Comparison of ^{111}In-DOTA-NOC and ^{111}In-DOTA-TATE Distribution in the Target and Dose-limiting Tissues: Conflicting Results *In Vitro* and *In Vivo*

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Abstract. *Background: In this study, some important biological characteristics of two radiolabelled somatostatin analogues ^{111}In-DOTA-1-Nal^3-octreotide (DOTA-NOC) and ^{111}In-DOTA-Tyr^3-octreotate (DOTA-TATE) were compared. Materials and Methods: Rats were used for in vivo biodistribution experiments and in vitro cell models (OK and AR42J cell lines) were used for simulating the internalization in the kidney and in subtype 2 somatostatin receptor (SSTR2)-positive tissues, respectively. Results: Significantly higher radioactivity concentrations in rat organs with high density of somatostatin receptors after ^{111}In-DOTA-NOC administration in comparison with ^{111}In-DOTA-TATE were observed. The predominant urine excretion was associated with accumulation of the radioactivity in the kidney, where higher retention of ^{111}In-DOTA-TATE compared to ^{111}In-DOTA-NOC was detected. In the OK cell line the opposite results were found. No significant differences in the in vitro internalization and externalization of radioactivity to AR42J cell line were found for either peptide suggesting their same affinity for SSTR2. Conclusion: Preclinical experiments indicated that ^{111}In-DOTA-NOC is a very promising peptide for somatostatin receptor-positive tumour visualization. The conflict between the in vitro and in vivo kidney handling showed that the transfer of results from in vitro to in vivo conditions and their interpretation should be performed very carefully because both types of experiments can be affected by different factors, making their simple comparison difficult.*

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Key Words: Somatostatin analogues, ^{111}In-DOTA-1-Nal^3-octreotide, ^{111}In-DOTA-Tyr^3-octreotate, tumour targeting, renal handling, biodistribution, AR42J cells, OK cells.

Radiolabelled somatostatin (SST) analogues have great potential for visualization and treatment of somatostatin receptor (SSTR)-positive tumours and their metastases that are predominantly of neuroendocrine origin.

A sufficiently long biological half-life, the possibility of labelling with various radionuclides and, in particular, the ability of SSTR-positive tumour cells to specifically bind and internalize radiolabelled SST analogues are among the crucial conditions for *in vivo* targeting of tumour cells with a high density of SSTR. Whereas some SST analogues labelled with gamma emitters such as indium-111-(^{111}In) pentetreotide (^{111}In-DTPA-octreotide, Octreoscan®) or technetium-99m(^{99m}Tc)-depreotide (NeoTect™) are commercially available and clinically successfully used for visualisation of different types of tumours and their metastases, therapeutic application of SST analogues labelled with β-emitting particles (lutetium-177, yttrium-90) is accompanied by occurrence of delayed radiation-induced toxicity to kidney, bone marrow and liver (1, 2). The kidney is considered to be the dose-limiting organ for treatment of SSTR-positive diseases (3). According to published studies, nephrotoxicity can be caused by proximal tubular cell reuptake by nonspecific endocytic megalin receptor after glomerular ultrafiltration and long-term retention of radioactivity in the kidney (4-6). However, compounds that are able to reduce renal uptake such as the amino acids lysine and arginine, polyglutamic acid (5 or more polyglutamic acids residues), or gelofusine (gelatine plasma expander) do not exclude the assistance of other transport systems participating in the reuptake in the kidney (6-10).

Because of the unequal distribution of SSTR subtypes (SSTR1-5) in SSTR-positive malignancies (SSTR2 and SSTR5 are the most frequent in neuroendocrine tumour cells) and the varying affinity of SST analogues for these receptor subtypes, variations in the structures of well-tried analogues (octreotide, lanreotide, octreotate) are made to increase the binding affinity for all of the five known receptor subtypes (11-13). In 2003, Reubi *et al.* reported 1,4,7,10-tetraazacyclododecane-*N, N', N''*,

N''', tetraacetic acid (DOTA)-1-Nal^3-octreotide (DOTA-NOC) to be a high affinity ligand of SSTR subtypes 2, 3 and 5 (IC_{50} 2.9±0.1 nM, 8±2 nM, 10.4±1.6 nM, respectively) (14). Because there is a possibility that changes in the structure can affect not only the specific binding to SSTRs but also the tissue distribution in the body, here we determined SSTR2 internalization as well as biodistribution characteristics (focused predominantly on the kidney) of the relatively new SST analogue ^{111}In-labelled DOTA-NOC. The results were compared with those of the well established ^{111}In-DOTA-Tyr^3-octreotate (DOTA-TATE). It is known that this SST analogue has excellent affinity to SSTR2 (IC_{50} of DOTA-Tyr^3-octreotate is 1.5±0.4 nM), but low or no affinity to other SSTRs (11).

