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Establishment and Characterization of in vitro transporter models

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Membrane transporters play an important role in cellular uptake and efflux of substances. The superfamily of uptake transporters namely solute carriers (SLC) is represented by organic anion transporting polypeptides (OATPs). One family member is OATP2B1 which is highly expressed in hepatocytes and the small intestine. Understanding the mechanism and substrate specificity of transporters is essential for optimal efficacy and safety of a drug. Species-specific differences of drug transporters have been described previously.
Aim of this study was to establish a cell line stable expressing the murine Oatp2b1 and to characterize its expression and function compared to the human orthologue. Western blot analysis of transfected Madin-Darby Kidney Canine (MDCK) II cells demonstrated a difference in expression comparing the isolated clones. However, functional analysis by transport assays using a radiolabelled compound showed contrary data. The uptake of estrone 3-sulfate (E1S), a known substrate of the human transporter, was tested in HeLa cells transiently transfected with Oatp2b1. Subsequent analysis of all MDCK II-mOatp2b1 clones revealed only one positive cell clone (#3) showing a concentration- and time-dependent uptake with strongest accumulation at 30 min. Moreover, accumulation of E1S was inhibited by the known OATP2B1 inhibitor atorvastatin. As the human OATP2B1 demonstrated a pH-dependency in E1S accumulation, cells were incubated with E1S using different pH's demonstrating no difference. Western blot analysis comparing the expression of the murine transporter demonstrated a high expression in liver and kidney. Furthermore, immunohistochemically staining of tissue sections demonstrated luminal localization of OATP2B1 and Oatp2b1 in human and mice small intestine, respectively.
Our study indicated an active murine transporter in MDCK II cells even though we could not detect the expression by Western blot analysis. Further studies are necessary to proof our observations and to compare the activity with the human orthologue.

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