SUMMARY

Doctoral thesis

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Placenta, which represents a unique link between the mother and fetus, fulfills many functions essential for normal course of pregnancy and uncomplicated development of the fetus. Nutrient supply, gas exchange and metabolic waste product removal belong to its main roles. In addition, placenta serves as an endocrine, metabolic, immune and protective organ, since during pregnancy mother may be, either unconsciously or deliberately, exposed to a wide range of substances toxic for the fetus. Originally, it was supposed that the physiological barrier between maternal and fetal circulation is created only by cellular layers of syncytiotrophoblast and endothelium of fetal capillaries. However, it has been demonstrated that besides this mechanical component of protection, activity of drug efflux transporters and metabolic enzymes localized in the polarized syncytiotrophoblast layer contribute considerably to the protective function of placental barrier.

Efflux transporters are ATP dependent membrane proteins capable of actively removing different molecules out of cells. So far, the best described drug efflux transporters are P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), significantly affecting kinetics of transplacental passage of various substances.

The main goal of this work was to study the expression of placental transporters Bcrp and P-gp and their effect on the transport of drugs across the placenta. In the first study the presence of Bcrp in the rat term placenta and placental cell line HRP-1 was demonstrated by RT-PCR, Western blotting and immunohistochemistry. Simultaneously, we detected the expression of P-gp in the rat placenta but not in HRP-1 cell line. We confirmed the presence of Bcrp in the HRP-1 cells also functionally using inhibitors, Ko143 and GF120918, which increased accumulation of a fluorescent BCRP substrate BODIPY FL prazosin to the cells by more than 100%. Consistently with the results of expression studies no activity of P-gp was observed in the HRP-1 cell line. Furthermore, we investigated the impact of Bcrp on the transplacental transfer of a model substrate cimetidine using dually perfused rat placenta. We observed a considerable asymmetry between the materno-fetal and feto-maternal transport of the substrate, which was partly decreased by BCRP inhibitors fumitremorgin C and GF120918 and entirely eliminated at high cimetidine concentrations. This study clearly demonstrated that BCRP not only reduces the passage of drugs from the mother to the fetus but also actively removes the drug already present in fetal blood, even against concentration gradient.

Although we described the protective activity of placental Bcrp at the end of gestation, its expression and function in the earlier stages of gestation remained unclear. In our following study, therefore, we analyzed the expression of placental Bcrp mRNA during the

course of pregnancy in rat and observed significantly higher amount of Bcrp transcripts on gestation day (gd) 15 compared to gd 12, 18 and 21. In the next part of this study we quantified a 7-fold higher level of Bcrp mRNA in fetal tissues at the end of gestation compared to the 12^{th} gd. Furthermore, we studied the function of placental and fetal Bcrp by fetal exposure to a model substrate, cimetidine, infused to the maternal circulation. The relative amount of drug that penetrated to the fetus was highest on gd 12 and decreased to one tenth thereafter. Moreover, we have revealed much lower penetration of cimetidine to the brain compared to the whole fetal tissue. Our results indicate that increasing expression of Bcrp in fetal tissues can strengthen the protective role of placental Bcrp as pregnancy proceeds.

Overlapping substrate specificity and similar tissue distribution shared by P-gp and BCRP suggest their common role in detoxication processes of the placenta. Therefore, in the final study we aimed to evaluate the effect of P-gp and Bcrp on the transplacental pharmacokinetics (PK) of their substrates using the model of dually perfused rat placenta. Specific inhibitors, various concentrations of model substrates and PK modeling have been applied to assess the efficacy of these proteins to hinder maternal-to-fetal (mf) and accelerate fetal-to-maternal (fm) transport. Using a dual substrate of P-gp and BCRP, BODIPY FL prazosin (BP), we tried to answer the question whether the number of transporters involved in the transfer of a substance is reflected in its 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 (cimetidine – 24.6; rhodamine – 11.0; glyburide – 11.2). When testing the elimination of the same substrates from the fetal compartment by fetal reservoir recirculation, similar results were obtained, suggesting that effect of physical-chemical properties, especially liposolubility, can outweigh the impact of P-gp and Bcrp on the transplacental transport of BP. This hypothesis was confirmed in our study by PK modeling. Based on the relationship between passive/active transport and liposolubility we conclude that a rise in lipid solubility increases the passive diffusion and, at the same time, decreases the efflux transporter effectiveness.

In conclusion, the results of our studies confirm expression and functional activity of Bcrp and P-gp in the rat placenta and quantify the role of these transporters in fetal protection and detoxication. However, the last study indicates that the relative effectiveness of efflux transporters in transplacental pharmacokinetics may be limited by other factors, such as drug lipid solubility.