The aim of this work was to compare the biological characteristics of the two somatostatin analogues DOTA-TATE and DOTA-NOC with the view to assess the merits of the latter peptide for tumour visualization and therapy. For biodistribution experiments, male Wistar rats were used. The organs bearing a high density of SSTRs (namely the pancreas and adrenals) served as an endogenous marker of the agent's affinity to SSTRs *in vivo*. For comparison of the SSTR internalization/externalization characteristics, a rat pancreatic tumour cell line, AR42J, that is known to express SSTR2 was used. To predict the potential risk of investigated peptides in the kidney, an uptake experiment in the opossum kidney (OK) cell line was carried out, in an established model for studying renal tubular transport (4, 15-18).

Materials and Methods

Reagents. RPMI-1640 medium, minimum essential medium (MEM), foetal calf serum (FCS), L-glutamine, nonessential amino acids (NEAA), ethylenediaminetetraacetic acid (EDTA)/trypsin, 3-(morpholino)-propanesulphonic acid (MOPS), *N*-(2-hydroxyethyl)-piperazine-*N*'-(2-ethanesulphonic acid) (HEPES), Triton®-X-100 and chemicals used for radiolabelling, high-performance liquid chromatography (HPLC) analysis and instant thin-layer chromatography on silica gel (ITLC-SG) were obtained from Sigma-Aldrich, Czech Republic. Peptides DOTA-TATE and DOTA-NOC were purchased from piCHEM, Gratz, Austria; OK and AR42J cell lines from ECACC, Salisbury, UK; and Indium [^{111}In] Chloride Solution was purchased from GE Healthcare, Amersham, UK. Octreotide (Sandostatin®) was a commercial product of Novartis, Basel, Switzerland. ITLC-SG was performed on commercial SG impregnated glass fibre sheets (Gelman Sciences, Michigan, USA). Bicinchoninic acid assay (BCA) kit was from (Pierce, Rockford, USA).

Ringer solution was composed of (mM): NaCl 122.5, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 0.8, Na_2HPO_4 0.8, NaH_2PO_4 0.2, glucose 5 and HEPES 10 (titrated to pH 7.4 by NaOH at 37˚C). Phosphate-buffered saline (PBS) was composed of (mM): NaCl 137, KCl 2.7, Na_2HPO_4 0.01 and NaH_2PO_4 0.01 (titrated to pH 7.4). Acid wash buffer was composed of 50 mM glycine buffer pH 2.8, and 0.1 M NaCl.

Radiolabelling and radiochemical purity analysis. Two hundred microlitres of 0.4 M acetate buffer (pH 5) with 0.24 M gentisic acid, 10 μg DOTA-TATE or DOTA-NOC in 10 μl of H_2O and 0.5 mCi $^{111}InCl_3$ in 0.04 M HCl were sequentially added to a vial. The solution was mixed well and then incubated at 90-95˚C for 25 min. For determination of radiochemical purity, RP-HPLC analysis (HPLC System Agilent 1100 Series with gamma radiation and UV detector) (Agilent Technologies Inc., Santa Clara, CA, USA) was conducted on a Waters RP18 Symmetry Shield column (Waters Corp., Milford, MA, USA) with gradient elution with 0.1% trifluoroacetic acid and 3% acetonitrile as mobile phase A. Mobile phase B contained 90% acetonitrile in water. The elution sequence was 0-5 min of 0% B, 5-25 min of 0-30% B, 25-30 min of 30% B, 30-33 min of 30-100% B, 33-38 min of 100% B and 38-40 min of 100-0% B. Samples for HPLC analysis were prepared by dissolving of 2 μl of radiolabelled peptide solution in 100 μl of mobile phase A together with 10 μl of 1×10^{-3} M DTPA. ITLC-SG analysis was used for eliminating the possibility of radioactive particulate contamination and was performed on silica gel impregnated glass fibre sheets with 10% ammonium acetate and methanol 1:1 as the eluent. Detection of ITLC-SG strips was carried out by TLC-analyzer (Raytest, Straubenhardt, Germany).

