Transplacental pharmacokinetics of glyburide, rhodamine 123 and BODIPY FL prazosin; effect of drug efflux transporters and lipid solubility

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Running Title Page

Running title: Efflux transporters in rat placenta

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Non-standard Abbreviations: ABC – ATP-binding cassette; ANOVA – analysis of variance; BCRP/Bcrp – human/rodent breast cancer resistance protein; P-gp – P-glycoprotein; GF120918 – N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide

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Abstract

Breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) are the most abundantly expressed 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.
Introduction

The need to medicate women for various reasons even during pregnancy is often inevitable. Current multi-centre study monitoring pregnancies from 1996 to 2000 in the USA revealed that 64% of all pregnant women met the prescription of a drug other than vitamin or mineral supplement during 270 days before delivery (Andrade et al., 2004). Moreover, 5 – 10% of them received FDA category D or X, classified as potential teratogens (Andrade et al., 2006). These findings emphasize the importance 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 ABC (ATP binding cassettes) transporter family have been originally investigated in association with the phenomenon of multidrug resistance in cancer therapy (Kavallaris, 1997; van der Kolk et al., 2002; Perez-Tomas, 2006) as they are capable of actively pumping their substrates out of cells even against a concentration gradient. Later on, some of these membrane-embedded proteins have also been 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 of 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 as well as 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 recently (Bremer et al., 1992; Doyle et al., 1998; Maliepaard et al., 2001; Pavek et al., 2001; Staud et al., 2006). In the human placenta, significantly higher expression was found for BCRP when compared to P-gp (Ceckova et al., 2006). Moreover,
we have revealed that the placental expression and transport activity of P-gp and Bcrp changes during pregnancy in rat (Novotna et al., 2004; Cygalova et al., 2008). Functional studies indicate that P-gp as well as BCRP transport a large variety of molecules, ranging from endogenous substrates to chemotherapeutic agents and environmental toxins (Schinkel and Jonker, 2003; Mao and Unadkat, 2005; Staud and Pavek, 2005). Considerable overlap in substrate recognition as well as 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 perfused rat placenta in open or closed perfusion setup (Staud et al., 2006) to evaluate the effect of P-gp and Bcrp on transplacental pharmacokinetics (PK) of their substrates. Concentration-dependent studies, specific inhibitors and PK modeling have been employed to assess the efficacy of these proteins to hinder maternal-to-fetal (mf) and accelerate fetal-to-maternal (fm) transport. BODIPY FL prazosin (BP), a common substrate of both P-gp and BCRP (Kimchi-Sarfaty et al., 2002; Hori 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 between lipid solubility of the molecules and their passive diffusion and/or active transport were investigated.
Methods

Reagents and Chemicals

Glyburide (GLB; 1-[[p-[2-(5-chloro-o-anisamido)-ethyl]phenyl]-sulfonyl]-3-cyclohexylurea), a BCRP substrate, and [cyclohexyl-2,3-$^3$H(N)]glyburide ([$^3$H]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-quinazoliny1)-4-[3-[5-[(3,5-dimethyl-2H-pyrrol-2-ylidene.-kappa.N)methyl]-1H-pyrrol-2-yl.-kappa.N]-1-oxopropyl]piperazinato]difluoro-, (T-4)-), a common BCRP and P-gp substrate, was purchased from Invitrogen (Carlsbad, CA). Rhodamine 123 (Rho123; 2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester), a P-gp substrate, was obtained from Sigma-Aldrich (St. Louis, MO). Antipyrine (AP; 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), a marker of passive diffusion, and [N-methyl-$^{14}$C]antipyrine ([$^{14}$C]AP) (55 mCi/mmol) were purchased from Sigma-Aldrich (St. Louis, MO) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Specific BCRP inhibitor fumitremorgin C (FTC; 9'R-(9'alpha(4S*),(R*)),9'abeta))-4-(2-(1-(Acetyloxy)-2-methylpropyl)-4-oxo-3(4H)-quinazolinyl)-1',3,4,9'a-tetrahydro-1'-hydroxy-2',2'-dimethylspiro(furan-2(5H),9'-(9H)imidazo(1,2-a) indole)-3',5(2'H)-dione) and a dual P-gp and BCRP inhibitor GF120918 (N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinnolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide) were from Alexis Corporation (Lausanne, Switzerland) and GlaxoSmithKline (Greenford, UK), respectively. All other compounds were reagent grade.

Animals

Pregnant Wistar rats were purchased from Biotest Ltd. (Konarovic, Czech Republic) and maintained in 12/12-h day/night standard conditions with pellets and water ad libitum.
Experiments were carried out on day 21 of gestation. Fasted rats were anesthetized 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, 1986).

**Dual Perfusion of the Rat Placenta**

The method of dually perfused rat term placenta was used in our study, as described previously (Staud et al., 2006). Briefly, one uterine horn was excised and submerged in heated Ringer’s saline. A catheter was inserted into 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 maternal reservoir at a rate of 1 ml/min. The uterine vein, including the anastomoses to other fetuses, was ligated behind the perfused placenta 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 using 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 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):
(i) 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 approximately 5-min stabilization period before sample collection started (time 0). Fetal effluent was sampled into preweighed vials in 5-min intervals and analyzed either fluorometrically for Rho123 and BP or radiometrically for $[^3]$HGLB and $[^{14}]$CAP.

