Modulation of P-glycoprotein Transport Activity in the Mouse Blood-Brain Barrier by Rifampin

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ABSTRACT
The objective of the present study was to examine the time course and concentration dependence of modulation of P-glycoprotein (P-gp) activity in the blood-brain barrier (BBB) with consequent influence on substrate uptake into brain tissue. Potential P-gp inducers (rifampin and morphine) were administered subcutaneously to P-gp-competent [mdr1a(+/+)] mice to induce P-gp expression in brain; the impact of rifampin pretreatment on brain penetration of verapamil also was evaluated with an in situ brain perfusion technique. In addition, the effect of single-dose rifampin on P-gp BBB transport activity was assessed with brain perfusion using verapamil and quinidine as model P-gp substrates. Chronic exposure to rifampin or morphine induced P-gp expression in mouse brain to a modest extent. However, single-dose rifampin treatment increased the brain uptake of verapamil and quinidine in mdr1a(+/-) mice in a dose- and concentration-dependent manner, consistent with P-gp inhibition. Maximum inhibition of P-gp-mediated efflux of verapamil by rifampin pretreatment in vivo (150 mg/kg) was ~55%, whereas there was only ~12% inhibition of P-gp-mediated efflux of quinidine at that rifampin dose. Coperfusion of rifampin at a concentration of 500 µM abolished P-gp-mediated efflux of verapamil at the BBB. However, only ~40% inhibition of P-gp-mediated efflux of quinidine was observed with coperfusion of rifampin, even at a 2-fold higher rifampin concentration (1000 µM). The present studies demonstrate that P-gp function at the BBB can be modulated by rifampin in a dose- and concentration-dependent manner. The degree to which rifampin inhibits P-gp-mediated transport is dependent on the substrate molecule.

P-glycoprotein (P-gp), a member of the ATP-binding cassette transporter superfamily, is the product of the multidrug resistance (MDR) genes (Gottesman and Pastan, 1993). In humans, only the MDR1 gene-encoded P-gp is capable of conveying resistance to a large number of compounds, whereas in rodents the drug transport function is shared between mdr1a and mdr1b (Thiebaut et al., 1987; Hsu et al., 1989). In contrast, the MDR2 gene encodes a phospholipid transporter, the involvement of which in drug absorption or disposition is unclear (Smit et al., 1993; Ruetz and Gros, 1994).

The importance of P-gp was first recognized with the occurrence of multidrug resistance during chemotherapy (Juliano and Ling, 1976). Tumor cells are protected against various cytotoxic agents due to overexpression of P-gp; the transporter reduces intracellular concentrations of P-gp substrates such as vinca alkaloids, anthracyclines, and taxol (Leveille-Webster and Arias, 1995). It is becoming increasingly clear that expression of P-gp in normal tissues plays an important role in the disposition and pharmacological activity of a broad range of compounds. P-gp is expressed constitutively in the epithelial cells lining the luminal surface of many organs associated with excretory or barrier functions, i.e., the hepatic bile canaliculus membrane, the renal proximal tubule, and the villus-tip enterocyte in the small intestine. In addition, P-gp is expressed in the endothelial cells that comprise the blood-brain barrier (BBB) and blood-testes barrier (Cordon-Cardo et al., 1989). The expression of P-gp in these tissues associated with drug absorption, distribution to sites of biological activity, and elimination from the body has led to the hypothesis that P-gp evolved as a protective mechanism against a wide range of potentially toxic substances, serving to limit distribution and facilitate elimination of substrates (Schinkel, 1997; Ambudkar et al., 1999).