Distribution studies in rats. Male Wistar rats weighing 200-250 g were used for biodistribution experiments. The animals were fasted overnight before the experiment (to empty the bowels) but had free access to water. The radiopharmaceutical was administered to rats intravenously in a volume of 0.2 ml (0.8 1 μg of the peptide per kg of body weight). For determination of receptor-mediated binding in the organs and tissues of rats, together with control samples, two groups of animals were pretreated by intravenous injection of 100 μg octreotide (Sandostatin®) per kg 15 min before ^{111}In-DOTA-TATE or ^{111}In-DOTA-NOC were administered. During the course of experiments, the animals were placed singly in cages. At selected times (5 min, 1 h, 2 h, 24 h and 48 h) after dosing, the carotid artery was exposed under ether anaesthesia and a blood sample was collected in glass tubes containing dry heparin. After exsanguination, the tissues and organs of interest were removed and weighed, and their radioactivity was measured. In the first pretreated group of animals, the distribution of radioactivity after 2 h of dosing was determined. The second group was used for elimination studies and for the determination of radioactivity distribution at 48 h after administration of the radiopharmaceuticals.

All animal experiments were approved by the Ethics Committee of the Faculty of Pharmacy, Charles University.

Internalization/externalization experiments in AR42J cells. AR42J cells were grown in RPMI-1640 supplemented with 2 mM L-glutamine and 10% FCS in a 5% CO_2 atmosphere at 37˚C. Subculturing was performed employing a (EDTA)/trypsin solution. Experiments were performed at passages 20-32.

For internalization studies, the cells were treated with (EDTA)/trypsin solution and concentrated to 1×10^6 cells per ml of internalization medium (RPMI 1640 supplemented with 2 mM L glutamine and 1% FCS) per microcentrifuge tube. Incubation was started by the addition of 1 ng of radiolabelled peptide per tube (0.66 nM). Cells were incubated at 37˚C in triplicates for both peptides for the indicated time periods. Cellular uptake was stopped by removal of the internalization medium and washing of the cells with ice-cold PBS (2x). Thereafter, the cells were incubated twice at ambient temperature in acid wash buffer for 5 min. The supernatant was removed and cells were lysed by treatment of 1 M NaOH and cell radioactivity collected (internalized radioligand fraction).

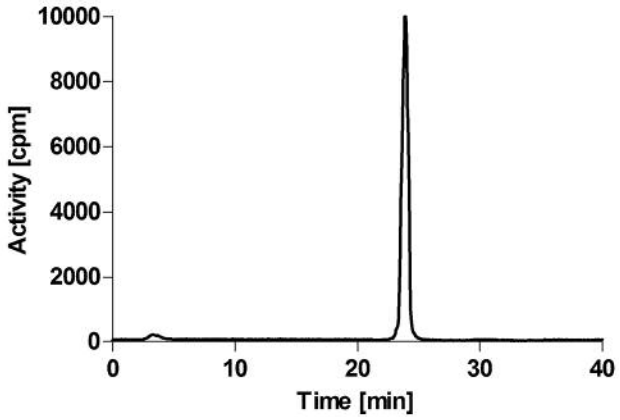

Figure 1. *HPLC radioactivity profile of ^{111}In-DOTA-NOC.*

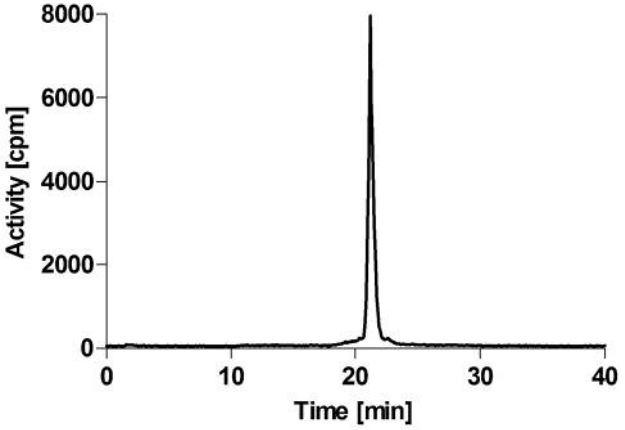

Figure 2. *HPLC radioactivity profile of ^{111}In-DOTA-TATE.*

For externalization studies AR42J cells (1×10^6) were incubated with 1 ng of radiolabelled peptide for 120 min. The medium was then removed and the cells were washed with ice-cold PBS. Thereafter the cells were incubated twice at ambient temperature in acid wash buffer for 5 min. Cells were then incubated again at 37˚C with fresh externalization medium (RPMI-1640 supplemented with 2 mM L-glutamine and 1% FCS). At indicated time points the external medium was removed for quantification of radioactivity and replaced with fresh externalization medium.

