Investigation of the Functional Role of P-Glycoprotein in Limiting the Oral Bioavailability of Lumefantrine

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In the quest to explore the reason for the low and variable bioavailability of lumefantrine, we investigated the possible role of P-glycoprotein (P-gp) in lumefantrine intestinal absorption. An in situ single-pass intestinal perfusion study in rats with the P-gp inhibitor verapamil or quinidine and an ATPase assay with human P-gp membranes indicated that lumefantrine is a substrate of P-gp which limits its intestinal absorption. To confirm these findings, an in vivo pharmacokinetic study was performed in rats. The oral administration of verapamil (10 mg/kg of body weight) along with lumefantrine caused a significant increase in its bioavailability with a concomitant decrease in clearance. The increase in bioavailability of lumefantrine could be due to inhibition of P-gp and/or cytochrome P450 3A4 in the intestine/liver by verapamil. However, in a rat intestinal microsomal stability study, lumefantrine was found to be resistant to oxidative metabolism. Further, an in situ permeation study clearly showed a significant role of P-gp in limiting the oral absorption of lumefantrine. Thus, the increase in lumefantrine bioavailability with verapamil is attributed in part to the P-gp-inhibitory ability of verapamil. In conclusion, lumefantrine is a substrate of P-gp, and active efflux by P-gp across the intestine partly contributed to the low/variable bioavailability of lumefantrine.

MATERIALS AND METHODS

Chemicals and reagents. Propanolol, digoxin, quinidine, sulfasalazine, and verapamil were purchased from Sigma-Aldrich Ltd. (St. Louis, MO). Lumefantrine and halofantrine (internal standard) were generous gifts from IPCA Laboratories Ltd. (Mumbai, India). Phenol red, methanol, and acetonitrile of high-pressure liquid chromatography (HPLC) grade were purchased from Sasico Research Laboratories (SRL) Pvt. Ltd. (Mumbai, India). Ammonium acetate, acetic acid, ammosium, sodium carboxymethyl cellulose (CMC), and polyethylene glycol (PEG) 400 were purchased from Sigma-Aldrich Ltd. (St. Louis, MO). Sodium dihydrogen ortho-phosphate (NaH2PO4·2H2O) and anhydrous disodium hydrogen ortho-phosphate (Na2HPO4) were purchased from Glaxo Laboratories Limited (Mumbai, India). Sodium chloride (NaCl) was purchased from Ranbaxy Laboratories Limited (Punjab, India). A human P-gp ATPase assay kit was purchased from BD Gentest (Woburn, MA). Ethanol was purchased from Merck (Germany). Milli-Q pure water was obtained from a Millipore Elix water purification system purchased from Millipore India Pvt. Ltd. (New Delhi, India). All other chemicals were of analytical reagent grade.

Animals and legal prerequisite. Young, adult male Sprague-Dawley (SD) rats weighing 220 ± 25 g were procured from the National Laboratory Animal Center, CSIR-CDRI (Lucknow, India), and housed in well-ventilated cages at room temperature (24 ± 2°C) and 40 to 60% relative humidity.
humidity while on a regular 12-h light and 12-h dark cycle. The animals were acclimatized for a minimum period of 3 days prior to the experiment. Approval from the institutional animal ethics committee (IAEC) of CDRI was sought, and the study protocols were approved before the commencement of the studies.

**In situ SPIP studies.** Preliminary experiments revealed that no considerable adsorption of the digoxin, lumefantrine, or propranolol took place on the tubing and syringe used in the single-pass intestinal perfusion (SPIP) experimental setup. Single-pass intestinal perfusion studies in rats were performed using established methods adapted from the literature (18–21). Briefly, male SD rats were fasted overnight for 12 to 16 h with free access to water, anesthetized using an intraperitoneal injection of urethane (1.5 g/kg of body weight), and placed on a heated pad to keep normal body temperature. Upon verification of the loss of pain reflex, a midline longitudinal abdominal incision was made, and the lumen of the jejunum (10 cm) was flushed with 10 ml of saline warmed to 37°C. The proximal end of the lumen was catheterized with an inlet polypropylene tube, which was connected to a perfusion pump (Perist; pump; Atto, Tokyo, Japan). The distal end of the jejunum was also catheterized with an outlet polypropylene tube to collect intestinal effluent. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. The entire excised area was covered with an absorbable cotton pad soaked in normal saline. Perfusion buffer containing digoxin (20 μM; digoxin is a well-known P-gp substrate), propranolol, and phenol red (20 μM) was infused, with or without the presence of the well-known P-gp inhibitor verapamil (200 μM) or quinidine (200 μM), at a flow rate of 0.2 ml/min. Phenol red was used as a nonabsorbable marker for measuring water flux and to correct for changes in the water flux across the incised intestinal segment. Propranolol was used as a high-permeation marker. After allowing 30 min to reach steady-state outlet concentrations, outlet perfusate samples were collected every 15 min for a 120-min perfusion period. At the end, the length of the segment was measured without stretching, and finally, the animal was euthanatized with an overdose of ether anesthesia. Samples were stored at −20°C until analysis. A similar procedure was followed for ileal permeation of digoxin in the ileum region for validation of the suitability of the in situ permeation model for the P-gp substrate study.

