Role of the Multidrug Transporter Proteins ABCB1 and ABCC2 in the Diaplacental Transport of Talinolol in the Term Human Placenta

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ABSTRACT:
Placental syncytiotrophoblasts are known to express the efflux transporter proteins P-glycoprotein (ABCB1) and multidrug resistance-associated protein 2 (ABCC2), which are supposed to be a functional part of the human placental barrier. With advancing gestational age, expression of ABCB1 decreases progressively, whereas ABCC2 is more expressed. To evaluate to which extent they contribute to placental barrier function at term, permeability of talinolol, a substrate of both carriers, was measured using a validated human placenta perfusion model. We identified in randomized, crossover experiments a unidirectional transfer of talinolol in the fetomaternal direction because the maternofetal transfer was significantly lower (0.663 ± 0.188 versus 0.394 ± 0.067 relative to creatinine permeability, \( p = 0.012 \)). Maternofetal permeability was increased by the ABCC2 inhibitor probenecid (0.59 ± 0.15 versus 0.68 ± 0.13, \( p = 0.028 \)) and the nonspecific inhibitor verapamil (0.53 ± 0.09 versus 0.66 ± 0.16, \( p = 0.028 \)) but was not influenced by the ABCB1 inhibitor valsapar (PSC833) (0.48 ± 0.11 versus 0.46 ± 0.09, \( p = 0.345 \)). Genetic polymorphisms of ABCB1 and ABCC2 lacked significant influence on expression of the carriers and permeability of talinolol, respectively. In conclusion, maternofetal transfer of talinolol is restricted by a unidirectional process that is influenced by inhibitors of ABCC2.

The human placenta brings the maternal and fetal blood circulations closely together, separated only by the single cell layer of the syncytiotrophoblast. It regulates the supply of nutrients and gases, the elimination of fetal waste products, and the exposure to exogenous substances, including drugs. There is growing evidence that energy-dependent transporter processes are involved in the maternofetal exchange of endogenous and xenobiotic substances. Meanwhile, more than 30 transport proteins were identified that are expressed to the maternal-facing brush-border apical membrane or to the fetal-facing basolateral membrane of the syncytiotrophoblast (Marzolini and Kim, 2005; Evseenko et al., 2006; Myllynen et al., 2007). There is evidence for some of them to be significantly involved in drug disposition, such as the maternal-facing efflux carriers P-glycoprotein (ABCB1) and the multidrug resistance-associated protein 2 (ABCC2), a second member of the ABC transporter family (St. Pierre et al., 2000; Marzolini and Kim, 2005; Ceckova-Novotna et al., 2006; Evseenko et al., 2006). ABCB1 and ABCC2 share a wide overlapping substrate spectrum and may be coregulated, e.g., in the small intestine (Fromm et al., 2000; Jedlitschky et al., 2006). Information on their function in the human placenta, however, is contradictory and limited so far to ABCB1. Because placental ABCB1 expression decreases with advancing gestation age whereas ABCC2 undergoes gestational maturation, we hypothesized that ABCC2 in term placentas may be more important than ABCB1 for the transfer of drugs that are substrates of both carriers (Meyer zu Schwabedissen et al., 2005; Sun et al., 2006). A suitable probe drug to evaluate function of ABCB1 and ABCC2 in humans is the nonmetabolized \( \beta_1 \)-selective blocker talinolol, which is a high affinity substrate of ABCB1 as evidenced by in vitro experiments and pharmacokinetic studies in humans and of ABCC2 as concluded from major changes in disposition in Abcc2-deficient GY/TR rats (Spahn-Langguth et al., 1998; Gramatte and Oertel, 1999; Westphal et al., 2000; Bernsdorf et al., 2003). \( \beta_1 \)-Selective blockers are used in the treatment of hypertension during pregnancy (Magee et al., 2000).

We provide evidence from perfusion experiments using the dually perfused human placenta model that the placenta serves as a functional barrier for talinolol as caused by a maternal-directed efflux transport. Furthermore, we evaluated the influence of the most common ABCB1 and ABCC2 polymorphisms and of the inhibitors PSC833 (for ABCB1), probenecid (for ABCC2), and verapamil (nonspecific) on the maternofetal transfer of the probe drug (Horikawa et al., 2007).