Uptake experiments in OK cells. Renal OK cells were grown in plastic 75 cm^2 culture flasks in MEM supplemented with 2 mM L-glutamine, 1% NEAA and 10% FCS in a 5% CO_2 atmosphere at 37˚C. Confluent monolayers were split 1:6 twice a week using (EDTA)/trypsin solution. For experiments, OK cells (passage number 55 62) were grown to confluency on plastic Petri dishes (6 cm diameter). Confluent monolayers were washed (2x) first with PBS and incubated simultaneously for indicated intervals (0, 15 min, 30 min, 1 h, 2 h and 3 h) with the same concentration (1 nM) of ^{111}In-DOTA-TATE and ^{111}In-DOTA-NOC in Ringer solution (37˚C). At the end of the incubation, the uptake buffer was discarded and the dishes with cell monolayers were rapidly rinsed with ice-cold PBS (6x). The cells were disintegrated by Triton® X-100 (0.1% v/v) in 10 mM MOPS. To elicit the measuring error caused by nonspecific sorption to the cells and Petri dishes, the radioactivity uptake at time 0 was used as a blank value where the internalization medium was discarded immediately after addition. The intracellular radioactivity was normalized to the cell protein content by the BCA method.

Measurement of ^{111}In activity. ^{111}In activity was measured by a gamma spectrometer 1480 Wizard™ 3˝ (Wallac, Finland). Radioactivity of all measured samples was compared with those of standard samples.

Results

Radiochemical purity of the peptides under study. The HPLC analysis of ^{111}In-DOTA-NOC and ^{111}In-DOTA-TATE showed single peaks with elution times of 23.8 min and 21.2 min, respectively (Figures 1 and 2). Radiochemical purity was over 98% in all cases.

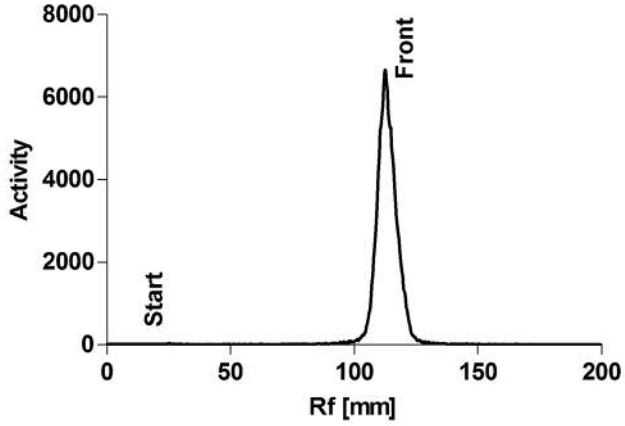

Figure 3. *An example of ITLC-SG radioactivity profile (^{111}In-DOTA-TATE).*

In the ITLC-SG analysis, respectively in the system that was used for this purpose, labelled peptide moved with the solvent and the unbound radiometal remained at the start of the chromatograph (Figure 3). Radiochemical purity determined by this method was greater than 98% , identical to the results measured by HPLC.

Distribution studies in rats. The distribution (% administered dose per g of organ or tissue) of ^{111}In-DOTA-TATE and ^{111}In-DOTA-NOC in blood and selected organs of rats at intervals from 5 min to 48 h is summarized in Tables I and II. Each value represents the mean±standard deviations of a minimum of four animals.

Both agents exhibited rapid radioactivity clearance from the blood and most organs and tissues (spleen, testes, brain, thyroid, lung, femur, skin, abdominal fat, muscle). The long-

Table I. *Distribution of ^{111}In-DOTA-NOC in selected organs and tissues of rats. Data are expressed as mean±SD.*

