Abstract

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Title of doctoral thesis:	Mechanisms of membrane transport of radiolabeled receptor-specific peptides in the kidney

Radiolabelled receptor-specific peptides are a useful tool for radiodiagnostic and radiotherapy of some neuroendocrine tumours. Despite many recent developments in this radiopharmaceutical group, there is still a lack of relevant information about the mechanisms determining the pharmacokinetics and describing interactions with membrane transport systems. These transporters may be responsible for undesirable renal accumulation and subsequent radiotoxic kidney damage, which significantly limited the use of radiopeptides in nuclear medicine.

The aim of our work was to assess the role of several transport mechanisms that can be potentially involved in the transmembrane transport of radiopeptides in the renal tissue. We mainly focused on studying the role of megalin mediated active endocytosis, fluid phase endocytosis and important human renal SLC transporters - hOAT1 and hOCT2. Simultaneously, the potential transport of selected radiometabolites of these peptides was studied *in vitro* and their biodistribution and renal excretion were studied *in vivo*. The project also included a pilot testing of potential interactions of radiopeptides with the important efflux transporters P-glycoprotein and BCRP.

The study analysed *in vitro* transport mechanisms of six radiopeptides from the group of somatostatin, gastrin, and bombesin analogs and two model radiopeptide metabolites - ^{177}Lu-DOTA-*D*Glu-Ala-Tyr, which is degradation product of ^{177}Lu-DOTA-minigastrin 11, and ^{177}Lu-DOTA-*D*Phe, which is the potential metabolite of somatostatin analogs such as ^{177}Lu-DOTA-NOC. Using cell lines, an inhibition study was carried out to reveal the transport mechanisms with megalin ligands, rottlerin as fluid phase inhibitor and low incubation temperature. The role of SLC transporters was tested by accumulation studies with transiently transfected cells overexpressing hOAT1 or hOCT2. *In vivo* study compared biodistributional

and elimination characteristics of two parent radiopeptides and their potential radiometabolites in rats after i.v. administration.

Ligands of endocytic receptor megalin reduced significantly the accumulation of all studied radiopeptides. Incubation with rottlerin caused concentration-dependent inhibition of cell accumulation of all studied peptides with only one exception.

Accumulation studies demonstrated no contribution of hOCT2 or hOAT1 to intact radiopeptides transport. Unlike metabolite ^{177}Lu-DOTA-*D*Glu-Ala-Tyr, which also showed no interaction with SLC transporters, in the case of ^{177}Lu-DOTA-*D*Phe uptake by hOCT2 was demonstrated *in vitro*. ^{177}Lu-PCTA-[Lys^3]bombesin weakly interacted with the BCRP transporter, peptides chelated DOTA showed no interaction with efflux transporters. The pharmacokinetics of both studied radiometabolites in rats has been characterized by relatively rapid elimination of radioactivity from the blood and body with predominant renal excretion. Uptake of the radiometabolites in receptor-positive tissues was expectable lower than that of the intact radiopeptides. Long-term retention of smaller radiometabolite ^{177}Lu-DOTA-*D*Phe was lower than in the case of tripeptidic metabolite ^{177}Lu-DOTA-*D*Glu-Ala-Tyr. Renal accumulation of radiometabolite ^{177}Lu-DOTA-*D*Glu-Ala-Tyr was comparable to parent peptide, accumulation of ^{177}Lu-DOTA-*D*Phe was significantly lower compared to intact peptide ^{177}Lu-DOTA-NOC.

The results have proved the decisive role of active transport mechanisms in the accumulation of all tested intact radiopeptides in the used cell lines. Fluid endocytosis may also participate in cellular uptake besides active endocytosis mediated by megalin system. Tested SLC transporters have probably no significant role in this process. The experimental data we have obtained the first insight into the systemic disposition of the radiometabolites of receptor-specific radiopeptides in the body and show that the SLC transporters may participate in renal transport of their advanced degradation products. In contrast, the results of pharmacokinetic studies we have performed suggest that the undesirable renal accumulation and retention of extrarenal metabolites could be lower compared to parent radiopeptides. The experimental data may suggest a negative correlation between the degree of peptide chain degradation and renal retention of radiometabolites.