Since the discovery of the drug efflux activity of P-gp, numerous attempts have been made to inhibit P-gp-mediated drug efflux. Initial investigations used existing compounds, such as calcium channel blockers (e.g., verapamil), immunosuppressive agents (e.g., cyclosporine A), and antiarrhythmic drugs (e.g.,

ABBREVIATIONS: P-gp, P-glycoprotein; MDR, multidrug resistance; BBB, blood-brain barrier; DMSO, dimethyl sulfoxide; CLup, uptake clearance; HPLC, high-performance liquid chromatography; PSC833, valspodar; H33342, 2-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5-bi-1H-benzimidazole trihydrochloride.
Radiolabeled Chemicals (St. Louis, MO). Verapamil hydrochloride and quinidine. However, because of undesirable pharmacological effects or limited in vivo inhibition of transport, more specific and potent "second-generation" P-gp modulators have been developed, such as the acridine carboxamide GP120918 (Hyafil et al., 1993) and a nonimmunosuppressive analog of cyclosporin A, PSC833 (Lemaire et al., 1996).

Several recent studies have shown that P-gp expression can be up-regulated in normal tissues as well as in tumor cells. Morphine increased P-gp content approximately 2-fold in rat brain after a 5-day treatment (Aqualante et al., 2000). The immunosuppressant cyclosporine A has been shown to increase P-gp in both liver and intestine (Prince et al., 1996). Rifampin was able to induce P-gp in both in vivo studies in humans (Greiner et al., 1999) and in vitro in human colon carcinoma cells (Schuett et al., 1996). Dexamethasone rapidly increased P-gp expression more than 4.5- and 2-fold in rat liver and lung, respectively, whereas P-gp expression was decreased 40% in kidney (Demeule et al., 1999).

Based on the broad substrate specificity and tissue distribution of P-gp, modulation of P-gp activity may result in significant alterations in the pharmacokinetics and, potently, the pharmacodynamics of P-gp substrates. For example, the brain/blood distribution ratio of the opioid peptide [n-Pen²⁵,³⁷]-enkephalin was increased ~4-fold, and the EC₅₀ was decreased ~10-fold, in mdr1a(-/-) mice compared with FVB controls (Chen and Pollack, 1998). Similarly, mdr1a(-/-) mice evidenced enhanced brain accumulation and antinociceptive effect of morphine compared with their gene-competent counterparts (Zong and Pollack, 2000). Although not as well studied, induction of P-gp in normal tissue also is likely to be of clinical importance. For example, P-gp induction in response to rifampin administration was implicated in the reduced pharmacodynamic response to morphine in a study of 10 healthy human volunteers (Fromm et al., 1997). Similarly, induction of P-gp has been proposed as a possible mechanism of resistance to antiretroviral agents (Lee et al., 1998).

Although several experiments have shown that modulation of P-gp activity can influence drug disposition and action, the dynamics of P-gp modulation have yet to be addressed, especially with respect to the impact on BBB transport per se. The present studies were designed to evaluate modulation of P-gp transport activity in the murine BBB using an in situ brain perfusion model.

Materials and Methods

Materials. [³H]Verapamil (85 Ci/mmol) and [¹⁴C]Inulin (2.21 mCi/g) were purchased from PerkinElmer Life Sciences (Boston, MA). [³H]Quinidine (20 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [(-)]-Verapamil hydrochloride and quinidine sulfate were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of the highest grade available from the commercial sources.

Animals. Adult CF-1 mice [mdr1a(-/-) and mdr1a(+/-), 30–45 g] were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and maintained in a breeding colony in the School of Pharmacy (The University of North Carolina, Chapel Hill, Chapel Hill, NC). Animals were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle and had free access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. All experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC, 1996).

Induction of P-gp Expression by Morphine and Rifampin in Mice. Morphine (10, 20, and 30 mg/kg s.c. three times/day on days 1, 2, and 3, respectively; saline (10 µg/kg) as a control) or rifampin (200 mg/kg i.p. once daily for 4 days; DMSO (4 µg/kg) as a control) was administered to CF-1 mice [mdr1a(-/-) and mdr1a(+/-), n = 3/group, 35–45 g]. After a 24-h washout, animals were decapitated and brain samples were harvested. Western blot analysis was performed to assess P-gp expression in the brain.