ABBREVIATIONS: ABCB1, P-glycoprotein; ABCC2, multidrug resistance-related protein 2; PSC833, valsapar; HPLC, high-performance liquid chromatography; AUC, area under the concentration-time curve; OATP, organic anion transport polypeptide.
ABCC2 genotypes were as follows: newborn body weight 2920–4470 g, Apgar score 7–10). The ABCB1 and ABCC2 on the placental transfer of the probe drug talinolol, we used the human placenta perfusion model as initially described by Schneider et al. (1972). Seventy-five human placentas from healthy parturient women after noncomplicated vaginal or cesarean delivery and written informed consent were prepared for perfusion. Per protocol, analysis was possible with 26 of them (gestation 38–41 weeks, placenta weight 416–996 g, placental protein quantification.

Study Protocols. The experiments were performed randomized, controlled, two-period, crossover with 30-min washout period. In the first study using eight placentas, permeability of talinolol in the fetomaternal and maternofetal directions was compared. After the stabilization period, talinolol (0.8 mM, Sigma, Steinheim, Germany), and creatinine (1.3 mM, Arcos Organics, Geel, Belgium) were added randomly either to the maternal or fetal circuit (final concentrations). Samples (2 ml) were taken from both circuits after 5, 10, 15, 20, 30, 45, 60, 90, 120, and 150 min after administration and substituted by perfusion medium. In our second experiment, the maternofetal permeability of talinolol, antipyrine, and creatinine was measured without and in the presence of verapamil (30 mM; Sigma), PSC833 (1.8 μM; Novartis, Basel, Switzerland), or probenecid (10 mM; Sigma) using six placentas in each case. All the inhibitors were added to the perfusion medium of the maternal and fetal circulation in concentrations that have been shown in former studies to modulate the efflux carrier proteins (Pauli-Magnus et al., 2000; Narahashi et al., 2002; Mölsa et al., 2005).

Genotyping, mRNA Expression, and Protein Content of ABCB1 and ABCC2. The ABCB1 polymorphisms 2677G>T/A and 3435C>T and ABCC2 –24C>T, 1249G>A, and 3972C>T were screened by polymerase chain reaction/restriction fragment length analysis. ABCB1 and ABCC2 mRNA expression was quantified by real-time reverse transcription-polymerase chain reaction analysis (Gießmann et al., 2004). Placental protein levels of ABCB1 and ABCC2 were measured by Western blot analysis using for ABCB1 the monoclonal C219 (1:1000) and for ABCC2 the M2III-6 (1:500) antibodies (Alexis Biochemicals, Grünberg, Germany).

Assays for Glucose, Lactate, Creatinine, Talinolol, and Antipyrine. Glucose and lactate concentrations were measured amperometrically using the Super GL Ambulance (Ruhrtal Labor Technik, Möhnessen, Germany), and creatinine was measured using the kit Dimension CREA (Dade Behring, Marburg, Germany). Talinolol was quantified with a high-performance liquid chromatography (HPLC) method as described recently for human serum (Westphal et al., 2000). The method was validated between 0.005 and 1.0 μg/ml perfusion medium. Within-day accuracy of the method was between 2.8 and 8.3% of the nominal concentrations and precision 2.2 to 6.5% of means. The following between-day variability was assessed with quality control samples containing 0.025, 0.25, and 0.75 μg/ml talinolol: accuracy −2.9 to 4.1%, precision 4.3 to 10.6% of the nominal and mean values, respectively. Antipyrine in concentrations between 0.5 and 50 μg/ml was assayed by isocratic HPLC. In brief, 100 μl of perfusion medium was mixed with 400 μl of distilled water, 100 μl of 4 N sodium hydroxide, and 100 μl of internal standard solution (0.027 mg/ml phenacetin) and extracted twice with 3 ml of diethyl ether. After evaporation to dryness, the residue was dissolved in 140 μl of the mobile phase (triethylammonium phosphate buffer, pH 3.0, mixed with 35% methanol). Fifty microliters was injected into the HPLC (Merck-Hitachi, Düsseldorf, Germany) equipped with the column Merck LiChroCart 125-4 HPLC cartridge filled with LiChrospher 100 RP 18e (temperature 30°C, flow 1 ml/min). The following quality parameters were obtained: within-day accuracy and precision −7.8 to 9.5% and 2.8 to 11.2%, respectively; between-day accuracy −0.4 to 2.0% and precision 5.2 to 11.4% of the nominal and mean values, respectively.