Upon validation of the in situ permeation model using the well-known P-gp substrate digoxin, a similar procedure was followed for lumefantrine (20 μM), along with propranolol and phenol red (20 μM), for jejunal and ileal permeability determinations with or without verapamil or quinidine. Since there is a potential for verapamil to inhibit other efflux transporters, such as multidrug resistance-associated protein 2 (MRP2) and breast cancer resistance protein (BCRP) in the intestine, we evaluated the effect of verapamil (200 μM) on the jejunal perfusion of sulfasalazine (100 μM), a well-known substrate of MRP2 and BCRP, using a similar procedure.

**HPLC analysis of SPIP study samples.** The samples used for analysis of digoxin permeation were analyzed by using HPLC with photodiode array (PDA) detection at 220 nm after separation through a Waters Symmetry Shield C18 column (50 by 4.6 mm, 5.0 μm) with a mobile phase consisting of acetonitrile–methanol (50:50, vol/vol) and 0.01 M ammonium acetate (pH 5.0) in a ratio of 50:50 (vol/vol) and at a flow rate of 0.5 ml/min. The samples used for analysis of sulfasalazine permeation were analyzed by using HPLC with PDA detection at 350 nm after separation through a Zorbax SB100 C18 column (100 by 4.6 mm, 5.0 μm) with a mobile phase consisting of acetonitrile–methanol (50:50, vol/vol) and 0.01 M potassium dihydrogen phosphate (pH 5.0) in a ratio of 40:60 (vol/vol) and at a flow rate of 0.8 ml/min. The samples used for analysis of lumefantrine permeation were analyzed by using HPLC with PDA detection at 300, 220, and 420 nm, respectively, for lumefantrine, propranolol, and phenol red, after separation through a Waters Symmetry Shield C18 column (50 by 4.6 mm, 5.0 μm) with a mobile phase consisting of (i) methanol and (ii) 0.01 M ammonium acetate (pH 5.0) in gradient mode and at a flow rate of 1.5 ml/min in a ratio of 52:48 (vol/vol) for 3 min and then 95:5 (vol/vol) up to 9 min.

**P-gp ATPase assay.** A human P-gp ATPase assay kit was purchased from BD Gentest (Woburn, MA), and drug-stimulated P-gp ATPase activity was estimated by measuring the inorganic phosphate released from ATP according to the manufacturer’s protocol. Briefly, 20 μg of human P-gp membrane (20 μl of 1 mg/ml) was preincubated at 37°C for 5 min in a 40-μl reaction mixture with each test compound in the absence or presence of 300 μM sodium orthovanadate in 96-well plates. The reaction was initiated by the addition of 20 μl of 12 mM Mg ATP solution and was terminated 20 min later by the addition of 30 μl of stop solution (10% sodium dodecyl sulfate solution). Two hundred microliters of detection reagent (8% ascorbic acid, 0.8% ammonium molybdate, 3 mM zinc acetate) was added, and the mixture was incubated at 37°C for 20 min. The inorganic phosphate complex was detected by its absorbance at 800 nm and was quantitated by comparing the absorbance with that of a phosphate standard. The validity of the assay was assessed by using verapamil and propranolol as positive and negative controls, respectively. The vanadate-sensitive ATP hydrolysis was determined by subtracting the value obtained with the vanadate-coincubated membrane fraction from that obtained with the vanadate-free membrane fraction.

