Publication

Generation and Functional characterization of an OATP2B1 overexpressing MDCK II cell model

Thesis (Dissertationen, Habilitationen)

ID 4627648
Author Nageleisen, Sandra
Author at UniBasel Meyer zu Schwabedissen, Henriette ;
Year 2015
Title Generation and Functional characterization of an OATP2B1 overexpressing MDCK II cell model
Type of Thesis Masterarbeit;
Start of thesis 12.01.2015
End of thesis 05.06.2015
Name of University University of Basel
Name of Faculty Philosophisch-Naturwissenschaftliche Fakultät;
Supervisor(s) / **Fachvertreter/in** Meyer zu Schwabedissen, Henriette ;
Membrane transporters facilitate cellular uptake and/or efflux of compounds thereby contributing to pharmacokinetic mechanisms such as absorption. A membrane transporter that plays a pivotal role in drug transport is the organic anion transporting polypeptide (OATP) 2B1 assumed to be ubiquitously expressed. However, significant levels of OATP2B1 have been observed in the apical membrane of human enterocytes suggesting that OATP2B1 significantly contributes to the intestinal absorption of substrate drugs. For in vitro research several cell models were used to assess the involvement of drug transporters to intestinal absorption. To understand species differences in drug absorption as observed in preclinical animal models such as mouse and rat cellular models for the murine and rat Oatp2b1 would be helpful. It was aim of this study to generate functional MDCK II cell lines overexpressing these transporters. Therefore, eukaryotic vectors containing the coding sequences of Oatp2b1 were generated and used for transfection of MDCK II cells. Positive single cell clones where characterized for their expression of Oatp2b1 and functionality was determined by transport studies. As verified by Western blot analysis, immunofluorescence, and LC-MS/MS we generated a cell line overexpressing rat Oatp2b1 whereas we failed to overexpress mouse Oatp2b1. However, uptake experiments using the model substrate estrone 3-sulfate demonstrated a functionality of rat Oatp2b1. When comparing rat Oatp2b1 and human OATP2B1 we identified equal transport rates characterized by Km and Vmax. In addition, we identified known substrates and/or inhibitors of OATP2B1 involving atorvastatin, cerivastatin, bromosulphophtalien (BSP), or cyclosporine A as inhibitors of estrone 3-sulfate uptake (E1S) in rat Oatp2b1.
Taken together we were able to generate a cell line overexpressing rat Oatp2b1 suggesting it as a suitable system in pharmaceutical research that might serve as a model for functionality of the human OATP2B1.