafil were almost constant across the 8-fold range (2.5–20 mg).⁴⁾ To understand the clinical contribution of P-gp to the intestinal absorption of the two drugs, further studies are warranted.

In conclusion, we demonstrated for the first time that tadalafil is a substrate for P-gp. The present study may promote understanding of not only the transport properties of tadalafil but also one possible cause of variability in its pharmacokinetics.

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Conflict of Interest The authors declare no conflict of interest.

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