**Permeation data analysis.** The single-pass intestinal perfusion experiment is based on reaching steady state with respect to the diffusion of the compound across the intestine. Steady state is confirmed by plotting the ratio of the outlet to the inlet concentrations (corrected for water transport) versus time. The outlet concentrations were corrected by multiplying the outlet concentration by [phenol red]_in/[phenol red]_out, where [phenol red]_in is the phenol red inlet concentration and [phenol red]_out is the phenol red outlet concentration. Calculations of permeation across the rat jejunum and ileum were performed from intestinal perfusate samples collected over 30 to 120 min (steady state). The effective permeability coefficient (P eff) was calculated using the following equation:

\[
P_{\text{eff}} = \frac{-Q_{\text{in}} \ln \left( \frac{C_{\text{out}}}{C_{\text{in}}} \right)}{2 \pi r l}
\]

where \( C_{\text{out}} \) is the corrected concentration of the permeant compound in the exiting perfusate; \( C_{\text{in}} \) is the concentration of permeant compound in the entering perfusate; \( Q_{\text{in}} \) is the flow rate of the entering perfusate (0.2 ml/min); \( r \) is the inner radius of the intestine, which is 0.18 cm (19); and \( l \) is the length of the intestine. All the results are shown as the mean ± standard deviation.

**Effect of coadministered verapamil on plasma pharmacokinetics of lumefantrine in rats.** We examined the effects of pretreatment with verapamil (a P-gp inhibitor) at a dose of 10 mg/kg on the oral plasma pharmacokinetics of lumefantrine (10 mg/kg) in healthy male SD rats. The dose of verapamil was selected on the basis of data previously reported in the literature (19, 22). The oral and i.v. doses of lumefantrine were selected on the basis of the sensitivity of the method and previous understanding about the pharmacokinetics of lumefantrine (9, 23). The vanadate formulation (solution) was prepared by dissolving it in distilled water, while the lumefantrine formulation (suspension) was prepared in 0.25% sodium CMC. Rats were randomized to receive the following different treatments (\( n = 5 \) per group): lumefantrine at 10 mg/kg by oral gavage without verapamil and lumefantrine at 10 mg/kg in combination with verapamil at 10 mg/kg by oral gavage. The P-gp inhibitor verapamil was administered 10 min prior to lumefantrine dosing. Blood samples were collected from the retroorbital plexus of the rats while they were under light ether anesthesia at times of 0.5, 2, 5, 8, 24, 30, 54, 72, and 120 h postdosing and placed into heparinized microcentrifuge tubes. The inv tro formulation (solution) of lumefantrine was prepared in dimethyl formamide–PEG 400–ethanol-water (2:5:2.5:1:1.5, parts) and finally filtered through a 0.22-μm-pore-size filter before administration. The solution of lumefantrine was administered to male SD rats (\( n = 4 \)) via a lateral tail vein as a bolus dose of 0.5 mg/kg. Blood samples were collected at times of 0.08, 0.25, 0.5, 2, 5, 8, 24, 30, 54, 72, and 120 h postdosing and placed into heparinized microcentrifuge tubes. Plasma was harvested by
centrifuging the blood at 2,000 × g for 5 min and stored frozen at −70°C until analysis. Sample preparation and liquid chromatography-tandem mass spectrometry analyses of pharmacokinetic study samples were done by using the validated method published from our lab (9, 24).

**Pharmacokinetic analysis.** Plasma data were subjected to noncompartamental pharmacokinetics analysis using the WinNonlin program (version 5.1; Pharsight Corporation, Mountain View, CA). The observed compartmental pharmacokinetics analysis using the WinNonlin program and absence of verapamil or quinidine. The coadministration of verapamil or quinidine with digoxin resulted in significant (P < 0.01; Fig. 1) increases in its jejunal (2.2-fold with quinidine, 2.3-fold with verapamil) and ileal (4.3-fold with quinidine, 4.4-fold with verapamil) permeation. The P of propranolol in the absence and presence of verapamil or quinidine was found to be statistically insignificant (P > 0.01; Fig. 1). The coadministration of verapamil or quinidine with lumefantrine resulted in significant (P < 0.01; Fig. 1) increases in its jejunal (2.2-fold with quinidine, 2.3-fold with verapamil) and ileal (4.3-fold with quinidine, 4.4-fold with verapamil) permeation. The P of propranolol in the absence and presence of verapamil or quinidine was found to be statistically insignificant (P > 0.01; Fig. 1).

