Transplacental Pharmacokinetics of Glyburide, Rhodamine 123, and BODIPY FL Prazosin: Effect of Drug Efflux Transporters and Lipid Solubility

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ABSTRACT

Breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) are the most abundantly expressed ATP-binding cassette (ABC) drug transporters in the placenta. They recognize a large, partly overlapping spectrum of chemically unrelated compounds and affect their transplacental passage. In this study we investigate the effect of Bcrp and P-gp on the transplacental pharmacokinetics of their specific and common substrates employing the technique of dually perfused rat placenta. We show that the clearance of rhodamine 123 (P-gp substrate), glyburide (BCRP substrate) and BODIPY FL prazosin (P-gp and BCRP substrate) in fetal-to-maternal direction is 11, 11.2 and 4 times higher, respectively, than that in the maternal-to-fetal direction. In addition, all of these substances were found to be transported from the fetal compartment even against concentration gradient. We thus demonstrate the ability of placental ABC transporters to hinder maternal-to-fetal and accelerate fetal-to-maternal transport in a concentration-dependent manner. However, by means of pharmacokinetic modeling we describe the inverse correlation between lipid solubility of a molecule and its active transport by placental ABC efflux transporters. Therefore, in the case of highly lipophilic substrates, such as BODIPY FL prazosin in this study, the efficacy of efflux transporters to pump the molecule back to the maternal circulation is markedly limited.

The need to medicate women for various reasons, even during pregnancy, is often inevitable. A recent multicenter study monitoring pregnancies from 1996 to 2000 in the United States revealed that a drug other than a vitamin or mineral supplement was prescribed for 64% of all pregnant women during 270 days before delivery (Andrade et al., 2004). Moreover, 5 to 10% of them received Food and Drug Administration category D or X drugs, classified as potential teratogens (Andrade et al., 2006). These findings emphasize that it is important to understand the pharmacokinetics of the transport of these medications across the placental barrier and to assess their possible risk for the developing fetus.

Drug efflux transporters of the ATP-binding cassette (ABC) transporter family were originally investigated in association with the phenomenon of multidrug resistance in cancer therapy (Kavallaris, 1997; van der Kolk et al., 2002; Pérez-Tomás, 2006) because they are capable of actively pumping their substrates out of cells even against a concentration gradient. Later on, some of these membrane-embedded proteins were also localized in “normal” tissues, such as the liver, kidney, intestine, brain, or placenta, affecting body disposition of many xenobiotic compounds (Schinkel and Jonker, 2003; Leslie et al., 2005). In the placenta, the best described and most important drug efflux transporters seem to be P-glycoprotein (P-gp) (Ceckova-Novotna et al., 2006) and breast cancer resistance protein (BCRP) (Mao, 2008). Their placental expression in humans and in some experimental animals has been found to be much higher than in most other tissues (Bremer et al., 1992; Doyle et al., 1998; Maliepaard et al., 2001; Leazer and Klaassen, 2003; Wang et al., 2006). Expression, localization, and functional activity of P-gp and BCRP in the human and rat placenta has been described (Bremer et al., 1992; Doyle et al., 1998; Maliepaard et al., 2001; Pávek et al., 2001; Staud et al., 2006). In the human placenta, significantly higher expression was found for BCRP compared with P-gp (Ceckova et al., 2006). Moreover, we have revealed that the placental expres-
sion and transport activity of P-gp and Bcrp changes during pregnancy in the rat (Novotna et al., 2004; Cygalova et al., 2008). Functional studies indicate that P-gp and BCRP transport a large variety of molecules, ranging from endoge-
nous substrates to chemotherapeutic agents and environ-
mental toxins (Schinkel and Jonker, 2003; Mao and Unad-
kat, 2005; Staud and Pavek, 2005). Considerable overlap in
substrate recognition and in tissue distribution between
BCRP and P-gp presumes their shared effect in placental
detoxication processes.

