Together with enzymes membrane transporters have substantial influence on movement and fate of drugs in the organism. Their spectrum, expression extent and localization on cytoplasmic membranes have an effect on basic pharmacokinetic processes of absorption, distribution, metabolism and excretion (ADME).

Thematically, this thesis can be split into two fields according to the organs whose transport processes were investigated. The first four original studies deal with the role of efflux transporters P-glycoprotein and BCRP in human and rat placenta and in BeWo cell line derived from human choriocarcinoma. In the last study transporters that could participate in B22956/1 uptake in liver or in cell line derived from liver were investigated.

In the first study the functional expression of P-glycoprotein (*Abcb1*) in the rat placenta was confirmed at the end of pregnancy. By use of in vivo dually perfused rat placenta the ability of P-gp to actively pump its substrates from the fetal blood back to the maternal circulation was demonstrated.

At the mRNA level the expression of both genes coding for P-gp, *Abcb1*a and *Abcb1*b, was investigated using relative quantification by real-time RT-PCR method and compared to expression of a housekeeping gene *beta-2-microglobulin*. Using of Western blotting the placental P-gp was detected at the protein level. Immunohistochemistry was employed to localize P-gp in rat placenta.

Our study revealed the presence of mRNA transcripts of both genes encoding P-gp as soon as on the 11th day of gestation, which corresponds with the day when the definitive features of chorioallantoic rat placenta are well-presented and the organ begins to fulfill its physiological roles. The expression of both genes further increased up to the term. By means of Western-blotting, P-gp was detected from the 13th up to the 22nd day of pregnancy. Immunohistochemical analysis confirmed the presence of P-gp in the developing labyrinth zone of placenta of 13th gestation day; starting from 15th gestation day up to the term immunopositivity for P-gp was observed in the trophoblast layer. Our data thus indicate that P-gp is expressed in the placenta soon after its development and, therefore, can play a role in transplacental pharmacokinetics during the whole period of pregnancy.

In our subsequent study we aimed to examine the recently discovered BCRP drug efflux transporter encoded by *ABCG2* gene. Its localization and functional expression was studied employing human placental cell line BeWo as an in vitro model of placental barrier. At the level of mRNA transcripts we revealed a high endogenous expression of the transporter in

BeWo cells, as well as in human term placenta. The presence of BCRP was further confirmed in BeWo cells and in human term placenta using Western blotting with BXP-21 monoclonal antibody. Functional accumulation studies with mitoxantrone (a substrate of P-gp and BCRP) and inhibitors of BCRP and P-gp demonstrated significant activity of BCRP in pumping its substrates out of the cells. Employing indirect immunofluorescence microscopy, we further studied the subcellular localization of BCRP in BeWo cells. BCRP appears to be localized predominantly at the microvillous apical membrane of the cells, which corresponds to the part of trophoblast layer that is in direct contact with maternal blood. Since BeWo cells represent a model for trophoblast cells it seems plausible that BCRP is able to pump its substrates out of the trophoblast layer the feto-maternal direction. Imunohistochemical localization of BCRP in the human placenta confirmed high expression in the trophoblast layer, however, the immunopositivity for BCRP was also apparent in the endothelial cells of fetal vessels. The above data indicate that BCRP seems to provide (similarly to P-gp) protective role to fetus while pumping potentially toxic drugs and xenobiotics back to the maternal circulation. These findings were further confirmed in experiments on *in situ* perfused rat placenta in which functional activity of placental BCRP was described using cimetidine as a model substrate.

Using absolute quantitative real-time RT-PCR we futher studied the expression levels of *ABCG2* (coding for BCRP) and *ABCB1* (coding for P-gp) in mature human placenta. Surprisingly, the number of *ABCG2* transcripts exceeded ten times the number of *ABCB1* transcripts ten times. On the basis of these results we assume that BCRP is at least as important drug transporter as P-gp.

The aim of the last study, which was carried out on the basis of international cooperation between Centro Studi Fegato, University of Trieste and Department of Pharmacology and Toxicology of our faculty, was to investigate the molecular mechanisms of hepatic transport of MRI contrast agent B22956/1 in hepatic cellular models. Gadocoletic acid trisodium salt (B22956/1) is a new contrast agent showing high biliary excretion and thus is potentially advantageous in hepatobiliary imaging. The transport parameters of this agent were compared with taurocholate, as a typical endogenous compound that is excreted into the bile.

Uptake experiments were performed in Chang Liver and HepG2 cell lines that are considered as in vitro models of tumoral and non-tumoral cells. These experiments showed that transport parameters of taurocholate are very similar in both cell lines, while B22956/1 is taken up 3,5 times quicker by Chang Liver cells than by HepG2 cells.

Inhibition studies further demonstrated that the uptake of B22956/1 into Chang Liver cells is inhibited by taurocholate and bromosulphophthalein in contrast to HepG2 cells, which were not influenced by these inhibitors. In addition the uptake of B22956/1 into Chang Liver cells was greatly inhibited by cholecystokinin-8 (CKK8), which is specific inhibitor of OATP-8 (SLCO1B3) transporter.

Because no information is available about transport proteins involved in uptake mechanisms of B22956/1 in liver, we have performed absolute quantitative real-time RT-PCR analysis PCR in cell lines HepG2 and Chang Liver and healthy human liver tissue to evaluate the expression genes for: OATP-A, OATP-B, OATP-C, OATP-D, OATP-E, OATP-8, OAT2, OAT3, OCT1, NTCP, PEPT-1, PEPT-2. The results have shown that compared to normal liver, the expression of OATP-C (SLC1B1), OATP-D (SLCO3A1) and OATP-8 (SLCO1B3) was greatly repressed in HepG2 cells, while expression of OATP-B (SLCO2B1), OAT2 (SLC22A7) a OAT3 (SLC22A8) was either maintained or increased. On the contrary, in Chang liver cells, transcripts of OAT2 (SLC22A7) and OAT3 (SLC22A8) were undetectable, while the expression of OATP-D (*SLCO3A1*), OATP-E (*SLCO4A1*) and OATP-8 (*SLCO1B3*) was similar to normal liver.

We conclude that B22956 is transported by OATP-8 (*SLCO1B3*) with a relatively high affinity as demonstrated by the inhibition studies. Other transporters such as OATP-D (*SLCO3A1*) and OATP-E (*SLCO4A1*) seem to be involved in Chang liver cells, although with lower affinity. In HepG2 cells, the relatively low expression of OATP-8 (*SLCO1B3*) seems to be sufficient to account for B22956 transport; OATP-B (*SLCO2B1*) and OAT2 (*SLC22A7*) are other transporters that could also be involved in the process. Considering that OATP-8 (*SLCO1B3*) is a transporter expressed exclusively in the liver and its expression is greatly repressed in liver tumors, it may be assumed that B22956/1 will be accumulated with lower extent into hepatic tumors and, therefore, it could serve as a useful diagnostic agent for discrimination between tumoral and intact liver tissue.