**Human P-gp ATPase assay.** The affinity of lumefantrine, verapamil (positive control), and propranolol (negative control) for human P-gp was assessed by an ATPase activity assay. Figure 2A shows the vanadate-sensitive ATPase activities of these compounds. Verapamil at 20 μM and lumefantrine at 40 μM showed 7.59- and 7.19-fold increases in basal activity, respectively, compared to that for the drug-free membrane, while the basal fold activity in the presence of the negative control (propranolol) was only 0.94.

**Effect of coadministered verapamil on plasma pharmacokinetics of lumefantrine in rats.** The mean plasma concentration-time profiles of lumefantrine administered orally (10 mg/kg) alone or in combination with verapamil (10 mg/kg) in rats and upon intravenous (0.5 mg/kg) administration are shown in Fig. 2B. As shown in Table 2, the presence of verapamil significantly (P < 0.05) increased the AUC and C of orally administered lumefantrine compared to those for lumefantrine alone. Consequently, the oral bioavailability (F; in percent) of lumefantrine in the presence of verapamil was increased (79.62%) compared to that of lumefantrine alone. The clearance (CL/F) of lumefantrine decreased (37.70%) significantly (P < 0.05) in the presence of verapamil compared to that of lumefantrine alone.

**RESULTS**

**SPIP study.** Table 1 summarizes the P of digoxin in the presence and absence of verapamil or quinidine. The coadministration of verapamil or quinidine with digoxin resulted in significant (P < 0.01; Fig. 1) increases in its jejunal (2.6-fold with quinidine, 5-fold with verapamil) and ileal (2.6-fold with quinidine, 5-fold with verapamil) permeation. The change in the P of sulfasalazine in the absence and presence of verapamil was found to be statistically insignificant (P > 0.01; Fig. 1). The coadministration of verapamil or quinidine with lumefantrine resulted in significant (P < 0.01; Fig. 1) increases in its jejunal (2.2-fold with quinidine, 2.3-fold with verapamil) and ileal (4.3-fold with quinidine, 4.4-fold with verapamil) permeation. The P of propranolol in the absence and presence of verapamil or quinidine was found to be statistically insignificant (P > 0.01; Fig. 1).

**FIG 1** (A) Effect of the P-gp inhibitor verapamil or quinidine on P-gp-mediated efflux and the intestinal permeation of digoxin, lumefantrine, sulfasalazine, and propranolol in the rat jejunum and ileum. Values represent means ± standard deviations (n = 5). *, P < 0.01, in comparison to the P of the control (without P-gp inhibitor).

<table>
<thead>
<tr>
<th>Compound(s) (concn [μM])</th>
<th>P (cm/s [10^-5])</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin (20)</td>
<td>0.14 ± 0.02</td>
<td>0.17 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Digoxin (20) + quinidine (200)</td>
<td>1.61 ± 0.26*</td>
<td>1.79 ± 0.39*</td>
<td></td>
</tr>
<tr>
<td>Digoxin (20) + verapamil (200)</td>
<td>1.51 ± 0.41*</td>
<td>1.47 ± 0.31*</td>
<td></td>
</tr>
<tr>
<td>Lumefantrine (20)</td>
<td>3.56 ± 0.51</td>
<td>1.82 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Lumefantrine (20) + quinidine (200)</td>
<td>7.95 ± 1.49*</td>
<td>7.87 ± 0.88*</td>
<td></td>
</tr>
<tr>
<td>Lumefantrine (20) + verapamil (200)</td>
<td>8.06 ± 1.35*</td>
<td>7.97 ± 0.91*</td>
<td></td>
</tr>
<tr>
<td>Sulfasalazine (100)</td>
<td>1.49 ± 0.21</td>
<td>1.52 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Sulfasalazine (100) + verapamil (200)</td>
<td>6.40 ± 1.17</td>
<td>5.72 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>Propranolol (20)</td>
<td>6.10 ± 1.14</td>
<td>6.31 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>Propranolol (20) + quinidine (200)</td>
<td>6.29 ± 0.93</td>
<td>6.18 ± 1.06</td>
<td></td>
</tr>
<tr>
<td>Propranolol (20) + verapamil (200)</td>
<td>6.40 ± 1.17</td>
<td>5.72 ± 0.76</td>
<td></td>
</tr>
</tbody>
</table>

* Data are means ± standard deviations (n = 5). *: the result is significantly different (P < 0.01) in comparison to that for the control group (without inhibitor).
trine alone. The V/F and T_max of lumefantrine were not altered significantly in the presence of verapamil.