In a separate experiment, CF-1 mice [mdr1a(+/-), n = 4/group, 35–45 g] were pretreated with rifampin (200 mg/kg/day i.p.) or DMSO (4 µg/kg) for 4 days. After a 24-, 48-, or 72-h washout, mice were anesthetized and prepared for brain perfusion as described below to assess P-gp function in the BBB using brain uptake of verapamil as an index of P-gp function. Mice receiving an acute dose of rifampin (200 mg/kg i.p.) 2 h before perfusion also were included in the P-gp functional test.

Inhibition of P-gp in the BBB by Pretreating Mice with a Single Dose of Rifampin. The results of the preceding experiment indicated that any induction of BBB P-gp that might have occurred in response to rifampin pretreatment was masked by concomitant P-gp inhibition. To further evaluate the dynamics of the inhibitory process, mdr1a(+/-) CF-1 mice (30–45 g, n = 4/group) were pretreated with rifampin (50, 75, 100, and 150 mg/kg i.p.). P-gp-deficient animals received a 100 mg/kg i.p. dose of rifampin to control for any nonspecific effects of rifampin on processes other than P-gp-mediated efflux, and DMSO (4 µg/kg) was administered as a vehicle control. At 1 h postdose, mice were anesthetized and prepared for brain perfusion to assess P-gp function. In this experiment, the brain uptake of both verapamil and quinidine was used as independent indices of P-gp activity. A blood sample was obtained at the time of perfusion to determine the circulating concentration of rifampin to develop relationships between transport inhibition and rifampin concentration.

Inhibition of P-gp-Mediated Efflux of Verapamil and Quinidine by Coperfusion with Rifampin. CF-1 mice (30–45 g, n = 4/group) were anesthetized and prepared for brain perfusion. Verapamil and quinidine brain uptake was measured to assess P-gp function during coperfusion with differing concentrations of rifampin (50, 200, and 500 µM for verapamil; 500 and 1000 µM for quinidine).

Inhibition of P-gp-Mediated Efflux of Quinidine by Coperfusion with Verapamil. CF-1 mice (30–45 g, n = 4/group) were anesthetized and prepared for brain perfusion. Quinidine brain uptake was measured to examine P-gp function during coperfusion with various concentrations of verapamil (50, 200, and 1000 µM).

Western Blot Analysis. Freshly isolated brain tissue was processed to obtain membrane homogenate using a procedure modified from the method of Bergwerk et al. (1996). Briefly, brain tissue was rinsed and homogenized with a glass dounce in 4 volumes (w/v) of buffer A (1 mM NaHCO₃ and 50 mM phenylmethylsulfonyl fluoride) at 4°C. The homogenate was diluted to a final volume of 10.6 ml/g by addition of buffer B (buffer A with 1 mM EDTA). Aliquots (1.5 ml) of the homogenate then were extracted with 30 ml of buffer C (0.1 M Na₂CO₃, 50 mM phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, and 1 µg/ml leupeptin) at 4°C for 15 min, followed by centrifugation at 100,000 g for 1 h. The resulting pellet was reconstituted in buffer C. Protein content of the membrane preparations was determined by the method of Lowry et al. (1951). Plasma membrane preparations were resuspended in NuPAGE sample buffer and aliquots (20 µl) of sample (30 µg of protein) were loaded in triplicate onto a 4 to 12% NuPAGE Bis-Tris gel. Plasma membrane preparations from P-gp-overexpressing intestinal cells were used as a positive control. SDS-PAGE electrophoresis was conducted on ice for 2 h at 160 V (constant) under reducing conditions. After electrophoresis, the samples were transferred onto polyvinylidene difluoride membranes for 1 h at 25 V (constant). Nonspecific binding sites were
blocked with 5% nonfat dry milk in TBS-Tween 0.05% after an
overnight transfer. The membranes were incubated with P-gp anti-
body mdr(Ab-1) (rabbit IgG, 1:1500 dilution) (Oncogene Science,
Cambridge, MA) in TBS-Tween for 1 h. The membranes then
were washed with TBS-Tween (3 × 10 min) and incubated with a horse-
radish peroxidase-linked goat anti-rabbit antibody (1:3000 dilution)
in TBS-Tween for 1 h, followed by rinsing with TBS-Tween (3 × 10
min). The membrane was exposed to Amersham ECL detection
agent, and band intensity was determined by densitometric analysis.