Biometrical Evaluation. Permeability (P) of talinolol, creatinine, and antipyrine was calculated according to the equation P = C × [AUCperfusion − AUCblood] with C to be the concentration in the fetal circuit at the end of perfusion, AUCperfusion the area under the concentration-time curve (AUC) in the maternal circuit, AUCblood the AUC in the fetal circuit, and weight the wet weight of the cotyledon (Bajoria and Fisk, 1998). Ratios of the talinolol permeability over the creatinine permeability were calculated to normalize for individual differences caused by paracellular transfer (Brownbill et al., 2000). Means ± S.D. or medians, minimums, and maximums are given. Sample statistics were done using the Wilcoxon and Mann-Whitney tests and Spearman rank correlation as appropriate.

Results

Method Validation. Placental carbohydrate metabolism remained unchanged during the time of perfusion and was also not influenced by talinolol, PSC833, verapamil, or probenecid as confirmed by monitoring glucose consumption and lactate production. Furthermore, the passive placental transport was also not significantly influenced by the experimental conditions as verified by the permeability data for creatinine, a surrogate for paracellular transfer, and for antipyrine, a measure for nonionic simple diffusion (Table 1) (Schneider et al., 1972; Brownbill et al., 2000). Because of the lower perfusion rate in the fetal circulation, antipyrine permeability in the maternal direction was expectedly lower than in the fetal direction (p = 0.069). Therefore, permeability of talinolol in the maternal direction might have been underestimated.

Unidirectional Transfer of Talinolol. We identified a significant unidirectional placental transfer of talinolol in the fetomaternal direction. The permeability of talinolol from the maternal to the fetal circulation was significantly lower than that from the maternal side of the placenta (0.006 ± 0.002 ml × min⁻¹ × g⁻¹ versus 0.013 ± 0.007 ml × min⁻¹ × g⁻¹, p = 0.012). Similarly significant differences in permeability were obtained after considering the differences in paracellular transfer by normalization of the data to creatinine permeability (0.39 ± 0.07 versus 0.66 ± 0.19, p = 0.012) (Fig. 1).

Unidirectional Transfer of Talinolol Is Influenced by PSC833, Probenecid, and Verapamil. The maternofetal permeability of talinolol normalized to creatinine permeability was slightly but significantly increased in the presence of probenecid (0.68 ± 0.13 versus 0.59 ± 0.15, p = 0.028) and verapamil (0.66 ± 0.16 versus 0.53 ± 0.09, p = 0.028); the verapamil effect seemed to be stronger than that of probenecid. PSC833 did not significantly influence talinolol permeability (0.48 ± 0.11 versus 0.46 ± 0.09, p = 0.345), although we observed an increase of the maternofetal transfer in five of our six experiments with PSC833 (Fig. 2).

Placental Expression of ABCB1 and ABCC2 and Permeability of Talinolol. There were no significant correlations between placental...
mRNA expression and protein content of ABCB1 and ABCC2, respectively. Expression of the transporters on the mRNA and protein level was not correlated to maternofetal permeability of talinolol. Evaluation of our data with reference to the haplotypes of ABCB1 and ABCC2 showed that genetic polymorphisms did neither influence mRNA and protein expression of the efflux carriers nor permeability of talinolol (data are not shown).

**Discussion**

We provided valid experimental data on unidirectional placental transfer of talinolol using dually perfused human placentas that were obtained from carefully selected healthy women. The metabolic conditions during the perfusion for 7 h were stable as oxygen consumption and lactate production have not changed. Nevertheless, we used randomized, controlled, crossover designs to minimize study-related intrasubject differences such as time-dependent changes in expression of the transporters. Furthermore, we corrected our data with talinolol to the permeability of creatinine to minimize influence of paracellular transfer (Brownbill et al., 2000). In all our maternofetal transfer studies, there were also no significant differences in antipyrine permeability, which is an accepted surrogate to characterize placental passive diffusion (Schneider et al., 1985). As expected because of the lower perfusion rate in the fetal circulation, antipyrine permeability in the maternal direction was somewhat lower even though not statistically different. Therefore, permeability of talinolol in the maternal direction may have been underestimated if perfusion is also rate-limiting for the transfer of talinolol.