^{111}In-DOTA-NOC (% administered dose/g organ or tissue)							
Organ	5 min	1 h	2 h	2 h Pretreated	24 h	48 h	48 h Pretreated
Blood	1.15±0.16	0.28±0.05	0.06±0.01	0.05±0.01	≤0.01	≤0.01	≤0.01
Plasma	2.09±0.36	0.51±0.09	0.11±0.02	0.09±0.03	≤0.01	≤0.01	≤0.01
Pancreas	**6.17±1.37**	**12.02±2.50**	**9.02±3.49**	**1.33±0.23**	**6.01±1.25**	**4.38±0.60**	**1.11±0.23**
Liver	0.59±0.15	0.38±0.06	0.23±0.05	0.19±0.06	0.25±0.02	0.17±0.03	0.19±0.04
Adrenals	**12.52±0.91**	**32.01±12.89**	**22.49±2.96**	**2.60±0.39**	**18.51±5.68**	**14.44±2.72**	**5.24±2.28**
Lung	1.04±0.21	0.34±0.02	0.12±0.02	0.08±0.02	0.07±0.03	0.04±0.02	0.02±0.01
Kidneys	**6.73±2.56**	**2.84±0.28**	**2.13±2.29**	**1.84±0.60**	**2.21±0.16**	**2.10±0.34**	**2.30±0.46**
Heart	0.62±0.13	0.17±0.03	0.04±.001	0.03±0.01	0.02±0.00	0.01±0.00	0.01±0.01
Spleen	0.59±0.20	0.23±0.08	0.17±0.16	0.07±0.03	0.24±0.18	0.09±0.04	0.07±0.01
Stomach	1.05±0.21	1.08±0.14	1.47±1.01	0.28±0.20	0.89±0.05	0.47±0.22	0.20±0.05
Intestine	0.51±0.20	0.50±0.26	0.75±0.67	0.25±0.22	0.25±0.03	0.17±0.01	0.06±0.02
Colon	0.35±0.30	0.29±0.08	0.25±0.09	0.07±0.02	0.88±0.24	0.40±0.11	0.22±0.07
Testes	0.13±0.03	0.09±0.01	0.03±0.00	0.02±0.01	≤0.01	≤0.01	≤0.01
Skin	0.49±0.06	0.19±0.03	0.07±0.02	0.05±0.02	0.04±0.00	0.02±0.00	0.02±0.00
Muscle	0.25±0.09	0.06±0.01	0.02±0.00	0.01±0.01	≤0.01	≤0.01	≤0.01
Thyroid	1.08±0.27	0.44±0.09	0.20±0.05	0.07±0.03	0.07±0.01	0.05±0.00	0.03±0.01
Brain	0.05±0.01	0.02±0.00	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
Body fat	0.41±0.18	0.08±0.01	0.03±0.01	0.02±0.01	0.02±0.01	≤0.01	0.01±0.01
Femur	0.44±0.12	0.43±0.10	0.23±0.04	0.29±0.39	0.33±0.03	0.20±0.02	0.09±0.03

Table II. *Distribution of ^{111}In-DOTA-TATE in selected organs and tissues of rats. Data are expressed as mean±SD.*

^{111}In-DOTA-TATE (% administered dose/g organ or tissue)							
Organ	5 min	1 h	2 h	2 h Pretreated	24 h	48 h	48 h Pretreated
Blood	0.98±0.19	0.15±0.01	0.04±0.01	0.04±0.02	≤0.01	≤0.01	0.04±0.002
Plasma	1.86±0.27	0.27±0.03	0.08±0.01	0.07±0.03	≤0.01	≤0.01	0.07±0.03
Pancreas	**4.90±0.88**	**7.82±0.84**	**7.63±0.98**	**1.64±0.19**	**2.97±0.25**	**3.05±0.34**	**1.64±0.19**
Liver	0.26±0.04	0.11±0.02	0.09±0.01	0.07±0.01	0.05±0.00	0.07±0.01	0.07±0.01
Adrenals	**6.74±4.62**	**17.64±3.22**	**16.94±1.66**	**1.80±0.41**	**10.44±2.60**	**9.39±2.27**	**1.80±0.41**
Lung	0.67±0.12	0.14±0.01	0.07±0.01	0.05±0.01	0.03±0.00	0.02±0.01	0.05±0.01
Kidneys	**7.42±1.75**	**3.08±0.42**	**3.08±0.42**	**3.03±0.73**	**2.84±0.67**	**2.80±0.73**	**3.03±0.73**
Heart	0.31±0.21	0.07±0.01	0.02±0.01	0.02±0.01	≤0.01	≤0.01	0.02±0.01
Spleen	0.26±0.05	0.07±0.01	0.12±0.07	0.04±0.01	0.04±0.02	0.08±0.05	0.04±0.01
Stomach	1.07±0.18	0.65±0.40	1.67±1.46	0.18±0.04	0.59±0.08	0.39±0.06	0.18±0.04
Intestine	0.42±0.06	0.30±0.06	1.03±1.09	0.11±0.03	0.17±0.02	0.13±0.02	0.11±0.03
Colon	0.17±0.12	0.17±0.11	0.23±0.03	0.06±0.01	0.74±0.14	0.34±0.25	0.06±0.01
Testes	0.12±0.01	0.06±0.00	0.02±0.01	0.02±0.01	≤0.01	≤0.01	0.02±0.01
Skin	0.38±0.08	0.08±0.05	0.04±0.01	0.03±0.01	0.02±0.00	≤0.01	0.03±0.01
Muscle	0.16±0.10	0.03±0.00	0.01±0.00	0.01±0.00	≤0.01	≤0.01	0.0±0.003
Thyroid	0.88±0.15	0.26±0.11	0.10±0.00	0.05±0.01	0.04±0.01	0.04±0.01	0.05±0.01
Brain	0.04±0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
Body fat	0.14±0.13	0.06±0.02	0.01±0.00	0.02±0.01	≤0.01	≤0.01	0.02±0.01
Femur	0.26±0.03	0.20±0.03	0.17±0.03	0.07±0.01	≤0.01	0.12±0.01	0.07±0.01

term retention and high radioactivity concentrations for both compounds under study were found in the kidneys, and in organs with a high density of SSTRs, such as the pancreas and adrenals, as well as in stomach and intestine. The distribution of ^{111}In-DOTA-NOC in crucial organs was (nearly two-fold) higher in comparison with that of ^{111}In-DOTA-TATE. In order