**In Situ Mouse Brain Perfusion.** CF-1 mice [mdr1a(+/+)] and
mdr1a(−/−), 30–45 g, n = 4/group were prepared for in situ brain
perfusion according to the method of Dagenais et al. (2000). Briefly,
mice were anesthetized with ketamine/xylazine (140/8 mg/kg i.p.)
and the right common carotid artery was catheterized (polyethylene
tubing, 0.30 mm i.d. × 0.70 mm o.d.) after ligation of the external
branch. The cardiac ventricles were severed immediately before
brain perfusion with Krebs-bicarbonate buffer via a syringe pump
(60 s, 2.5 ml/min, pH 7.4 with 95% O2 and 5% CO2, 37°C) containing
1 μM [l-threo3H]verapamil (0.1 μCi/ml) or 1 μM [3H]quinidine (0.15
μCi/ml). 3H]Inulin (0.3 μCi/ml) was added as a vascular space
marker. The perfusion was terminated by decapitation and the brain
was dissected on ice. The right hemisphere (140 mg) and perfusate
(140 mg) were collected and weighed in tared 8-ml glass scintilla-
tion vials. Brain tissue was digested with 0.7 ml of Solvable
(PerkinElmer Life Sciences) at 50°C overnight. Samples were mixed
with 5 ml of scintillation cocktail (Ultimate Gold; PerkinElmer Life
Sciences). Total radioactivity (3H and 14C) was determined simulta-
nously in a PerkinElmer 1600TR liquid scintillation analyzer.

**Quantitation of Rifampin by HPLC.** All samples were stored
at −20°C before analysis. Rifampin concentrations in serum were
determined by reversed-phase capillary HPLC with UV detection
(Agilent 1100 series). After addition of internal standard (sulindac,
10 μl of a 1 mg/ml solution) to the serum sample (50 μl), acetonitrile
(100 μl) was added to precipitate proteins (centrifugation at 14,000
rpm for 3 min). The supernatant then was evaporated to dryness
under nitrogen and reconstituted in 100 μl of mobile phase [aceto-
nitrile/5 mM ammonium acetate, pH 4.0, 38:62 (v/v)], of which 10
μl of a 1 mg/ml solution was injected onto the HPLC. Chromatographic separation was
achieved on a Zorbax C8 column (1 × 150 mm, particle size 5 μm)
under isocratic conditions (50 μl/min). The absorbance of column
eluent was monitored at 254 nm. Standard curve was linear between
1.6 and 200 μg/ml when 50 μl of serum was extracted.

**Calculation of BBB Transport Parameters.** Parameters re-
lated to the in situ brain perfusion were calculated based on the
method described by Smith (1996). Brain vascular volume (V_{brain},
ml/100 g) was estimated from tissue distribution of l-threo3H]inulin,
which is known to diffuse very slowly across the BBB, according to
the following equation:

\[ V_{brain} = \frac{X^b}{C_{perf}} \]

where \( X^b \) and \( C_{perf} \) represent [3H]inulin in the brain (dpm/100 g) and
perfusate (dpm/ml), respectively. Apparent brain distributional vol-
umes of substrates (V_{brain}, ml/100 g) were calculated as follows:

\[ V_{brain} = \frac{X_{brain}}{C_{perf}} \]

where \( X_{brain} \) is substrate in the brain (dpm/100 g) corrected for
vascular contamination (\( X_{total} - V_{vac} \cdot C_{perf} \)) and \( C_{perf} \) is substrate
concentration in perfusate (dpm/ml). Initial uptake clearance of sub-
strates (CL_{up}, ml/100 g/min) were calculated as follows:

\[ CL_{up} = \frac{X_{brain} \cdot T}{C_{perf}} \]

where \( T \) is the perfusion time (minutes). The P-gp effect on brain
uptake was defined as the ratio of uptake clearance in the absence
versus the presence of P-gp (Dagenais et al., 2001):

\[ \text{BBB P-gp effect} = \frac{CL_{up, mdr1a(-/-)}}{CL_{up, mdr1a(+/+)} \times \text{DMSO}} \]

**Data Analysis.** Data are presented as mean ± S.D. Student’s t
or analysis of variance, where appropriate, were used to deter-
mine the statistical significance of difference between experimen-
tal groups. Statistical significance was defined as \( p < 0.05 \).