In our inhibition experiments, only the maternofetal transfer was measured using a crossover design because this is the clinically relevant transport route. However, it is recommended for future studies to evaluate whether inhibition of efflux carriers in the syncytiotrophoblast leads to decrease of the fetomaternal permeability.

It is largely accepted that endogenous and xenobiotic compounds are exchanged via the human placenta by active transporters localized to the basal and apical membrane of the syncytiotrophoblast such as the efflux pumps ABCB1, ABCC1 (MRP1), ABCC2, and ABCG2 (BCRP) or certain members of the organic anion transport polypeptides (OATP1A2, OATP2B1), the organic cation transporters (OCT1, OCT1, OCTN2), or the organic anion transporters (OAT1, OAT3) (Marzolini and Kim, 2005; Evseenko et al., 2006). Their function in the human placenta, particularly the interplay between uptake and efflux carriers to initiate unidirectional substance transfer, however, is so far nearly unknown. The only exception is ABCB1, which was shown to be involved in the maternal directed efflux of saquinavir, methadone, paclitaxel, and quetiapine (Mölsä et al., 2005; Nanovskaya et al., 2005; Rahi et al., 2007). Therefore, ABCB1-mediated efflux seems to be the mechanism behind that what is called “placenta barrier” for many drugs that reach much lower blood concentrations in the fetus than in the mother despite low plasma protein binding, e.g., digoxin, calcium

TABLE 1

<table>
<thead>
<tr>
<th>Protocol</th>
<th>n</th>
<th>Glucose Consumption</th>
<th>Lactate Production</th>
<th>Antipyrine Permeability</th>
<th>Creatinine Permeability</th>
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<tr>
<td></td>
<td></td>
<td>(μmol × min⁻¹ × g⁻¹)</td>
<td>(μmol × min⁻¹ × g⁻¹)</td>
<td>(ml × min⁻¹ × g⁻¹)</td>
<td>(ml × min⁻¹ × g⁻¹)</td>
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<tr>
<td>Maternofetal versus fetomaternal permeability</td>
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<tr>
<td>Maternofetal</td>
<td>8</td>
<td>0.21 (0.14, 0.28)</td>
<td>0.45 (0.30, 0.60)</td>
<td>0.10 (0.07, 0.12)</td>
<td>0.115 (0.041, 0.18)</td>
</tr>
<tr>
<td>Fetomaternal</td>
<td>8</td>
<td>0.21 (0.13, 0.28)</td>
<td>0.39 (0.29, 0.49)</td>
<td>0.07 (0.04, 0.10)</td>
<td>0.08 (0.014, 0.26)</td>
</tr>
<tr>
<td>Study (maternofetal permeability in presence of inhibitors)</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>0.32 (0.03, 0.62)</td>
<td>0.59 (0.05, 1.12)</td>
<td>0.09 (0.003, 0.18)</td>
<td>0.038 (0.001, 0.075)</td>
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<tr>
<td>PSC833</td>
<td>6</td>
<td>0.32 (0.10, 0.54)</td>
<td>0.66 (0.04, 1.27)</td>
<td>0.11 (0.05, 0.17)</td>
<td>0.042 (0.010, 0.075)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.29 (0.15, 0.43)</td>
<td>0.55 (0.30, 0.80)</td>
<td>0.09 (0.04, 0.15)</td>
<td>0.021 (0.016, 0.026)</td>
</tr>
<tr>
<td>Probenecid</td>
<td>6</td>
<td>0.36 (0.12, 0.61)</td>
<td>0.79 (0.32, 1.27)</td>
<td>0.07 (0.04, 0.11)</td>
<td>0.019 (0.014, 0.024)</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.27 (0.17, 0.37)</td>
<td>0.54 (0.20, 0.87)</td>
<td>0.10 (0.05, 0.15)</td>
<td>0.025 (0.018, 0.031)</td>
</tr>
<tr>
<td>PSC833</td>
<td>6</td>
<td>0.26 (0.18, 0.34)</td>
<td>0.48 (0.28, 0.68)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.025 (0.021, 0.028)</td>
</tr>
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**Arithmetic means and 95% confidence intervals (in parentheses) are given.**

**FIG. 1.** Permeability of talinolol normalized to creatinine permeability in the maternofetal versus fetomaternal direction (n = 8). Wilcoxon signed rank test was used to evaluate differences.