(ii) 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 substance 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 concentration of the tested substance was measured. This experimental setup ensures steady substance concentration on the maternal side of placenta and enables investigations of fetal-to-maternal concentration ratio; any net transfer of the drug implies transport against concentration gradient and provides an evidence of active transport.

In order to standardize the perfusion experiments, AP with trace amount of $[^{14}]$CAP was infused to maternal or fetal side of the placenta in concentration of 0.25 or 100 µM and transplacental clearances were calculated.

**Effect of Substrate Concentration on Transplacental Clearance 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 $[^3]$HGLB were added to the maternal or fetal reservoir, respectively, in the following 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,
concentration of 0.25 µM was examined only, because of 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 feto-maternal 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 non-saturating and saturating, respectively. In particular, Rho123 was infused at a concentration of 0.5 µM or 100 µM; GLB was infused at a concentration of 0.2 µM or 100 µM. BP, due to 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 demonstrate their effect on fetal-to-maternal equilibrium.

Pharmacokinetic Analysis of Efflux Transport Activity in the Placenta

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_{fm} and Cl_{mf}, respectively) is a function of passive diffusion (Cl_{pd} - governed by Fick’s law)
and efflux transporter activity ($Cl_{efflux}$ - governed by saturable kinetics). As efflux transport in the placenta runs in fetal-to-maternal direction only, $Cl_{efflux}$ is added to $Cl_{pd}$ in fm direction and subtracted from $Cl_{pd}$ in mf direction as follows:

$$Cl_{fm} = Cl_{pd} + Cl_{efflux}$$ (1)

and

$$Cl_{mf} = Cl_{pd} - Cl_{efflux}$$ (2)

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

$$Cl_{efflux} = \frac{V_{max}}{K_m + C_{ma(fa)}}$$ (3)

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

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

$$Cl_{fm} = Cl_{pd} + \frac{V_{max}}{K_m + C_{fa}}$$ (4)

and

$$Cl_{mf} = Cl_{pd} - \frac{V_{max}}{K_m + C_{ma}}$$ (5)

Data were fitted using reciprocal weighting and the numerical module of SAAM II (SAAM Institute, Seattle, WA).

Total maternal-to-fetal transplacental clearance ($Cl_{mf}$) normalized to placenta weight was calculated according to equation 6.
where $C_{fv}$ 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 ($Cl_{fm}$) was calculated according to equation 7.

$$Cl_{fm} = \frac{C_{fa} - C_{fv}}{Cl_{mf} / w_p}$$

where $C_{fa}$ 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 ($Cl_{fm}/Cl_{mf}$) 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 ANOVA 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

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 vs. inflowing substrate concentrations revealed sigmoid curves in both mf and fm directions (Figs. 1, 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. $Cl_{efflux}$ on the other hand is a concentration-dependent parameter. At high substrate concentration, the transporter becomes saturated, $Cl_{efflux}$ approaches zero and equations 4 and 5 transform into simple linear processes, i.e. $Cl_{mf} = Cl_{fm} = Cl_{pdl}$.

Fetal-to-maternal vs. 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 fm direction was observed compared to that in the opposite direction (Fig. 3). Addition of GF120918 caused 2.4, 5.3 and 1.6-fold decrease in the $Cl_{fm}/Cl_{mf}$ 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, 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 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 around 40 min of perfusion was observed; this decline was blocked by co-infusion 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 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 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 employed. cLogP (logarithm of the partition coefficient between n-octanol and water) values were calculated by ChemBioOffice 2008 (CambridgeSoft, Cambridge, MA), which exploits increment system adding contributions of every atom based on its atom type. Data concerning cimetidine were taken from our previous paper (Staud et al., 2006).

$Cl_{fm}/Cl_{mf}$ at low substrate concentration was considered as a parameter illustrating transporter effectiveness (Fig. 3) while $Cl_{pd}$ 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 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 (Utoguchi et al., 2000; Ceckova et al., 2006; Evseenko et al., 2006), perfused human (Kraemer et al., 2006) or rat placenta (Pavek et al., 2001; Staud et al., 2006). It is obvious, that these transporters limit mf and possibly also augment fm passage of many xenobiotics. On the other hand, 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) using 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 paper 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 last 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 (Elliott et al., 1991; Elliott et al., 1994). Similarly, 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) have given 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 afterwards other studies suggested interaction of GLB with placental BCRP using specific inhibitors of a group of various transporters (Gedeon et al., 2006; Gedeon et al., 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 non-linearity of mf and fm transport of the compound. Fitting the placental clearances vs. 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 set-up in which the fetal-to-maternal concentration ratio of GLB towards the end of the experiment was 0.2; addition of inhibitor (GF120918 2µM) 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) placentas and confirm GLB interaction with rat placental BCRP. In addition, using 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 dual perfusion system on the isolated human placental lobules (Pollex et al.,
Our results thus confirm extensive impact of BCRP on GLB transport across the rat placenta; however, 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 paper (Pavek et al., 2003), Rho123 was shown to interact with placental P-gp. Here we confirm these findings using a wide range of inflow Rho123 concentrations in both mf and fm directions as well as using 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 nonlinear relationship between clearance and drug concentration, as observed in studies with GLB in this study or recently with cimetidine (Staud et al., 2006). Low concentration plateau of the sigmoid line represents combined effect of passive clearance and efflux transporter activity; high concentration plateau delineates clearance of passive diffusion alone. In addition, we show that the concentration of Rho123 in 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 since it is a highly lipophilic compound transported by both P-gp and BCRP (Kimchi-Sarfaty et al., 2002; Hori et al., 2004). Using 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, 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, while in the case of cimetidine, the increase
was 45% (Staud et al., 2006). Similarly, 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, whilst 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 $Cl_{pd}$ and $Cl_{fm}/Cl_{mf}$ 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, 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, 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