Table III. *Cumulative excretion of ^{111}In-DOTA-NOC and ^{111}In-DOTA-TATE in rats without pretreatment with octreotide.*

	2 h	24 h		48 h	
	Urine	Urine	Faeces	Urine	Faeces
^{111}In-DOTA-NOC	32.9±19.4	50.8±17.7	11.3±4.8	67.3±19.8	16.1±3.7
^{111}In-DOTA-TATE	60.5±12.3	67.5±13.1	8.1±6.4	69.6±13.1	13.2±7.7

Data are expressed as a percentage of the administered dose (mean±SD).

Table IV. *Cumulative excretion of ^{111}In-DOTA-NOC and ^{111}In-DOTA-TATE in rats which were pretreated with octreotide.*

	2 h	24 h		48 h	
	Urine	Urine	Faeces	Urine	Faeces
^{111}In-DOTA-NOC	55.9±24.5	67.8±29.5	5.1±0.3	81.2±35.4	9.2±4.5
^{111}In-DOTA-TATE	58.6±22.4	75.8±6.0	4.9±1.8	77.5±6.4	8.3±3.4

Data are expressed as a percentage of the administered dose (mean±SD).

to partially block somatostatin receptor-mediated distribution, octreotide (unlabelled somatostatin analogue) was injected intravenously 15 min before ^{111}In-DOTA-TATE and ^{111}In-DOTA-NOC administration. Activity in the organs with a high density of somatostatin receptors (pancreas and adrenals) was significantly reduced by this pretreatment with unlabelled octreotide (Tables I and II). Significant decreases in radioactivity in the gastrointestinal tract (stomach and bowels) after receptor blockade were also determined both 2 h and 48 h after dosing (Tables I and II).

Cumulative urinary/faecal excretion of both agents is summarized in Table III (control group) and Table IV (pretreated group). Both investigated peptides were excreted predominantly in the urine; excretion of ^{111}In-DOTA-NOC in control animals was delayed compared to ^{111}In-DOTA-TATE. The receptor blockade with octreotide reduced the fraction of radioactivity excreted in the faeces, intensifying the urinary excretion. The highest activity of both studied radiolabelled peptides in the kidneys was found shortly after their administration. Kidney retention of ^{111}In DOTA TATE was higher than that of ^{111}In DOTA NOC at all indicated time intervals.

Internalization/externalization experiments in AR42J cells. The results from internalization studies of the SSTR2 positive AR42J cell line are expressed in Figure 4 and show that both investigated peptides were internalized at nearly the same range. Presented values are expressed as specific internalization (% administered dose to 1 million cells at 0.66 nM concentration). The values from externalization experiments were similar for both peptides under study and