**Results**

**P-gp Induction in Mice.** Western blot analysis (Fig. 1)
revealed modest increases in mouse brain P-gp expression by
morphine or rifampin treatment. Densitometric analysis of the
bands showed that P-gp content associated with mor-
phine (A) or rifampin (B) treatment. Lane 1, positive control from
morphine-treated intestinal cell homogenate; lanes 2 to 4, vehicle-
treated mdr1a(+/+); lanes 5 to 7, morphine-treated (A) or rifampin-
treated (B) mdr1a(+/+); lanes 8 to 10, morphine-treated (A) or
rifampin-treated (B) mdr1a(−/−).

![Fig. 1. Representative immunodetection of P-gp in mouse brain after morphine (A) or rifampin (B) treatment. Lane 1, positive control from P-gp-overexpressing intestinal cell homogenate; lanes 2 to 4, vehicle-treated mdr1a(+/+); lanes 5 to 7, morphine-treated (A) or rifampin-treated (B) mdr1a(+/+); lanes 8 to 10, morphine-treated (A) or rifampin-treated (B) mdr1a(−/−).](image-url)
phine pretreatment did not achieve statistical significance, further experiments were conducted only with rifampin.

Functional Evaluation of P-gp after Multiple-Dose Rifampin Pretreatment. The initial brain uptake clearance \((\text{CL}_\text{up})\) of verapamil in mice after pretreatment with rifampin is shown in Fig. 2. Unexpectedly, verapamil \(\text{CL}_\text{up}\) was significantly higher (consistent with inhibition, rather than induction, of P-gp-mediated transport) 24 h after treatment in rifampin-treated mice compared with controls. The effect of rifampin was not entirely diminished until 72 h after the last rifampin dose. Further study with an acute rifampin dose (200 mg/kg i.p.) indicated a significant inhibitory effect of rifampin on P-gp function in the BBB.

Dose-Dependent Inhibition of P-gp by Rifampin. Single-dose rifampin treatment increased both verapamil and quinidine initial brain uptake in \(\text{mdr1a}(+/+)\) mice in a dose-dependent manner (Fig. 3). Rifampin was much less potent in inhibiting of P-gp-mediated quinidine efflux compared with verapamil efflux. Maximum inhibition of P-gp-mediated efflux of verapamil by rifampin was approximately 55% (with 100% inhibition defined as uptake in \(\text{mdr1a}(-/-)\) mice), whereas there was only about 12% inhibition of P-gp-mediated efflux of quinidine at the highest rifampin dose tested. A 100-mg/kg dose of rifampin produced no effect on brain uptake of verapamil or quinidine in \(\text{mdr1a}(-/-)\) mice compared with the DMSO-treated controls \((p > 0.05)\). As was the case for the dose-response profile, the relationship between percentage of inhibition of quinidine efflux and rifampin blood concentration also was shifted rightward compared with that of verapamil efflux (Fig. 4).

Influence of Rifampin Coperfusion on Brain Uptake of Verapamil and Quinidine. Coperfusion of rifampin resulted in a concentration-dependent increase in verapamil uptake clearance in \(\text{mdr1a}(+/+)\) mice (Fig. 5). The apparent \(\text{IC}_{50}\) value for inhibition by rifampin was about 220 \(\mu\text{M}\). Rifampin at a concentration of 500 \(\mu\text{M}\) was able to inhibit almost completely P-gp-mediated efflux of verapamil in the BBB [verapamil \(\text{CL}_\text{up}\) of 120 \(\pm\) 41 ml/100 g/min in rifampin-treated \(\text{mdr1a}(+/+)\) mice versus 136 \(\pm\) 6 ml/100 g/min in DMSO-treated \(\text{mdr1a}(-/-)\) mice, \(p > 0.05\)].

