Summary:

The main limiting factor of distribution of the drugs in the body is the presence of physiological barriers. Placental and blood-testis-barrier are two barriers that were studied in this thesis. The placenta is an important endocrine organ bringing maternal and fetal blood into proximity, allowing exchange of nutrients and waste products. In addition, the placenta acts as the barrier between the mother and fetus, and plays an important role in fetal protection. The key rate-limiting layer in the placenta is the single layer of syncytiotrophoblast. The placental trophoblast contains multiple drug transporters and metabolizing enzymes that control maternofetal exchange of nutrients and hormones, or which form placental "metabolic" barrier. Moreover, several members of the nuclear receptor superfamily are expressed in placental trophoblasts. They regulate transcription of the genes involved in nutrient transport, mineral metabolism, proliferation and differentiation. Moreover it was suggested that they control expression of placental drug transporters and xenobiotic metabolizing enzymes. Germ cells in the testis are protected mainly by the presence of blood testis barrier, formed by the tight or adherens junctions between Sertoli cells. Moreover, intercellular junctions between Sertoli and germ cells are pivotal for spermatogenesis.

In this dissertation we focused on the expression and activity of **VDR** in human placenta, isolated trophoblast and in choriocarcinoma cell lines BeWo and JEG3. VDR is expressed (at the mRNA and protein level) in the human placenta and in the cultures of primary trophoblasts as well. However, its function in placental physiology is still unknown. We revealed that BeWo and JEG-3 choriocarcinoma cell lines express low levels of VDR in comparison with isolated cytotrophoblasts or human placenta. In order to elucidate the mechanism of VDR gene suppression in JEG-3 and BeWo cell lines, we used several approaches to stimulate differentiation of the cytotrophoblast cell lines and to restore expression of VDR in the placental cell lines. We used prototype differentiation agents forskolin and 17β-estradiol, histone deacetylase inhibitors sodium butyrate and trichostatin A and DNA methylase inhibitor 5-deoxy-3´-azacytidin.

We showed that VDR mRNA expression is restored after treatment of BeWo and JEG3 cells with sodium butyrate and 5-deoxy-3´-azacytidine. It means that VDR expression is suppressed epigenetically in choriocarcinoma cell lines, which results in low transactivation activity of VDR in the cells. Finally, we observed a non-genomic effect of $1\alpha,25(OH)_2D_3$ in JEG-3 cells and the activation of the extracellular signal-regulated kinase (ERK) signaling pathway. We revealed that $1\alpha,25(OH)_2D_3$ stimulates phosphorylation of ERK1/2 kinases in

JEG3 cells, what could affect gene expression independently on VDR expression. These results should be considered in future studies regarding the biological function of VDR and $1\alpha,25(OH)_2D_3$ in the normal and tumour placental trophoblast.

The next study was focused on the expression and function of **BCRP** in transplacental pharmacokinetics of rat. Employing real-time RT-PCR and Western blotting established expression of BCRP in rat placenta and in rat placental cell line HRP-1. Immunohistochemical analysis confirmed the presence of BCRP in trophoblast of labyrinth zone of the placenta. Our data thus indicate that BCRP is expressed in the placenta and therefore can play a role in transplacental pharmacokinetics. Using dually perfused rat placenta, we confirmed that BCRP (similarly to P-gp) reduces the passage of its substrates from mother to the fetus and also removes the drug already present in the fetal circulation. The rat placental barrier is morphologically and functionally similar to human placenta, the results obtained here might have a high predictive value for the transplacental pharmacocinetics in humans.

The next study was focused on the expression of **P-cadherin** in normal, busulphan treated and cryptorchid rat testis. The pattern of expression of P-cadherin in the seminiferous epithelium changed with the stage of the seminiferous epithelium. Our experiments revealed that the busulphan treatment and cryptorchism led to destructive changes in the structure of seminiferous tubules, together with the decrease of the P-cadherin expression. We suggest that P-cadherin participates in the architecture of adherens junctions in testis, plays an important role in maintaining normal spermatogenesis and that cryptorchism and busulphan treatment lead to adherens junction disintegration.

In conclusion, the efflux drug transporter **BCRP** expressed in placenta is the important factor affecting penetration of drugs from mother to fetus. Knowledge of its substrate specifity and functional activity could be used in optimalization of pharmacotherapy of pregnant women. The study concerned with the expression of **VDR** in placental cell lines could help to other investigators to elucidate the role of VDR in placental physiology. The aim of next study will be to verify if VDR transcriptionally regulates the expression of biotransformation enzymes (CYP3A4) or transporters (P-gp). Presently we are working on the study of transcriptional regulation of CYP3A4 via VDR, PXR and CAR using placental, hepatic and intestinal cell lines. Elucidation the role of **P-cadherin** in normal and injured spermatogenesis could help us to clarify some of the reasons and mechanisms of man infertility, especially in connection with a study of other proteins participating in forming

intercellular junctions (tight, adherens, communicative junctions, cytoskeleton or basement membrane.