

Comparison of renal accumulation of [DOTA⁰, 1-Nal³]-octreotide labelled with selected radiometals

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SUMMARY

Comparison of renal accumulation of [DOTA⁰, 1-Nal³]-octreotide labelled with selected radiometals

Radiolabelled somatostatin receptor-specific peptides are a useful tool in the diagnosis and/or radiotherapy of somatostatin-positive neuroendocrine tumours. However, significant renal uptake of the radiopeptides may result in radiotoxicological injury of the kidney. The aim of the study was to analyze the undesirable renal accumulation of a newly developed somatostatin receptor-specific peptide, [DOTA⁰, 1-Nal³]-octreotide (DOTA-NOC), labelled with ¹⁷⁷Lu, ¹¹¹In or ⁹⁰Y. To quantify the accumulation rate of the radiopeptides, the renal uptake of ¹⁷⁷Lu-DOTA-NOC, ¹¹¹In-DOTA-NOC and ⁹⁰Y-DOTA-NOC was compared with the accumulation of selected compounds known to be intensively concentrated in the renal cells, such as albumin or ^{99m}Tc-MAG3. The investigation studied the renal uptake *in vitro* at the cellular level using freshly isolated rat renal cells. The found results did not reveal a significant influence of the used radiolabel on the uptake in the rat renal cells. The studied radiopeptides exerted a lower renal uptake than the selected comparators. Nevertheless, the rate of accumulation of the studied radiopeptides in the renal rat cells seemed to be quantitatively relatively high. The results of the experiments confirmed that the used *in vitro* method might serve as a helpful tool for comparative studies or analyses of transport mechanisms in radiopharmaceuticals.

Key words: somatostatin analogues – radiodiagnostic – radiotherapeutics – renal elimination

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SOUHRN

Srovnání ledvinné akumulace [DOTA⁰, 1-Nal³]-oktreotidu značeného vybranými kovovými radionuklidy

Radioaktivně značené receptorově specifické peptidy jsou užitečným prostředkem při radiodiagnostice a radioterapii neuroendokrinních tumorů exprimujících somatostatinové receptory. Avšak jejich výrazný záchyt v ledvinách může vést k radiotoxickému poškození ledvinné tkáně. Cílem této studie bylo analyzovat tuto nežádoucí ledvinou akumulaci u nově vyvinutého somatostatinového analogu [DOTA⁰, 1-Nal³]-oktreotidu (DOTA-NOC) značeného ¹⁷⁷Lu, ¹¹¹In nebo ⁹⁰Y. Pro kvantifikaci míry akumulace radiopeptidů bylo vychytávání ¹⁷⁷Lu-DOTA-NOC, ¹¹¹In-DOTA-NOC a ⁹⁰Y-DOTA-NOC v ledvinách porovnáváno s akumulací vybraných látek, které jsou výrazně koncentrovány v ledvinných buňkách, jako jsou albumin nebo ^{99m}Tc-MAG3. Zkoumání probíhalo na buněčné úrovni *in vitro* s využitím čerstvě izolovaných ledvinných buněk potkana. Získané výsledky neprokázaly významný vliv použitého radionuklidu na vychytávání v potkaních ledvinných buňkách. Zkoumané radiopeptidy vykazovaly nižší míru záchytu než zvolené komparátory. Míra vychytávání studovaných radiopeptidů v ledvinných potkaních buňkách se přesto jeví jako poměrně vysoká. Výsledky experimentů potvrdily, že použitá *in vitro* metoda může sloužit jako užitečný prostředek při srovnávacích studiích nebo analýze transportních mechanismů radiofarmak.

Klíčová slova: somatostatinové analogy – radiodiagnostika – radioterapeutika – ledvinná eliminace

