Why does genetic modification of the drug uptake transporter OATP2B1 affect the expression of CYP3A in liver but not the *in vivo* metabolism of the CYP3A-phenotyping drug midazolam - a mechanistic study

It is assumed that there is a functional interplay of drug-metabolizing enzymes and drug transporters in drug disposition. The cytochrome P450 enzyme CYP3A4 is of major relevance in drug metabolism considering its high abundance in the liver and the wide range of substrate drugs. In rats, there are two orthologues of CYP3A4 namely rCYP3A1 and rCYP3A2, which are both present in the liver and small intestine of the animals. Assessing the expression of rCYP3A1 in rats that are genetically modified to express the human OATP2B1 (*SLCO2B1*$^{+/+}$) or rats that lack the gene of the transporter (*rSlco2b1*$^{-/-}$), we observed higher levels in the liver of the humanized animals. Going one step further, we wanted to verify that changes in the hepatic expression of the enzyme translate into changes of its *in vivo* activity. For that reason, we proceeded to a phenotyping study using the known CYP3A-probe substrate midazolam. Surprisingly, we observed no changes when comparing the formation of the midazolam metabolites α-hydroxymidazolam (1-OH MDZ) and 4-hydroxymidazolam (4-OH MDZ) in *SLCO2B1*$^{+/+}$ *and rSlco2b1*$^{-/-}$ *rats*.

The aim of the proposed master thesis project is to shed light on the unexpected observation in order to acquire more information about OATP2B1 and how it may affect midazolam's disposition. The master student will be exposed to various *in vitro* and *ex vivo* techniques including: isolation of microsomal membranes from liver and small intestine, *ex vivo* enzyme activity measurements with microsomes, *in vitro* transport experiments, and Western blot analysis for protein quantification. Finally the master student will gain a comprehensive hands-on experience with quantification of small molecules using LC-MS/MS.

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