In the present study we used the technique of dually per-
fused rat placenta in an open or closed perfusion setup (Staud
et al., 2006) to evaluate the effect of P-gp and Bcrp on trans-
placental pharmacokinetics (PK) of their substrates. Concen-
tration-dependent studies, specific inhibitors and PK model-
ing have been used to assess the efficacy of these proteins to
hinder maternal-to-fetal (mf) and accelerate fetal-to-mater-
nal (fm) transport. BODIPY FL prazosin (BP), a common
substrate of both P-gp and BCRP (Kimchi-Sarfaty et al.,
2002; Horí et al., 2004), was used to test whether the number
of transporters involved in the drug transfer is reflected in its
transplacental pharmacokinetics. Finally, correlations be-
tween lipid solubility of the molecules and their passive dif-
fusion and/or active transport were investigated.

Materials and Methods

Reagents and Chemicals. Glyburide (GLB; 1-[p-[2-(5-chloro-o-
anisamido)-ethyl]phenyl]-sulfonfyl-3-cyclohexylurea), a BCRP
substrate, and [cyclohexyl-2-3H]glyburide ([3H]GLB (50.2 Ci/mmol)
were obtained from Sigma-Aldrich (St. Louis, MO) and from
PerkinElmer Life and Analytical Sciences (Boston, MA), respectively.
BODIPY FL prazosin [BP; boron, [1-(4-amino-6,7-dimethoxy-2-
quinazolinyl)-4-[3-[5-(3,5-dimethyl-2H-zyrro-2-ylidine-kN)methyl-
1H-pyrryl-2-yl-kN]-1-oxopropyl]piperazinato(difluoro-, (T-4)], a com-
mon BCRP and P-gp substrate, was purchased from Invitrogen
(Carlsbad, CA). Rhodamine 123 [Rho123; 2-(6-amino-3-imino-3
hydroxy-2-furan-2(5H)-yl-1-oxopropyl]piperazinato]difluoro-, (T-4), a
common BCRP and P-gp substrate, was obtained from Sigma-Aldrich. Antipyrine (AP; 2,3-dimethyl-1-phenyl-3-
pyrazolin-5-one), a marker of passive diffusion, and [N-methyl-
[14C]antipyrine ([14C]AP) (55 mCi/mmol) were purchased from Sigma-
Aldrich and American Radiolabeled Chemicals (St. Louis, MO,
respectively. Specific BCRP inhibitor fumitremorgin C (FTC; 9
H-(9H-(9H-(45R)))-9a3,4,9a-tetrahydro-1-hydroxy-2,2'-dimethylspiro-
(furan-2/5H)-9Himidazo(1,2-a indole)-3'-5/2'H-dione) and a dual
P-gp and Bcrp inhibitor GF120918 were from Alexia Corporation
(Lausanne, Switzerland) and GlaxoSmithKline (Greenford, UK), re-
spectively. All other compounds were reagent grade.

Animals. Pregnant Wistar rats were purchased from Biotest Ltd. (Konarovic, Czech Republic) and maintained in
12:12 day/night standard conditions with pellets and water ad libitum. Experiments were carried out on day 21 of gestation. Fasted rats were anesthe-
tized with pentobarbital (40 mg/kg; Nembutal; Abbott Laboratories, Abbott Park, IL) administered into the tail vein. All experiments were approved by the Ethical Committee of the Faculty of Pharmacy in Hradec Kralove (Charles University in Prague, Czech Republic) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (1996) and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, France, 1986).

Dual Perfusion of the Rat Placenta. The method of dually per-
fused rat term placenta was used in our study, as described previously (Staud et al., 2006). In brief, one uterine horn was excised and submersed in heated Ringer’s saline. A catheter was inserted
to the uterine artery proximal to the blood vessel supplying a
selected placenta and connected with the peristaltic pump. Krebs’
 perfusion liquid containing 1% dextran was brought from the mater-
nal reservoir at a rate of 1 ml/min. The uterine vein, including the
anastomoses to other fetuses, was ligated behind the perfused pla-
centa and cut so that maternal solution could leave the perfused
placenta. The selected fetus was separated from the neighboring
fetuses by ligatures. The umbilical artery was catheterized by use of
a 24-gauge catheter connected to the fetal reservoir and perfused at
a rate of 0.5 ml/min. The umbilical vein was catheterized in a similar
manner, and the selected fetus was removed. Before the start of each
experiment, the fetal vein effluent was collected into preweighed
glass vial to check for a possible leakage of perfusion solutions from
the placenta. In the case of leakage, the experiment was terminated.
Maternal and fetal perfusion pressures were maintained at levels
close to physiological values and monitored continuously throughout
the perfusion experiments as described previously (Pavek et al.,
2001).

