Abstract

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Title of diploma thesis: Using of genetically modified cells lines to study transport processes in *in vitro* kidney models

Transport processes in renal cells are mostly secured by proximal tubule transporters. These transporters mediate excretion and reabsorption of wide variety of endogenous and exogenous substances, including xenobiotics. Within the broad spectrum of many drug-transporting proteins, we have chosen the glycoprotein megalin (LRP2), a receptor involved in the reabsorption of albumin and other endogenous substances, such as insulin or hemoglobin. Peptide and aminoglycoside antibiotics represented by e.g., gentamicin and belongs between LRP2 ligands.

The diploma thesis deals with the development and utilization of the *LRP2* knockout cell lines in accumulation studies. The experiments were performed using human cell lines naturally expressing LRP2, the JEG-3 and HK-2. Several genetically modified cell lines expressing unfunctional LRP2 and consequently exerting harmed ability of model ligand internalization were prepared using the CRISPR/Cas9 technique. The effect was confirmed by accumulation studies with FITC-albumin. Significant decrease of FITC-albumin accumulation was detected in the modified cell lines.

Another part of the experiments is represented by toxicity studies based on reducing of cytotoxicity of gentamicin due to harmed *LRP2* gene resulting in harmed protein with harmed function and thus decreased accumulation of the antibiotic in the cells. The cytotoxicity studies were performed using modified cells compared to the parent JEG-3 and HK-2 cell lines. Edited cells with reduced ability to internalize LRP2 ligands showed significantly higher viability compared to unmodified cell lines.