**DISCUSSION**

In recent years, there has been much interest in the potential role of P-gp, which, by its action of pumping drugs out of epithelial cells back into the intestinal lumen, may limit the oral bioavailability of a wide range of drugs. Several drugs have been shown to have low bioavailability due to the P-gp-mediated efflux occurring in the small intestine (25–28). Lumefantrine, the first-line drug for the treatment of uncomplicated malaria (29). Lumefantrine is reported to be metabolized by CYP3A4 into desbutyl-lumefantrine (14). From the literature, it is evident that P-gp and CYP3A4

**TABLE 2** Pharmacokinetic parameters of lumefantrine after oral administration with or without verapamil and lumefantrine administered i.v. as a bolus injection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC_0–t (ng · h/ml)</th>
<th>AUC_0–/H11009 (ng · h/ml)</th>
<th>T_max (h)</th>
<th>C_max (ng/ml)</th>
<th>t_1/2 (h)</th>
<th>V/F (liters/kg)</th>
<th>CL/F (liters/h/kg)</th>
<th>F (%)</th>
<th>k_el (1/h)</th>
<th>k_d (1/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumefantrine control (oral, 10 mg/kg)</td>
<td>14,115.41</td>
<td>2,995.16</td>
<td>15.46</td>
<td>24,080</td>
<td>1.50</td>
<td>1.50</td>
<td>2,005</td>
<td>58.76</td>
<td>0.019</td>
<td>0.006</td>
</tr>
<tr>
<td>Lumefantrine (oral, 10 mg/kg) and verapamil (10 mg/kg)</td>
<td>25,364.15</td>
<td>2,735.57*</td>
<td>14,315.12</td>
<td>28,063.08*</td>
<td>1.50</td>
<td>1.50</td>
<td>2,005</td>
<td>58.76</td>
<td>0.019</td>
<td>0.006</td>
</tr>
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<td>Lumefantrine (oral, 10 mg/kg) and verapamil (10 mg/kg)</td>
<td>14,115.41</td>
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<td>1.50</td>
<td>2,005</td>
<td>58.76</td>
<td>0.019</td>
<td>0.006</td>
</tr>
<tr>
<td>Lumefantrine (i.v., 0.5 mg/kg)</td>
<td>9,189.76</td>
<td>1,372.42</td>
<td>27.12</td>
<td>9,529.47</td>
<td>4.81</td>
<td>4.81</td>
<td>2,40</td>
<td>0.002</td>
<td>0.022</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*a Lumefantrine was administered orally (10 mg/kg) with or without (control) verapamil (10 mg/kg) (n = 5 per group) and as an i.v. bolus injection (0.5 mg/kg) (n = 4 per group). Data are the means ± standard deviations. *, the result is significantly different (P < 0.05) in comparison to that for the lumefantrine control group.
are colocalized and share most of their substrates (5). Further, previous studies have shown that several BCS class II drugs, like carbamazepine, levodopa, glibenclamide, and phenytoin, are P-gp substrates (22).

Based on these pieces of evidence, we hypothesized that lumefantrine could be a P-gp substrate, which may be one of the reasons for its low and variable bioavailability. To test our hypothesis, we carried out in situ single-pass intestinal perfusion (SPIP) and in vivo studies in rats. Further ATPase assays were conducted to test if lumefantrine is a substrate for human P-gp.

In situ perfusion of intestinal segments of rodents (rats or mice) and rabbits is frequently used to study the permeation and absorption kinetics of drugs (19–21, 30, 31). The method is highly accurate for prediction of absorption, if the compound under study is susceptible to chemical or enzymatic degradation in the lumen and/or brush border membrane. ATPase values can be overestimated. Therefore, it is necessary to study the intestinal stability of the compounds under study. Digoxin, sulfasalazine, lumefantrine, and propranolol were found to be stable for up to 4 h in unperfused and perfused buffer.