Two types of perfusion systems were used in this study (Staud
et al., 2006):

1. For pharmacokinetic analysis of concentration-dependent
transplacental passage both maternal and fetal sides of the placenta
were perfused in open-circuit systems, without recirculation of the
perfusate. The tested substance was added to the maternal (in mf
studies) or fetal (in fm studies) reservoir immediately after successful
surgery followed by an approximately 5-min stabilization period
before sample collection started (time 0). Fetal effluent was sampled
into preweighed vials at 5-min intervals and analyzed either fluoro-
metrically for Rho123 and BP or radiometrically for [3H]GLB and
[14C]AP.

2. To investigate the potential of Bcrp and P-gp in removing their
substrates from fetal circulation, both maternal and fetal sides of the
placenta were infused with equal concentrations of the tested sub-
stance and after 5-min stabilization period, the fetal perfusate (10
ml) was recirculated for 60 min. Samples (250 μl) were collected
every 10 min from the maternal and fetal reservoirs, and concentra-
tion of the tested substance was measured. This experimental setup
ensures steady substance concentration on the maternal side of the
placenta and enables investigations of fetal-to-maternal concentra-
tion ratio; any net transfer of the drug implies transport against
concentration gradient and provides an evidence of active transport.

To standardize the perfusion experiments, AP with trace amount of
[14C]AP was infused to the maternal or fetal side of the placenta in
concentrations of 0.25 or 100 μM, and transplacental clearances were
calculated.

Effect of Substrate Concentration on Transplacental Clear-
ance in the Presence or Absence of Inhibitors. To investigate the effect of various concentrations of Rho123 and GLB on mf and fm
clearances, Rho123 or GLB with a trace amount of [3H]GLB were
added to the maternal or fetal reservoir, respectively, in the follow-
ing concentrations: 0.05, 0.1, 0.5, 1, 10, 30, or 100 μM for Rho123 and
0.01, 0.2, 1, 10, 100, 500, or 1500 μM for GLB. In the case of BP, only
the concentration of 0.25 μM was examined because of the low
solubility of the compound in water. The inflowing concentration of
the substances was maintained constant during the experiment.
Transplacental clearances of the aforementioned substances were
calculated for every concentration from all measured intervals as
described below.

BCRP-specific inhibitor FTC (2 μM), or P-gp and BCRP common
inhibitor GF120918 (2 μM) were added to both the maternal and
fetal reservoirs at the beginning of the perfusion to study the effect
of BCRP and P-gp on the transplacental movement of the
substances.

Effect of P-gp and Bcrp on Fetal-to-Maternal Equilibrium
of Their Substrates. To examine the effect of Bcrp and P-gp on the
fetomaternal concentration ratio at equilibrium, both maternal and
fetal sides of the placenta were infused with equal concentrations of
the investigated compound. Low and high substrate concentrations
of each substrate were used; these concentrations were taken from sigmoid curves of concentration-dependent studies described above and considered nonsaturating and saturating, respectively. In particular, Rho123 was infused at a concentration of 0.5 or 100 μM; GLB was infused at a concentration of 0.2 or 100 μM. BP, because of its low solubility in water, was infused only at a low concentration of 0.25 μM. FTC (2 μM) or GF120918 (2 μM) were added to both maternal and fetal reservoirs to inhibit the transporters and to demonstrate their effect on fetal-to-maternal equilibrium.