Footnotes

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Legends for Figures

**Fig. 1.** Transport of rhodamine 123 across the dually perfused rat placenta in fetal-to-maternal (A) and maternal-to-fetal (B) direction. Changes of clearance with increasing Rho123 concentration confirm 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).

**Fig. 2.** Transport of glyburide across the dually perfused rat placenta in fetal-to-maternal (A) and maternal-to-fetal (B) direction. Changes of clearance with increasing GLB concentration confirm 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).

**Fig. 3.** Ratio of clearances between fetal-to-maternal (fm) and maternal-to-fetal (mf) directions at low (left column) and high (right column) substrate concentrations. In the case of Rho123 (A), GLB (B) and BP (C) administered at low concentrations, significantly higher clearances in fm than in mf direction were observed. Addition of GF120918 to maternal and fetal circulations significantly decreased this asymmetry. At high concentrations, no differences between fm and mf clearances were detected. In the case of AP (D), a marker of passive diffusion, it is evident that its transplacental pharmacokinetics is not concentration-dependent and, therefore, not affected by either P-gp or Bcrp. Numbers in parentheses show ratio of fm-to-mf clearance; bars are means ± S.D of at least three experiments; Student’s t-test was used; *p < 0.05, **p < 0.01 vs. control.
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 partly inhibited by GF120918 (2 µM) or fumitremorgin C (2 µM). Concentrations of AP (D) remained stable in both circulation 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; ■ - maternal concentration; ○ - fetal concentration with GF120918; ◊ - fetal concentration with fumitremorgin C; □ - fetal concentration without inhibitor; Student’s t-test or one-way ANOVA 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.

Fig. 6. Effect of the n-octanol/water partition coefficient (cLogP) on clearance of passive diffusion (Cl_od) (open symbols) and on efflux transporter effectiveness (expressed by C_{fud}/C_{mf} clearance ratio) (closed symbols). Cimetidine (●, ○), Rho123 (■, □), GLB (▲, △) and BP (♦). Data concerning cimetidine were taken from our previous paper (Staud et al., 2006). This figure suggests that the higher the lipid solubility of a compound, the higher the clearance of passive diffusion and the lower the effect of transport proteins on transplacental passage.
Tables

**TABLE 1**

Pharmacokinetic parameters of transplacental passage of Rho123 and GLB. Pharmacokinetic parameters were obtained by fitting experimental data with eqs. 4 and 5 for fm and mf transport, respectively. Data are presented as mean ± S.D.

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<td>$Cl_{pd}$ (ml/min/g)</td>
<td>0.125 ± 0.022</td>
<td>0.129 ± 0.015</td>
</tr>
<tr>
<td>$V_{max}$ (mmol/min/g)</td>
<td>0.97 ± 0.12</td>
<td>6.56 ± 0.47</td>
</tr>
<tr>
<td>$K_{m}$ (µM)</td>
<td>1.42 ± 0.19</td>
<td>88.0 ± 16.8</td>
</tr>
</tbody>
</table>
Fig. 1.

(A) Fetal-to-maternal Rho123 clearance (ml/min/g) against Rho123 concentration (μM).

(B) Maternal-to-fetal Rho123 clearance (ml/min/g) against Rho123 concentration (μM).
Fig. 2.

A

Fetal-to-maternal GLB clearance (ml/min/g)

GLB concentration (μM)

B

Maternal-to-fetal GLB clearance (ml/min/g)

GLB concentration (μM)
Fig. 4.

A

Rho123 concentration (µM)

Time (min)

B

GLB concentration (µM)

Time (min)

C

BP concentration (µM)

Time (min)

D

AP concentration (µM)

Time (min)
Fig. 5.

A

Rho123 concentration (µM)

0 10 20 30 40 50 60
Time (min)

B

GLB concentration (µM)

0 10 20 30 40 50 60
Time (min)

C

AP concentration (µM)

0 10 20 30 40 50 60
Time (min)
Fig. 6.

\[ y = -3.48x + 23.34 \]
\[ R = 0.93 \]

\[ y = 0.06x + 0.01 \]
\[ R = 0.98 \]