**FIG. 2.** Permeability of talinolol normalized to creatinine permeability in the maternofetal versus fetomaternal direction (n = 6). Wilcoxon signed rank test was used to evaluate differences.
channel blockers, β-receptor blockers, antidepressants, and antiretroviral drugs (Marzolini and Kim, 2005; Everseenko et al., 2006). We provide for the first time evidence that the multidrug transporter ABC2 may be also a functional part of the “placenta barrier.” In late pregnancy, ABC2 may be more important than ABCB1 because it is increasingly expressed with advancing pregnancy, which is contrary to ABCB1, for which a progressive 2-fold decrease in expression was observed between the early pregnancy and term (Meyer zu Schwabe-dissen et al., 2005; Sun et al., 2006). This hypothesis is confirmed by our data with talinolol, which is a substrate of ABCB1 and ABC2 (Sapan-Langguth et al., 1998; Bernsdorf et al., 2003). Placental transfer of talinolol at term was in our study more influenced by ABC2 than ABCB1 as concluded from its higher permeability in the fetal direction in the presence of the ABC2 inhibitor probenecid but not in the presence of the ABCB1 inhibitor PSC833 (Horigawa et al., 2002; Modok et al., 2006).

Obviously, ABC2 dominates drug efflux in late pregnancy to a higher extent than ABCB1. This is supported by observations with digoxin, which is a substrate of ABCB1 but not of ABC2 (Lowes et al., 2003). Therefore, the transplacental transfer of digoxin at term was not influenced by the ABCB1 inhibitors verapamil and quinidine in the dually perfused placenta model (Holcberg et al., 2003). One may speculate that ABC2 contributes also to placental barrier functions for saquinavir and paclitaxel, which are substrates of ABCB1 and ABC2. For both drugs it was already shown by competition experiments with specific ABCB1 inhibitors that at least ABCB1 is involved (Janneh et al., 2005; Mösä et al., 2005; Nanovskaya et al., 2005; Lagas et al., 2006).

However, the unidirectional placental transfer of drugs seems to be an extremely complex process as also evidenced by our results. In the presence of the widely used ABCB1 inhibitor verapamil, the maternofoetal talinolol permeability was significantly increased, even to a higher extent than in the presence of probenecid, although the specific modulator PSC833 lacked marked influence (Naito and Tsuruo, 1989). R-Verapamil is also known to modulate ABCB1 and OATP as confirmed for the uptake of fexofenadine (Cvetkovic et al., 1999; Perrotton et al., 2007). ABCB1 seems to be localized to the basal and brush-border membrane of the syncytiotrophoblast and to the blood vessel endothelia, and it undergoes gestational maturation. The OATP transporters OATP2B1 and OATP1B3 are localized to the basal membrane of the syncytiotrophoblast and are obviously involved in uptake of steroid sulfates and unconjugated bilirubin (Everseenko et al., 2006). Functional interaction of basolateral uptake transporters (e.g., OATP2B1, OATP1B3) with apical efflux carriers (e.g., ABCB1, ABC2, ABCG2) may be the way steroids, bilirubin, and drugs pass the placenta in the fetomaternal direction. This conception is in line with the recent observation that the expression of OATP2B1 and ABCG2 in the human placenta is significantly correlated (Grube et al., 2004; Meyer zu Schwabedissen et al., 2005). The statistical power in our study, however, was too low to confirm functional relevance of this genetic variability for placental talinolol transfer because of the low sample size (n = 26) and the high intersubject variability of the permeability (35%).

Conclusion

The maternofoetal transfer of talinolol is restricted by a unidirectional process that is influenced by inhibitors of ABC2. There is evidence that additional active transporters are involved. However, the efflux of talinolol seems to be low and obviously not of clinical relevance.

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References


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