Publication

Optimization of cell culture protocols of HK2 and RPTE cells as cellular models for renal proximal tubule

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The kidneys play a pivotal role in the body's clearance of watersoluble substances. The proximal tubule plays a central role as its main function is the reabsorption of the majority of the primary filtrate and secretion of harmful substances. These processes are mediated by transmembrane transport proteins. Transporters identified in the renal proximal tubule are amongst others organic anion transporters (OAT1, SLC22A6; OAT2, SLC22A7; OAT3, SLC22A8; OAT4, SLC22A11; and OAT10, SLC22A13), organic
cation transporters (OCT1, SLC22A1; OCT2, SLC22A2; and OCT3, SLC22A3), organic cation transporter,
novel, type (OCTN1, SLC22A4, OCTN2, SLC22A5), urate anion exchanger 1 (URAT1, SLC22A12), multidrug
and extrusion protein (MATE1, SLC47A1), Pglycoprotein (Pgp, MDR1, ABCB1), and multidrug resistance
associated protein (MRP2, ABCC2; MRP4, ABCC4).
The aim of this master thesis was to establish an optimized protocol for renal proximal epithelial tubule cells (RPTEC) and human kidney 2 (HK2) cells as a cellular model for the human renal proximal tubule with focus on drug transport. The idea originates in the wellestablished cell model intestine Caco2 for human intestine. We wanted to determine the quality of RPTE and HK2 cells as a good cellular model for
transport assays for the proximal tubule. Additionally we compared HK2 cells and RPTEC to Caki1, Caki 2, RCCEW, and HEK293 partial used as renal models. We conducted quantitative Realtime PCR, Western
blot, and immunofluorescence to compare the expression. In addition, Standard Operation Protocols (SOPs) were composed for TEER value measurements and transport assays and experiments were carried
out accordingly.
TaqManő analysis showed no significant differences in expression of transporter mRNA between RPTEC cultivated on PETmembrane (Transwellő inserts) and tissue culture treated plastic surface. We found no or marginal expressions of organic anion transporters and OCT2 in all investigated samples. OCT3, OCTN1

and 2, Pgp, and MRP2 showed moderate expression in RPTEC on both surfaces. OCT1 and MATE1 expression was rather low compared to the kidney and we measured a high expression of MRP4 especially
in RPTEC. Comparing the expression in cells cultivated on transwell or plastic surface showed that especially the expression of OCT1 and OCT3 was significantly higher in HK2 cells grown on normal cell culture material. Western Blot analysis and immunofluorescence assay verified results gained from TaqManő analysis. Determination of cell layer density as preliminary experiments for transport assays were determined by microscopy and TEER value. We observed that both RPTEC and HK2 cells reached a
monolayer on day 7 followed by formation of leaky multilayers. Therefore, transport assays were conducted on day 7 showing no active transport of the model substrates E1S, MPP+ and PAH through RPTEC and HK2 cells. We assumed that flux is mediated through the cell monolayer as a result of diffusion through the cell layer or the involvement of transport proteins that were not investigated in this study.
To conclude we can state that results of our investigations indicated that human kidney 2 cells are not an
ideal cell line used as a model for the proximal tubule. We further hypothesize that the most eligible candidate for further studies are primary renal proximal tubule epithelial cells further research is necessary with respect to increase confluence and to avoid the loss of transporter expression during cultivation.

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