Similar to the results obtained from the single-dose rifampin treatment experiment, coperfusion of rifampin was much less potent in inhibiting of P-gp-mediated quinidine efflux compared with verapamil efflux. The degree of inhibition of P-gp-mediated efflux of quinidine by coperfusion of rifampin was approximately 30 and 40%, respectively (Fig. 5).

Influence of Verapamil Coperfusion on Brain Uptake of Quinidine. Coperfusion of verapamil was able to inhibit P-gp-mediated quinidine efflux in the BBB (Fig. 6). However, the degree of inhibition was not significantly dif-
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Figure 1: Graph showing the effect of verapamil on P-gp-mediated efflux of quinidine and verapamil.

Figure 2: Graph showing the effect of rifampin on P-gp-mediated efflux of quinidine and verapamil.

Discussion

Due to the central role of P-gp in the absorption, distribution, and elimination of xenobiotics, an understanding of the dynamics of P-gp modulation is of importance from both a clinical and toxicological point of view. The present study was designed to investigate the influence of modulation of P-gp activity on brain transport of compounds.

Rifampin was selected to induce P-gp in mouse brain because it is a potent inducer of P-gp both in human duodenal biopsies (~3.5-fold) (Greiner et al., 1999) and in human colon carcinoma cell lines (Schuetz et al., 1996). Morphine has been shown to increase P-gp content about 2-fold in rat brain after a 5-day treatment (Aquile et al., 2000). However, in the present studies both agents induced P-gp expression in mouse brain to only a modest extent (~40 and ~50% increase with morphine and rifampin treatment, respectively; the changes associated with morphine treatment did not achieve statistical significance). This discrepancy may be due to species differences and/or tissue specificities in xenobiotic-mediated induction of P-gp expression in the brain. Such differences are not unusual. For instance, rifampin is an efficacious inducer of cytochrome P450 3A (CYP3A) in humans but not in rodents (LeCuyse, 2001). Similarly, dexamethasone increased P-gp expression more than 4.5- and 2-fold in rat liver and lung, respectively, whereas it decreased P-gp expression 40% in kidney (Demeule et al., 1999). Further studies are required to identify more effective inducers of brain P-gp to study the dynamics of P-gp induction in the BBB.

The results of functional tests after P-gp induction by rifampin were unanticipated. There was a significant increase in verapamil brain uptake, rather than the anticipated decrease in verapamil CLup, after pretreatment with rifampin (Fig. 2). This observation suggests that inhibition of P-gp by rifampin masks whatever increase in P-gp-mediated transport might occur secondary to rifampin pretreatment. It is possible that rifampin binds tightly to the brain capillaries due to its high lipophilicity, thus evidencing a prolonged...
residence time in the BBB or the brain parenchyma. Several early studies have shown that rifampin can inhibit P-gp activity in vitro (Fardel et al., 1995; Furusawa et al., 1997). Functional tests with an acute dose of rifampin indeed demonstrated a significant inhibitory effect on BBB P-gp (Fig. 2). Therefore, induced P-gp activity by multiple doses of rifampin might actually be masked by an inhibitory effect of rifampin itself.

Further assessment of the dynamics of P-gp inhibition by acute rifampin was conducted using verapamil and quinidine as model compounds because of their moderate to high P-gp effect in situ (Dagenais et al., 2001). These experiments revealed a dose-dependent inhibition of P-gp-mediated efflux, although the degree of rifampin-associated inhibition differed between the two substrates (Fig. 3). The maximum inhibition of P-gp-mediated verapamil efflux was ~55% at a rifampin dose of 150 mg/kg, whereas there was only a maximum inhibition of ~12% for P-gp-mediated quinidine efflux. These results are consistent with the fact that quinidine has a higher P-gp effect than verapamil at the murine BBB. The brain uptake of both verapamil and quinidine was unaffected by a 100 mg/kg i.p. dose of rifampin in mdr1a(−/−) mice compared with the controls, suggesting that transporters other than mdr1a isoform encoded P-gp were not involved.