Prior to investigating the role of P-gp in the intestinal absorption of lumefantrine using the SPIP model, this model was standardized using a well-documented P-gp substrate, digoxin, and known P-gp inhibitors, verapamil and quinidine. As shown in Fig. 1, the coperfusion of verapamil or quinidine along with digoxin resulted in a significant increase in the permeation of digoxin in both the jejunum and ileum. This demonstrates the suitability of the SPIP model for studying the role of P-gp in absorption.

In addition to P-gp, the intestine also expresses other efflux transporters, such as MRP2 and BCRP. Therefore, to prove that verapamil selectively inhibited P-gp in our SPIP experiment, we evaluated the effect of verapamil on sulfasalazine (a well-known substrate of MRP-2 and BCRP) permeation. Verapamil had no significant effect on sulfasalazine permeation, indicating that verapamil selectively inhibits P-gp at the maximum concentration tested of 200 μM in our experiment. These results are well in agreement with those previously reported by Dahan and Amidon (16).

It is well-known that the expression of P-gp increases from the jejunum to the ileum (32). Further, due to the low solubility of BCS class II drugs, the major site of absorption of such drugs shifts from the jejunum to the ileum. Therefore, lumefantrine permeation was determined in both the jejunum and the ileum. The concentration of the lumefantrine used for the SPIP study was selected on the basis of sensitivity determined by the HPLC method and the solubility of lumefantrine, while the concentrations of the inhibitors were based on previous reports in the literature (16, 19, 22, 30). As shown in Fig. 1, the coperfusion of verapamil or quinidine along with lumefantrine resulted in a significant increase in the P_app of lumefantrine in both the jejunum and the ileum. The results of the SPIP study indicate that P-gp limits the intestinal absorption of lumefantrine by effluxing it back into the intestine. Propranolol was used as a passive, highly permeant marker and as an indicator of major changes in mesenteric blood flow (19). As shown in Fig. 1, the difference in the permeation values of propranolol in the absence and presence of verapamil was found to be statistically insignificant (P > 0.01). These findings suggest that the increase in lumefantrine permeation in the presence of verapamil and quinidine is due to the inhibition of P-gp rather than a compromise in membrane integrity.

Further, the affinity of lumefantrine to human P-gp was assessed by the ATPase activity assay using a human P-gp membrane. The validity of the model was checked by taking verapamil as a positive control and propranolol as a negative control. The increased ATPase activity at the tested lumefantrine concentrations (Fig. 2A) indicates that it is a substrate of human P-gp.

To further address the role of P-gp in the in vivo disposition of lumefantrine, we examined the effects of verapamil (P-gp inhibitor) coadministration on its pharmacokinetics. We selected verapamil as the in vivo P-gp inhibitor on the basis of the drug-drug interaction guidance of the U.S. FDA and data reported in the literature (15, 22, 33). The oral administration of verapamil (10 mg/kg) along with lumefantrine caused a significant increase in the bioavailability of lumefantrine with a concomitant decrease in CI/F. The increase in the bioavailability of lumefantrine could be due to the inhibition of P-gp and/or CYP3A in the intestine/liver by verapamil. In a rat intestinal microsomal stability study, lumefantrine was found to be resistant to oxidative metabolism (data not shown). Further, lumefantrine is a drug with low clearance (0.03 liters/h/kg) and has an Fss (fraction of the dose escaping presystemic hepatic first-pass elimination) value of 0.99 (17); therefore, the major reasons for the low bioavailability of lumefantrine are efflux by transporters at the intestinal level and its limited solubility. Therefore, on the basis of in situ permeation results, which clearly showed a significant role of P-gp in limiting the absorption of lumefantrine, and in vivo data, the inhibition of intestinal P-gp by verapamil was considered to be one of the major reasons for the increased oral bioavailability of lumefantrine in rats. However, the oral bioavailability of lumefantrine in the presence of P-gp inhibitors is still low, i.e., 13.3%, which indicates that some other factors, like the poor solubility of lumefantrine in the physiological milieu (13) and the presence of other efflux transporters, limit its absorption. In conclusion, lumefantrine is a substrate of P-gp, and active efflux by P-gp across the intestine partly contributes to the low/variable bioavailability of lumefantrine. Since lumefantrine was found to be a substrate of human P-gp as well, the results obtained in this study can be extrapolated to humans as well.

ACKNOWLEDGMENTS

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