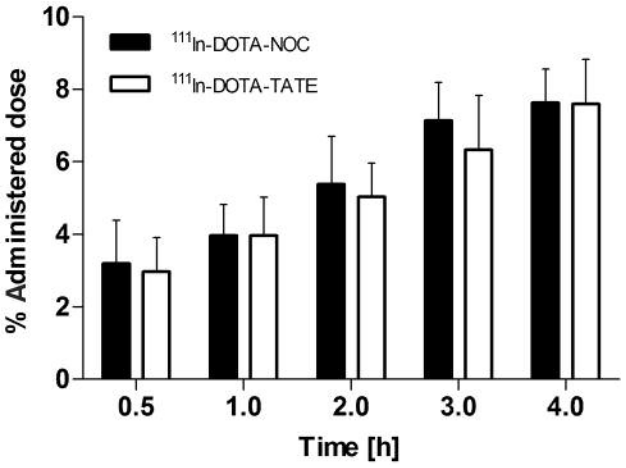

Figure 4. *Internalization in the AR42J cell line. Data expressed as mean±SD.*

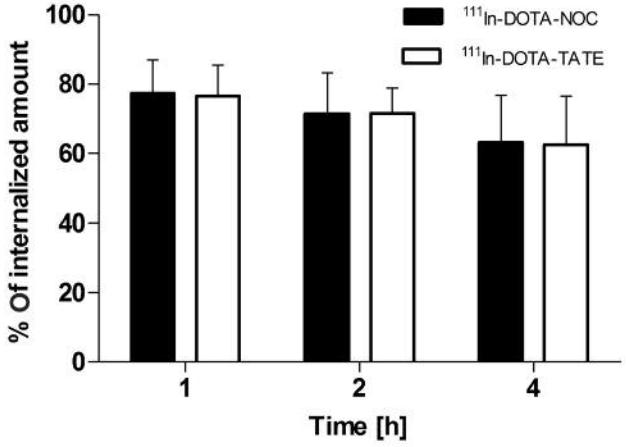

Figure 5. *Externalization in the AR42J cell line. Data expressed as mean±SD.*

are expressed in Figure 5. All values are results of three independent experiments with triplicates in each experiment.

Uptake experiments in OK cells. Results obtained from uptake experiments in the renal OK cell line are expressed in Figure 6. Uptake of ^{111}In-DOTA-NOC showed similar kinetics to that of ^{111}In-DOTA-TATE (maximum of uptake between 2 and 3 h of incubation) but was three-fold more effective. All values are the results of three independent experiments with triplicates in each experiment.

Discussion

Many of radiolabelled SST analogues have been developed for identification, localization and treatment of neuroendocrine tumours and metastases. The present work

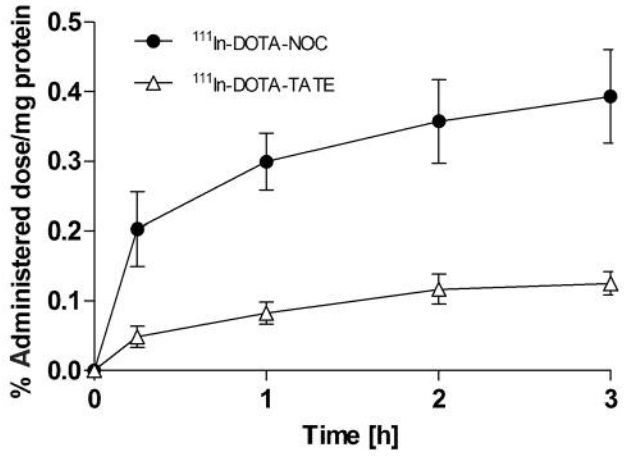

Figure 6. *Internalization in the OK cell line. Data expressed as mean±SD.*

focused on the analysis of some new characteristics of the SST analogue ^{111}In-DOTA-NOC that should possess better properties for targeting SSTR-positive tumours compared to others, especially through its larger binding spectrum to discrete SSTRs (14). Biological properties of ^{111}In-DOTA-NOC were compared with those of the well-established peptide ^{111}In-DOTA-TATE. Because there is the explicit tendency to obtain preclinical results using various *in vitro* techniques recently, we provided internalization experiments in two different cell lines (SSTR2-positive AR42J cell line and kidney megalin/cubilin-positive OK cell line) with the view to comparing the conclusions of these experiments with results obtained in rat biodistribution studies.

In rats, both studied peptides were quickly cleared from the blood and most organs and tissues. Nevertheless, long-term retention of radioactivity in SSTR-rich organs (the pancreas, adrenals, and some others) and also in the kidneys was detected. Our results showed that octreotide premedication significantly reduced the radioactivity uptake in SSTR-positive tissues and also in the gastrointestinal tract (GIT). This finding is different from that in the kidney, where no significant effect of somatostatin receptor blockade on renal uptake of radioactivity was found. The blockade reduced both radioactivity accumulation in the GIT and the fraction of radioactivity excreted in faeces. On the contrary, the fraction of urinary excreted radioactivity was higher in the pretreated group. In interspecies comparison, it has been confirmed that the gastric mucosa of rats contains primarily SSTR2 while in human intestine SSTR1 mainly is expressed (19). This means that the situation in man is different and receptor-mediated uptake of the agents under study in the GIT of man is negligible or slight.

On interdrug comparison, significantly higher (nearly two-fold) radioactivity concentrations in rat organs with a high density of somatostatin receptors (namely the adrenals and pancreas) after ^{111}In-DOTA-NOC in comparison with ^{111}In-DOTA-TATE were determined. These organs could serve as endogenous indicators of *in vivo* affinity of the agents to SSTRs. On the other hand, no significant differences in the *in vitro* internalization and externalization of radioactivity in the AR42J cell line were found for either peptide under study, in agreement with the similar affinity of both compounds for SSTR2 (AR42J cell line exhibits only SSTR2). This difference between *in vivo* and *in vitro* results can be attributed to the formerly reported enhanced affinity profile of ^{111}In-DOTA-NOC to SSTR subtypes in comparison with ^{111}In-DOTA-TATE and partial binding of the former agent to SSTR3 and -5 in rat SSTR-rich organs.