Pharmacokinetic Analysis of Efflux Transport Activity in the Placenta. The clearance concept has been adopted to describe transplacental pharmacokinetics of ABC substrates. Assuming that both passive and active transports are involved in the net transplacental passage of ABC substrates, total transplacental clearance in fm or mf direction (Cl\textsubscript{fm} and Cl\textsubscript{mf}, respectively) is a function of passive diffusion (Cl\textsubscript{pd}, governed by Fick's law) and efflux transporter activity (Cl\textsubscript{efflux}, governed by saturable kinetics). Because efflux transport in the placenta runs in the fetal-to-maternal direction only, Cl\textsubscript{efflux} is added to Cl\textsubscript{pd} in the fm direction and subtracted from Cl\textsubscript{pd} in the mf direction as follows:

\[ Cl_{fm} = Cl_{pd} + Cl_{efflux} \]  
\[ Cl_{mf} = Cl_{pd} - Cl_{efflux} \]

Because Cl\textsubscript{efflux} is a capacity-limited process, it can be expressed in terms of Michaelis-Menten kinetics:

\[ Cl_{efflux} = \frac{V_{max}}{K_m + C_{max}} \]  

where \( V_{max} \) is the maximal velocity of the transport, \( K_m \) is the concentration at which half the maximal velocity is reached, and \( C_{max} \) is substrate concentration in maternal (\( C_{ma} \)) or fetal (\( C_{fa} \)) circulation.

Adding eq. 3 to eqs. 1 and 2 gives the final equations, which were used to fit clearance versus inflow concentration data:

\[ Cl_{fm} = Cl_{pd} + \frac{V_{max}}{K_m + C_{fa}} \]  
\[ Cl_{mf} = Cl_{pd} - \frac{V_{max}}{K_m + C_{ma}} \]

Data were fitted by use of reciprocal weighting and the numerical module of SAAM II (SAAM Institute, Seattle, WA). Total fetal-to-maternal transplacental clearance (Cl\textsubscript{fm}) normalized to placenta weight was calculated according to eq. 6

\[ Cl_{mf} = \frac{C_{fa} \cdot Q_f}{C_{ma} \cdot W_p} \]  

where \( C_f \) is the drug concentration in the umbilical vein effluent, \( Q_f \) is the umbilical flow rate, \( C_{ma} \) is the concentration in the maternal reservoir, and \( W_p \) is the wet weight of the placenta. Total fetal-to-maternal clearance normalized to placenta weight (Cl\textsubscript{fm}) was calculated according to eq. 7.

\[ Cl_{fm} = \frac{(C_{fa} - C_{fc})Q_f}{C_{fa} \cdot W_p} \]

where \( C_{fc} \) is the drug concentration in the fetal reservoir entering the perfused placenta via the umbilical artery.

At very low substrate concentrations, the role of passive diffusion in net transplacental clearance is minimized. Therefore, the ratio between clearances in fetal-to-maternal and maternal-to-fetal direction (Cl\textsubscript{fm}/Cl\textsubscript{mf}) at low substrate concentrations was used in this study as a measure of transporter efficiency.

**Statistical Analysis.** For each group of placental perfusion experiments, the number of animals was \( n \geq 3 \). Student’s \( t \) test or one-way analysis of variance followed by Bonferroni’s test were used where appropriate to assess statistical significance. Differences of \( p < 0.05 \) were considered statistically significant.

**Results**

**Effect of Substrate Inflow Concentrations on Transplacental Clearance in Maternal-to-Fetal and Fetal-to-Maternal Direction.** The maternal or fetal side of the placenta was infused with various concentrations of Rho123 (0.05, 0.1, 0.5, 1, 10, 30, or 100 μM) or GLB (0.01, 0.2, 1, 10, 100, 500, or 1500 μM). In both mf and fm transport studies, increase in substrate concentration resulted in significant change in transplacental clearance; plotting transplacental clearances versus inflowing substrate concentrations revealed sigmoid curves in both mf and fm directions (Figs. 1 and 2) confirming involvement of capacity-limited mechanisms. Fitting experimental data with eqs. 4 and 5 provides description of passive and active components of transplacental passage (Table 1). It is evident that the passive movement across the placenta is comparable in both mf and fm directions for both substrates. Cl\textsubscript{efflux}, however, is a concentration-dependent parameter. At high substrate concentrations, the transporter becomes saturated, Cl\textsubscript{efflux} ap-

![Fig. 1](link)

**Fig. 1.** Transport of rhodamine 123 across the dually perfused rat placenta in the fetal-to-maternal (A) and the maternal-to-fetal (B) direction. Changes of clearance with increasing Rho123 concentration confirm the nonlinearity of the processes and involvement of a saturable mechanism. Experimental values are presented as means ± S.D. of at least three experiments; the line represents the best fit of these data to eqs. 4 (A) and 5 (B).
proaches zero, and eqs. 4 and 5 transform into simple linear processes, i.e., Cl_{lim} = Cl_{pot}.

