Abstract

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Title of diploma thesis:	Study of transport of protein molecules across cell membranes

Kidney transporters are found mainly in the proximal tubules, they participate in the secretion and reabsorption of endogenous and exogenous substances. Plenty of kidney transporters have been identified, however this diploma thesis is focused on megalin. Specifically, we dealed with determination of transport of peptides via this renal transporter in HK-2 and JEG-3 cell lines.

Megalin is a glycoprotein whose main function is tubular reabsorption of albumin filtered in glomerules and other high molecular weight substances. Another endogenous substrates of megalin are e.g. transcoblamine – vitamin B_{12}, insulin or light chains of immunoglobulins. It is also able to transport some drugs like aminoglycoside and polypeptide antibiotics.

Within this work, we investigated the influence of reduced expression of megalin on the transport of albumin and anti-VEGFR2 aptamer. Reduction of expression was achieved by transfection of cells with siRNA against the *LRP2* gene. Lower expression of *LRP2* led to reduction of transport of albumin and anti-VEGFR2 aptamer into cells, which was subsequently determined by accumulation studies. Although the accumulation of used peptides increased with time, it was in lower extent compared to control cells.

In the next part of the thesis, viability of the cell lines HK-2 and JEG-3 was examined after their exposure to increasing concentration of albumin and anti-VEGFR2 aptamer to exclude potential toxic effect of studied substances on the cells. The concentrations of tested substances, which were used in the first part of the experiment, are not toxic for both cell lines, IC_{50} values exceed tested concentration by two orders of magnitude.