# ABSTRACT

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Title of Doctoral Thesis **Organic Cation Transporter 3 (OCT3/SLC22A3) and Multidrug and Toxin Extrusion 1 (MATE1/SLC47A1) Protein in the Placenta: Expression, Localization and Function.**

The aim of the present study was to investigate the expression, localization, and function of organic cation transporter 3 (OCT3, Slc22a3) and multidrug and toxin extrusion protein 1 (MATE1, Slc47a1) in the rat placenta. Using qRT-PCR, Western blotting and immunohistochemical techniques, we demonstrated abundant expression of OCT3 on the basolateral, i.e., fetus-facing side of the placenta, and MATE1 on the apical, i.e., maternal side of the placenta. To investigate the role of these transporters in the transplacental pharmacokinetics, the in situ method of dually perfused rat term placenta was employed in open- and closed-circuit arrangements; 1-methyl-4-phenylpyridinium (MPP+) was used as a model substrate of both OCT3 and MATE1. We provide evidence that OCT3 and MATE1 cause considerable asymmetry between maternal-to-fetal and fetal-to-maternal transport of MPP+ in favor of fetomaternal direction. Using closed-circuit experimental setup, we further describe the capacity of OCT3 and MATE1 to transport their substrate from fetus to mother even against a concentration gradient. Additionally, employing a range of pH values (6.5, 7.3, and 8.5) on the maternal side of the placenta, we observed that the oppositely directed H+-gradient can drive the secretion of MPP+ from the placenta to mother, confirming MATE1 involvement in MPP+ elimination from trophoblast cells to the maternal circulation.

In the following part of our study, we hypothesized that changes in placental levels of Oct3/OCT3 and Mate1/MATE1 throughout gestation might affect the fetal protection and detoxication. We were able to detect Oct3/OCT3 and Mate1/MATE1 expression in the rat placenta as early as on gestation day (gd) 12 with increasing tendency toward the end of pregnancy. In contrast, comparing the first vs. third trimester human placenta, we observed stable expression of OCT1 and decreasing expression of OCT2,3 isoforms. Contrary to current literature, we were able to detect also MATE1,2 isoforms in the human placenta, however, with considerable inter- and intraindividual variability. Using infusion of MPP+ into pregnant dams we observed that the highest amount of MPP+ reached the fetus on gd 12 while from gd 15 onwards, maternal-to-fetal transport of MPP+ decreased significantly.

In the final part of this study, we investigated the transplacental passage of metformin, which is a substrate of both OCT and MATE transporters; in addition, it is used during pregnancy to treat gestational diabetes mellitus. We observed concentration-dependent transplacental clearance of metformin in both maternal-to-fetal and fetal-to-maternal direction and also the capacity of OCT3 and MATE1 to transport this compound from the fetal to maternal compartment even against its concentration gradient. Furthermore, employing pH values from 6.5 to 8.5 on the maternal side, we observed that the oppositely directed H+-gradient can drive the secretion of metformin from placenta to maternal circulation, confirming metformin elimination from trophoblast cells by MATE1.

We conclude that OCT3, in a concentration-dependent manner, takes up organic cations, such as MPP+ or metformin, from the fetal circulation into the placenta, whereas MATE1, on the other side of the barrier, is responsible for efflux of these compounds from placenta to the maternal circulation. Furthermore, we propose that increasing expression of Oct3/OCT3 and Mate1/MATE1 in the rat placenta during gestation, along with general maturation of the placental tissues results in significantly lower transport of organic cations from mother to fetus. In contrast, decreasing expression of OCT3 and MATE1 in human placenta indicates these transporters may play a role in fetal protection preferentially at earlier stages of gestation. OCT3 and MATE1, thus, form an efficient transplacental eliminatory pathway and play an important role in

the fetal protection and detoxication. This is the first time that OCT3/MATE1 vectorial pathway is described in the placenta.