6 Summary

It is known that *ABCB1* polymorphisms influence the bioavailability of drugs. However, numerous *in vivo* studies resulted in various outcome. Up to now, reasons could not be solved satisfactory by *in vitro* studies. We could express ABCB1 wildtype (893Ala) and its variants 893Ser and 893Thr in a heterologous *in vitro* expression system (baculovirus system). Using this system recombinant viruses can be generated after insertion of foreign DNA (e.g. *ABCB1* variants) in a suitable vector (e.g. pFastBac1 vector). Insect cells (e.g. HighFive cells) which are infected with these viruses produce the requested foreign protein. After preparation of membranes transport, expression and inhibition studies were carried out. It could be shown that the baculovirus system can be used to determine *in vitro* transport characteristics for ABCB1-893Ala (wildtype) and its genetic variants 893Ser and 893Thr.

Regarding the data reported here functional differences in the ABCB1-893Ala/Ser/Thr triple variant could be identified. Our results suggest that genetic polymorphisms in exon 21 2677 of *ABCB1* play an important role for the pharmacogenetic meaning of ABCB1. The transport activity of ABCB1 is grossly determined by the amount of expressed transporter and by functionality of the protein structure. Initial reports associated a silent SNP in *ABCB1* exon 26 3435 to an alteration in the expression level causing differences in transport activity [41]. The underlying molecular mechanism still remains unclear, although mRNA stability recently appeared a factor determining lower transcription of the 3435 T-allele [76] in human liver. However, many reports resulted in a considerable inconsistency or even discrepancy regarding the impact of this SNP [13;21]. The strong association of 3435C/T to polymorphisms in exon 21 (2677G/T/A) suggested that some of the observed differences in ABCB1 transport function might actually be attributed to the nonsynonymous polymorphism in exon 21. Indeed, we have now evidence that a SNP in 2677 by itself can cause differences in ABCB1 transport characteristics K_m and/or V_{max}. Effects of ABCB1-893 variants were dependent on substrate concentration, indicating that the dose administered in subjects and local drug loads are important parameters to be considered. From our inhibition studies we learned that the interaction with substrate drugs appeared to be dependent on the ABCB1-893 variant. A similar

observation was made showing that 893Ser did not affect the resistance to colchicine, but was associated with changes in resistance to doxorubicin and vinblastine [75]. This aspect could be important in the evaluation of drug-drug interactions in different ABCB1-893 variants.

ABCB1 was also indicated to interact with CYP3A4 on gene and protein level to set up barriers against compounds [23;25]. Furthermore *CYP3A4* gene activity seems to be influenced by *ABCB1* function [24]. Thus, ABCB1-893 variant-related transport function might also play a considerable role in CYP3A4 activity. As ABCB1 and CYP3A4 share a wide substrate disposition could also be affected by this route.

Our discovery provides a functional basis for ABCB1 haplotype-associated alterations in drug disposition and indicates SNPs in exon 21 2677 of this transporter as a causative parameter. Our findings give a plausible explanation for many conflicting results obtained from other studies. Moreover our *in vitro* transport assay might serve as a basis for the development of diagnostic tools to identify *ABCB1* coding variants in drug treatment and drug development.