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Introduction

Radiolabelled receptor-specific peptides are a relatively new group useful in several areas of nuclear medicine ^{1, 2}. Overexpression of somatostatin receptors on various tumour cells provided the molecular basis for the successful use of radiolabelled somatostatin analogues as tumour tracers. Several somatostatin analogues are used or tested as radiodiagnostics or targeted radiotherapeutics of somatostatin-positive neuroendocrine tumours ^{3, 4}. Analogues such as octreotide with enhanced binding to the somatostatin receptor and reduced metabolic degradation through peptidases were developed. Incorporation of a chelator moiety, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), in the octreotide molecule, a convenient compound enabling labelling with indium-111 or other radionuclides, was obtained. This group was used in connection with Tyr³-octreotide for the formulation of a newer potential radiodiagnostic agent from the group of somatostatin derivatives, [¹¹¹In-DOTA,Tyr³]-octreotide and [¹¹¹In-DOTA,Tyr³]-octreotate^{5, 6}. A further modification of the octapeptide octreotide in position 3 resulted in a high affinity ligand of somatostatin receptors, [DOTA⁰,1-Nal³]-octreotide (DOTA-NOC), useful for labelling with various radiometals ^{7, 8}. However, significant renal uptake of the radiopeptides may result in radiotoxicological injury of the kidney which limits the clinical use of the peptides ^{9, 10}. Recent experimental observations have suggested that the multiligand megalin/cubilin receptor complex could be the mechanism responsible for undesirable accumulation and retention of the radiolabelled peptides ^{11, 12}. However, a more detailed knowledge of renal handling of the radiopeptides is necessary to develop compounds possessing lower renal toxicity and to increase clinical effectiveness of the agents.

The aim of the study was to analyze the renal accumulation of a newly developed radiolabelled somatostatin receptor-specific peptide, [DOTA⁰, 1-Nal³]-octreotide (DOTA-NOC), labelled with ¹⁷⁷Lu, ¹¹¹In or ⁹⁰Y. The investigation studied the renal uptake *in vitro* at the cellular level using freshly isolated rat renal cells. The rate of the renal uptake of ¹⁷⁷Lu-DOTA-NOC, ¹¹¹In-DOTA-NOC and ⁹⁰Y-DOTA-NOC in the renal cells was compared with the accumulation rate of selected compounds known to be intensively concentrated in the renal cells.

EXPERIMENTAL PART

Chemicals

[DOTA⁰, D-Phe¹, 1-Nal³]-octreotide (DOTA-NOC) was purchased from piCHEM (Graz, Austria). ¹¹¹InCl₃ was purchased from GE Healthcare Ltd. (UK). ¹⁷⁷LuCl₃

and ⁹⁰YCl₃ were purchased from Perkin Elmer (Boston, USA). MAG3 (mercaptoacetylglycylglycylglycine) was obtained from Nuclear Research Institute (Řež, Czech Republic) and labelled with ^{99m}Tc according to the instructions of the producer for the kit.

Radiolabelling of peptides and quality control

DOTA-NOC labelled with ¹⁷⁷Lu was prepared using the following method: to 200 µl of 0.4 M acetate buffer (pH 5) with 0.24 M gentisic acid, 10 µg peptide in 10 µl of H₂O and ~ 20 MBq of ¹⁷⁷LuCl₃ in 0.04 M HCl were successively added. The solution was then incubated at 90–95 °C for 25 min. Radiolabelling of the peptide with ¹¹¹In or ⁹⁰Y was performed analogically. Radiochemical purity analysis using HPLC was performed on a column of LiChroCART 125-4 HPLC Cartridge Purospher RP18e, 5 mm (Merck), using a Pharmacia LKB system linked to a Gradient Master GP 962 (Institute of Organic Chemistry and Biochemistry, Prague) and operated with a UV detector and a radioactivity monitoring analyser. Mobile phases A (0.1% TFA) and B (CH₃CN) were used with the following gradient: 0–5 min 0% B, 5–25 min 0–30% B, 25–30 min 30% B, 30–35 min 30–100% B, 35–40 min 100% B. The flow rate was maintained at 0.5 ml/min.

Isolated rat renal cells

Male Wistar rats weighing 280–320 g were used. The rats were housed under standard conditions (tap water and standard diet, light cycle 12/12 h). Animals were fasting 18–24 h prior to the experiments.

The fresh rat renal cells were isolated from the kidney by means of the two-phase collagenase perfusion method as described by Jones et al. ¹³. The procedure of isolation was performed according to a method published previously ¹⁴.