**Fetal-to-Maternal Versus Maternal-to-Fetal Clearances: Effect of Concentration and Inhibition.** Comparing fm and mf clearances at low substrate concentrations (0.5 μM for Rho123, 0.2 μM for GLB, and 0.25 μM for BP), significantly higher transport of all substances in the fm direction was observed compared with that in the opposite direction (Fig. 3). Addition of GF120918 caused 2.4-, 5.3-, and 1.6-fold decrease in the Cl_{lim}/Cl_{lim} ratio of Rho123, GLB, and BP, respectively (Fig. 3). At high substrate concentrations (100 μM for Rho123 and 500 μM for GLB), mf and fm clearances reached similar values (Figs. 1 and 2), confirming saturation of transporting proteins and limited role of their efflux activity.

In contrast, no statistically significant differences between fm and mf clearances of AP, a model compound of passive diffusion, at either low or high concentrations (0.25 and 100 μM) were found (Fig. 3D). These observations demonstrate solely passive transplacental transfer of AP with no involvement of active transporters and validate the usefulness of our model.

**Effect of P-gp/Bcrp on Fetal-to-Maternal Equilibrium of their Substrates.** To investigate the potential of P-gp and Bcrp to remove their substrates from fetal circulation, Rho123, GLB, or BP were simultaneously infused to both the maternal and fetal side of the placenta at equal concentrations of 0.5 or 100 μM for Rho123, 0.2 or 100 μM for GLB, and 0.25 μM for BP. In this experimental setup, fetal perfusate was recirculated for 60 min. At low drug concentrations of all tested compounds, a steady decrease in the drug amount in the fetal reservoir with stabilization after approximately 40 min of perfusion was observed; this decline was blocked by coinfusion of P-gp and/or BCRP inhibitors GF120918 or FTC (Fig. 4). At high Rho123 and GLB concentrations (100 μM), maternal and fetal concentrations remained unchanged throughout the perfusion period with the fetal-to-maternal concentration ratio staying close to 1.0 (Fig. 5). Through these findings we demonstrate the capacity of P-gp and Bcrp to remove their substrates from the fetal compartment and to maintain a significant concentration gradient between maternal and fetal circulations.

As expected, in the case of AP no decrease in fetal drug concentration was observed at either low (0.25 μM) or high (100 μM) drug concentration with fetal-to-maternal concentration ratio values close to 1.0 (Figs. 4D and 5C).

**Effect of Lipid Solubility on Efflux Transporter Effectiveness in Transplacental Pharmacokinetics.** To investigate the effect of lipid solubility on efflux transporter effectiveness, the obtained PK parameters for both specific and common P-gp and BCRP substrates were used. cLogP (logarithm of the partition coefficient between n-octanol and water) values were calculated by ChemBioOffice 2008 (CambridgeSoft Corp., Cambridge, MA), which exploits the increment system adding contributions of every atom based on its atom type. Data concerning cimetidine were taken from our previous article (Staud et al., 2006).

Cl_{lim}/Cl_{lim} at low substrate concentrations was considered as a parameter illustrating transporter effectiveness (Fig. 3), whereas Cl_{lim} describes passive movement of drugs across the placenta. When plotting these two parameters against cLogP it is evident that a rise in lipid solubility increases passive diffusion and, at the same time, reduces the effect of the efflux transporter (Fig. 6).

**Discussion**

The role of placental ABC drug efflux transporters, especially P-gp and BCRP, in transplacental PK has become a widely discussed issue (Ceckova-Novotna et al., 2006; Mao, 2008). They have been localized and functionally described in many in vitro and in situ models, including BeWo cell line (Utoeguchi et al., 2000; Ceckova et al., 2006; Evseenko et al., 2006), and perfused human (Kraemer et al., 2006) or rat...
placenta (Pávek et al., 2001; Staud et al., 2006). It is obvious that these transporters limit mf and possibly also augment fm passage of many xenobiotics. However, these transporters are not omnipotent and the role of other factors, such as physical-chemical properties or plasma protein binding, in the placental transport must not be overlooked. We have previously confirmed the functional activity of placental drug efflux transporters, P-gp (Pavek et al., 2003) and Bcrp (Staud et al., 2006) by use of the model of dually perfused rat placenta. In the latter study, we proposed a pharmacokinetic model describing transplacental transport of ABC substrates that allows for separate quantification of both passive and active events of the process. The aim of the present article was to investigate and compare transplacental passage of several ABC substrates and quantify the effect of drug efflux transporters and/or lipid solubility.