 Pretreatment with rifampin was only able to partially abolish P-gp activity in the BBB in situ. This incomplete inhibition may be due to an inability to achieve sufficiently high rifampin concentrations in the BBB after systemic administration because of dose-limiting toxicity. Alternatively, rifampin may not be able to block completely P-gp activity in the BBB. The latter hypothesis was not supported because rifampin was able to inhibit almost completely P-gp activity in the BBB during coperfusion with verapamil (Fig. 5). Verapamil CL_{up} in mdr1a(+/-) mice coperfused with 500 µM rifampin was not statistically different from that in mdr1a gene-deficient mice (p > 0.05). More importantly, the degree of P-gp inhibition by pretreatment with rifampin was in good agreement with the results from the coperfusion study. When the data are plotted on the same set of axes (Fig. 7), the concentration-dependent change in verapamil efflux transport is nearly identical between the two experiments, suggesting that the method of treatment was not important (i.e., derived metabolites do not contribute to the inhibitory effect) and that the concentration of the inhibitor dictates the degree of inhibition. In addition, total concentrations of rifampin in the cerebral vasculature (i.e., unbound rifampin in the coperfusion study, in which the perfusate was protein-free; bound plus unbound rifampin after in vivo pretreatment) seem to serve as the driving force for P-gp inhibition. In contrast, rifampin seemed to be unable to inhibit completely P-gp-mediated quinidine efflux even at very high concentrations (i.e., 1000 µM in the perfusate; Fig. 5). The maximum inhibition based on the data from both pretreatment and coperfusion of rifampin studies was ~42% (Fig. 8), which was comparable with the ability of verapamil to inhibit quinidine transport (~63% inhibition; Fig. 6). These results were intriguing. As evidenced in the present study, rifampin was able to completely inhibit P-gp-mediated verapamil efflux in the BBB. In addition, quinidine has been shown to be able to restore verapamil brain uptake in mdr1a(+/-) mice to the level of mdr1a(−/−) mice (C. Dagenais, personal communication). However, both rifampin and verapamil were only able to partially block P-gp-mediated quinidine efflux in the BBB. It has been suggested that there are multiple binding sites on the P-gp (Shapiro et al., 1999; Martin et al., 2000). One possible explanation for the current observation is that verapamil and rifampin may bind to the same site(s) on P-gp, whereas quinidine may interact with other site(s) in addition to the verapamil/rifampin site. This hypothesis was further supported by observations reported by Wang et al.

Fig. 6. Effect of coperfusion with various concentrations of verapamil (VER; 0, 50, 200, 1000 µM) on brain uptake of quinidine (1 µM) in mdr1a(+/-) mice. Data are presented as mean ± S.D. (n = 4/poin). *, p < 0.05 versus mdr1a(−/−) controls.

Fig. 7. Concentration-dependent inhibition of P-gp-mediated verapamil efflux by rifampin. Line indicates the fit of a simple inhibitory model (γ = 1) to the coperfusion data (open triangles) shown in Fig. 5; closed circles indicate data from rifampin pretreatment experiment (Fig. 4). Kinetic parameters associated with the model are I_{max} (120 ± 21.7%) and IC_{50} (220 ± 94.9 µM).
They have shown that the P-gp substrate H33342 interact with quinidine in a noncompetitive manner, whereas it interacts with verapamil in a mixed mode of inhibition.

In conclusion, the present studies have shown that P-gp function in the BBB can be modulated by rifampin in a dose- and concentration-dependent manner. The results also suggest that perturbations in the disposition of P-gp substrates in the brain may be predicted based on the extent of P-gp modulation (e.g., inhibition or induction).

References


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