Standard incubations were carried out for 30 min at 37 °C using 1 ml of cell suspension containing 2.10⁶/ml renal cells. Uptake of the radiopeptide was determined by mixing 1 ml cell suspension and 10 µl of radiolabelled peptide (1 µg/ml). After 30 minutes of incubation, 4 ml of ice-cold Krebs-Henseleit buffer was added to the mixture and the cells were separated from the medium by centrifugation (1 min; 120 g; 4 °C). Following centrifugation, the supernatant was carefully aspirated and the cells washed again with buffer. Washing of the cells, centrifugation and aspiration of the supernatant were repeated four times under the same conditions. The radioactivity of the cell fractions was then measured as mentioned below. The uptake of the radiolabelled peptides in the renal cells was expressed as the percentage of radioactivity remaining in the cell fraction. In addition to the incubations at 37 °C, experiments at low incubation temperature (2 °C) were performed to test the role of active transport processes in the renal uptake of the tested radiopeptides.

The rate of accumulation of the radiopeptides in the isolated renal cells was compared to that of several model substances (albumin, ^{99m}Tc-MAG3) with known high uptake in the renal tubular cells. The uptake of the

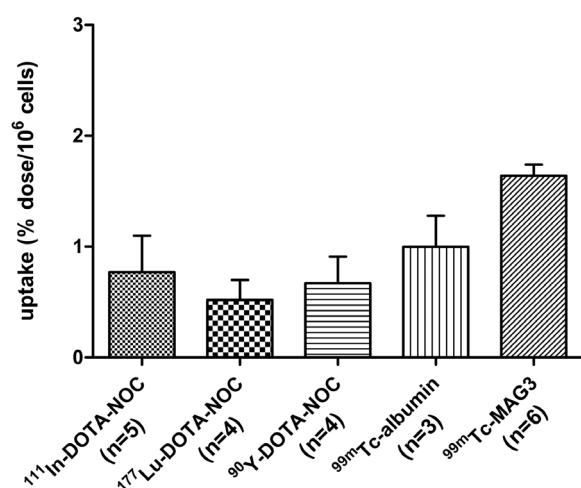


Fig. 1. Uptake of ¹¹¹In-DOTA-NOC, ¹¹¹Lu-DOTA-NOC, ⁹⁰Y-DOTA-NOC and comparative compounds in the isolated rat renal cells (mean $\pm$ S.D.)

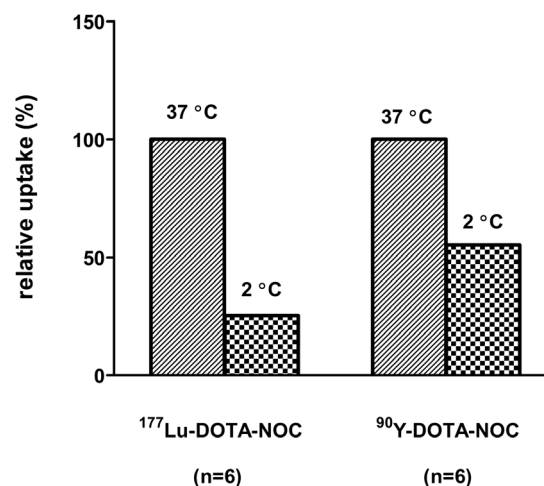


Fig. 2. Relative uptake of ¹⁷⁷Lu-DOTA-NOC and ⁹⁰Y-DOTA-NOC in the rat renal cells under normal (100%) or lower incubation temperature

model transport markers was tested and expressed using the same procedure as in the case of radiopeptides.

Radioactivity measurement

The ¹¹¹In-, ⁹⁰Y- and ¹⁷⁷Lu-activity in biological samples was measured by a gamma-counter Wallac 1480 Wizard 3 (Wallac, Turku, Finland).

RESULTS

The uptake of the studied radiopeptides in the isolated renal rat cells at 37 °C is compared in Figure 1. Although some differences in the rate of accumulation were found, the uptake seems to be comparable in all three radiopeptides. The found differences between renal accumulation of ¹⁷⁷Lu-DOTA-NOC, ¹¹¹In-DOTA-NOC and ⁹⁰Y-DOTA-NOC were not statistically significant. The found experimental data also documented that the rate of the cellular uptake of the radiolabelled somatostatin analogues was lower than that of both selected comparators, ^{99m}Tc-albumin and ^{99m}Tc-MAG3 (Fig. 1).