Glyburide is one of the sulfonylureas intensively studied during the past decade within the search for alternative treatment of gestational diabetes. In situ perfusions of human placental cotyledon have revealed that GLB crosses the placenta to fetal compartment in insignificant amounts (Elliot et al., 1991, 1994). Likewise, a randomized, controlled trial failed to detect measurable GLB levels in umbilical cord blood of infants born to mothers that were treated with the drug (Langer et al., 2000). This low permeability of the drug through the placenta was originally attributed to high plasma protein binding (99.8%) and short elimination half-life (Koren, 2001; Nanovskaya et al., 2006). Recently,
Kraemer et al. (2006) provided the first direct evidence, using in vitro close-circle perfusions of a human placental cotyledon, that GLB is actively effluxed by a transporter other than P-gp. Shortly afterward, other studies suggested

Fig. 4. Elimination of Rho123 (A), GLB (B), and BP (C) from fetal circulation by placental P-gp and/or Bcrp at low Rho123 (0.5 μM), GLB (0.2 μM), and BP (0.25 μM) concentrations. Fetal Rho123, GLB, and BP concentrations decreased to 0.207, 0.049, and 0.121 μM, respectively, and stabilized after 40 min of perfusion. This decline was, in part, inhibited by GF120918 (2 μM) or fumitremorgin C (2 μM). Concentrations of AP (D) remained stable in both circulations throughout the whole experiment, confirming lack of active transport of the molecule. Experimental values are presented as means ± S.D. of at least three experiments; ■, fetal concentration; □, maternal concentration; ○, fetal concentration with GF120918; △, fetal concentration with fumitremorgin C; □, fetal concentration without inhibitor; Student’s t test or one-way analysis of variance followed by Bonferroni’s test were used; *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with control (fetal concentration without inhibitor).

Fig. 5. Elimination of Rho123 (A), GLB (B), and AP (C) from fetal circulation at high concentration (100 μM). At this concentration, no decrease in fetal compartment was observed, suggesting saturation and limited activity of the transporters. Data are presented as means ± S.D. of at least three experiments. ■, maternal concentration; □, fetal concentration.

Kraemer et al. (2006) provided the first direct evidence, using in vitro close-circle perfusions of a human placental cotyledon, that GLB is actively effluxed by a transporter other than P-gp. Shortly afterward, other studies suggested
interaction of GLB with placental BCRP by use of specific inhibitors of a group of various transporters (Gedeon et al., 2006, 2008; Pollex et al., 2008; Zhou et al., 2008). In contrast to human studies, when tritium-labeled GLB was injected into pregnant rat, the fetal-to-maternal radioactivity ratio was 0.535, similar to diazepam (0.641) (Sivan et al., 1995). The authors concluded that GLB crosses the rat placenta and should be considered with caution in the treatment of gestational diabetes. This result was ascribed to interspecies differences (Langer et al., 2000), but was not confirmed in our study. In our experimental setup of dually perfused rat term placenta, albumin was replaced by dextran in the perfusion liquid to avoid contamination of results by plasma protein binding. Subsequently, a broad range of GLB concentrations was tested to unveil nonlinearity of mf and fm transport of the compound. Fitting the placental clearances versus drug concentrations to our PK model resulted in sigmoid curves for both directions, suggesting involvement of an active transport. Comparing fm and mf clearances at low GLB concentrations (0.2 μM), fm clearance was 11.2-fold higher than clearance in the opposite direction. Addition of GF120918 inhibitor reduced this asymmetry to 2.1. Strong effect of BCRP was observed also in the fetal-recirculation experimental setup in which the fetal-to-maternal concentration ratio of GLB toward the end of the experiment was 0.2; addition of inhibitor (2 μM GF120918) reversed this ratio to 0.92. Our data are thus in agreement with those obtained from human (Gedeon et al., 2006; Pollex et al., 2008) and mouse (Zhou et al., 2008) placental and confirm GLB interaction with rat placental Bcrp. In addition, by use of a fetal recirculation setup, we also evidently demonstrate the ability of Bcrp to transport GLB from fetus to mother even against a concentration gradient, which is in accord with the results obtained by a dual perfusion system on the isolated human placental lobules (Pollex et al., 2008). Our results thus confirm extensive impact of Bcrp on GLB transport across the rat placenta; however, the possible effect of other transporters cannot be excluded.

Rhodamine 123, a fluorescent dye, was established as a model compound for P-gp-mediating transport in various sites of the body (Masereeuw et al., 1997; van der Sandt et al., 2000). In our previous article (Pavek et al., 2003), Rho123 was shown to interact with placental P-gp. Here, we confirm these findings by use of a wide range of inflow Rho123 concentrations in both mf and fm directions and by use of a highly effective inhibitor GF120918 (de Bruin et al., 1999). Unlike Pavek et al. (2003), we have omitted albumin from the perfusion buffer so that the net transfer of Rho123 could be measured without any distorting effect of protein binding. Infusion of Rho123 to the maternal or fetal side of the placenta resulted in a nonlinear relationship between clearance and drug concentration, as observed in studies with GLB in this study, or recently with cimetidine (Staud et al., 2006). The low-concentration plateau of the sigmoid line represents the combined effect of passive clearance and efflux transporter activity; the high-concentration plateau delineates clearance of passive diffusion alone. In addition, we show that the concentration of Rho123 in the fetal compartment decreased by 70% within 60 min of the recirculation experiment, confirming the ability of P-gp to remove its substrate even against a concentration gradient.

BODIPY FL prazosin was included in this study because it is a highly lipophilic compound transported by both P-gp and BCRP (Kimchi-Sarfaty et al., 2002; Hori et al., 2004). With use of this “dual substrate” we aimed to test whether the number of transporters involved in drug transfer is reflected in the transplacental PK. Contrary to our expectations, however, the ratio of BP clearances between fm and mf direction (4.0) was the lowest among all tested substances (Rho123, 11.0; GLB, 11.2) indicating rather limited transporter effectiveness (Fig. 3). Furthermore, addition of a P-gp and BCRP inhibitor, GF120918, caused only 21% increase in mf transport of BP, whereas, in the case of cimetidine, the increase was 45% (Staud et al., 2006). Likewise, when testing the elimination of various substrates from the fetal compartment by fetal reservoir recirculation, BP concentration decreased by 2-fold after the stabilization period, whereas the concentration of GLB decreased by 4-fold. We therefore suggest that the number of efflux transporters involved in placental transport of a substrate does not necessarily correlate with its placental transfer. It seems plausible that other characteristics, such as physical-chemical properties, lipid solubility, in particular, may outweigh the effect of efflux transporters.

In our PK model (Staud et al., 2006) we hypothesized, that drugs with higher lipid solubility and therefore faster passive diffusion will be less affected in their placental passage by ABC transporters than drugs with low lipid solubility. This hypothesis has been demonstrated in this study; when plotting Cl_{pad} and Cl_{pad}/Cl_{mcf} ratio against cLogP it is evident that a rise in lipid solubility increases the passive diffusion and, at the same time, decreases the efflux transporter effectiveness (Fig. 6). This relationship provides a reasonable explanation for the transplacental passage of BP. Despite the fact that this substrate is transported by both P-gp and BCRP, the combined effect of these transporters on the transplacental passage of BP seems to be suppressed by high lipid solubility of the molecule and, therefore, rapid clearance by passive diffusion. Therefore, the simple statement that a compound is a substrate of one or more drug efflux transporter(s) is not sufficient to forecast its lower transport from mother to fetus.
In summary, the role of P-gp and Bcrp in the transplacental pharmacokinetics of Rho123, GLB, and BP has been described. These efflux transporters were confirmed to limit the entry of their substrates to fetal circulation and pump them from fetus to mother against concentration gradient. However, the effectiveness of drug efflux transporters is markedly reduced in highly lipophilic drugs.

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References


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