The results showed a considerable decrease in the accumulation rate of the radiopeptides in the case of incubation at 2 °C (Fig. 2). ¹⁷⁷Lu-DOTA-NOC uptake after a 30 minute incubation of the renal rat cells at 2 °C was only 25% of the values found in control samples incubated at 37 °C. In ⁹⁰Y-DOTA-NOC, 55% of the control values was observed in the cell fraction following incubation at 2 °C (Fig. 2).

DISCUSSION

Radiolabelled peptides potentially useful in radiodiagnosis or targeted radiotherapy such as

somatostatin analogues are in most cases excreted from the body via the kidney. Therefore, their concentrations in the urine and in the kidney may be relatively high. This pharmacokinetic behaviour is a base for possible undesirable uptake and retention of the radiolabelled peptides. A high accumulation in the kidney may result in a consequent radiotoxic damage of the renal tissue. Experimental studies on the radiopeptide accumulation in the kidney may be successfully performed *in vitro* using selected cell lines such as OK (opossum kidney) cells ¹⁵. The freshly isolated renal cells were used in toxicological *in vitro* studies or in experimental studies on drug metabolism. The present paper has used this method in an experimental study in radiopharmacy. In comparison to cell lines, the fresh isolated cells may have some advantages, e.g., they exerted very similar functions as the natural cells. On the other hand, a disadvantage could be a limited interval for the use of the cells following their preparation due to lowering of cell viability. According to a recently published interspecies comparison, rats appear to be a favourable species for the study of renal retention of radiolabelled peptides ¹⁶. The results of the previous experiments suggest that this method might serve as a helpful tool for comparative studies or analyses of transport mechanisms of radiopharmaceuticals.

The experimental results showed that all the tested radiopeptides are accumulated in the renal rat cells. ¹¹¹In-DOTA-NOC exerted a relatively highest and ¹⁷⁷Lu-DOTA-NOC the lowest accumulation in the rat renal cells. However, the found differences among the radiopeptides were not significant. This observation means that there is probably a relatively low influence of the type of the radiolabel on renal uptake. The mechanism of renal accumulation is usually explained as a process including several transport and metabolic steps. The radiolabelled peptides are primarily filtered in the glomeruli and subsequently partly reabsorbed into the cells of the proximal tubules. Thereafter, the

agents are transferred into lysosomes by means of pinocytosis, wherein they are degraded by proteolytic enzymes. Most often, breakdown products, namely radiolabelled chelate-conjugated amino acids, cannot leave the lysosomes and remain trapped in the proximal tubular cells^{17, 18)}. Low-molecular-weight peptides can be taken into the renal tubular cells by several mechanisms. The peptides may enter the cells by low efficient fluid-phase endocytosis or the highly efficient receptor-mediated endocytosis by the megalin/cubilin tandem receptor¹⁵⁾. Another potential mechanism might be the specific somatostatin receptor-mediated influx. Especially, the role of specific somatostatin receptor 2 (SSTR2) may be important since radiolabelled DOTA-NOC binds to this receptor with high affinity^{19, 20)}. Inhibition of energy-dependent transport processes at low temperature resulted in a significant decrease in the uptake in the DOTA-NOCs under study. This finding documents a participation of active transport processes in the influx of the radiopeptides into renal cells. However, temperature-dependent inhibition of the renal uptake of the radiopeptides was only partial. It means that a part of the radiopeptides enters the intracellular space via a passive transport mechanism(s).

The uptake of the radiopeptides in isolated rat renal cells was compared with the rate of uptake of two radiolabelled compounds with known transport mechanism into cells. Albumin is intensively reabsorbed in the renal proximal tubules and transported into renal cells by active endocytosis mediated by the megalin/cubilin system. Albumin presents a typical substrate of this endocytic receptor²¹⁾. ^{99m}Tc-MAG3 is a compound intensively accumulated in the renal tissue via a transport system for organic anions²²⁾. The studied radiopeptides exerted a lower renal uptake than ^{99m}Tc-albumine or ^{99m}Tc-MAG3. On the other hand, in general, the rate of the uptake of the DOTA-NOCs in the renal rat cells seems to be quantitatively not so different from that of the comparators used. This finding showed that the renal uptake of the radiopeptides *in vitro* could be in some cases relatively intensive and comparable with that of highly accumulated compounds.

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