Slower blood radioactivity-time decrease for ^{111}In-DOTA-NOC in comparison with ^{111}In-DOTA-TATE and also a slower elimination rate of radioactivity to urine could be explained by higher plasma protein binding of the former peptide due to its higher lipophilicity. The slower elimination rate of ^{111}In-DOTA-NOC from the central compartment also resulted in its higher radioactivity concentrations in other non SSTR positive organs and tissues with the exception of the kidneys. Considering the fact that the compounds under study are eliminated in the kidney predominantly by the mechanism of glomerular filtration, higher plasma binding also results in a slower renal elimination rate (given as a product of glomerular filtration rate and free peptide fraction in plasma).

In *in vivo* experiments, systematically lower (statistically significant only in the interval of 24 hours) radioactivity concentrations in the kidney after ^{111}In-DOTA-NOC in comparison with ^{111}In-DOTA-TATE were determined. Long term renal accumulation of radioactivity is a consequence of the peptide internalization by receptor-mediated endocytosis where the transmembrane scavenger-receptor system megalin/cubilin should participate in reabsorption of these radiolabelled peptides (4-6). To distinguish the potential risk of radionephrotoxicity after therapeutical use of the investigated radiolabelled peptides, the proximal tubule-derived OK cell line was also employed. However the results of these experiments surprisingly showed the tendency of ^{111}In-DOTA-NOC to internalize to a significantly higher extent than ^{111}In-DOTA-TATE in the OK cells (the uptake of ^{111}In-DOTA-NOC was three times higher than that of ^{111}In-DOTA-TATE).

These *in vitro* results lead to the opposite conclusions when compared to *in vivo* kidney radioactivity uptake studies. The reason for this is not quite clear yet. One can speculate about interspecies differences in the kidney transport of the peptides under study, the differences of transport mechanisms between isolated cells and the organ with intact architecture, the effect of the different lipophilicity of the agents to their transport across biological membranes in the whole kidney, the presence of other

transport/cotransport systems in the intact organ, amongst others. Nevertheless, this study showed that a transfer of results from *in vitro* to *in vivo* conditions and interpretation of the results between different species is not simple and should be performed very carefully.

Acknowledgements

This grant was supported by grant No. 305/07/0535 of the Grant Agency of the Czech Republic. Authors wish to thank Mrs. J. Hoderova and Mrs. E. Teichmanova for their excellent technical assistance.

References

1 Weiner RE and Thakur ML: Radiolabeled peptides in oncology: role in diagnosis and treatment. BioDrugs *19*: 145-163, 2005.
2 Forrer F, Valkema R, Kwekkeboom DJ, De Jong M and Krenning EP: Neuroendocrine tumors. Peptide receptor radionuclide therapy. Best Pract Res Clin Endocrinol Metab *21*: 111-129, 2007.
3 Forrer F, Rolleman E, Bijster M, Melis M, Bernard B, Krenning EP and de Jong M: From outside to inside? Dose dependent renal tubular damage after high dose peptide receptor radionuclide therapy in rats measured with *in vivo* ^{99m}Tc-DMSA-SPECT and molecular imaging. Cancer Biother Radiopharm *22*: 40-49, 2007.
4 Barone R, Van Der Smissen P, Devuyst O, Beaujean V, Pauwels S, Courtoy PJ and Jamar F: Endocytosis of the somatostatin analogue, octreotide, by the proximal tubule-derived opossum kidney (OK) cell line. Kidney Int *67*: 969-976, 2005.
5 De Jong M, Barone R, Krenning E, Bernard B, Melis M, Visser T, Gekle M, Willnow TE, Walrand S, Jamar F and Pauwels S: Megalin is essential for renal proximal tubule reabsorption of ^{111}In-DTPA-octreotide. J Nucl Med *46*: 1696-1700, 2005.
6 Melis M, Krenning EP, Bernard BF, Barone R, Visser TJ and De Jong M: Localisation and mechanism of renal retention of radiolabelled somatostatin analogues. Eur J Nucl Med Mol Imaging *32*: 1136-1143, 2005.
7 Rolleman EJ, Valkema R, De Jong M, Kooij PP and Krenning EP: Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. Eur J Nucl Med Mol Imaging *30*: 9-15, 2003.
8 Behe M, Kluge G, Becker W, Gotthardt M and Behr TM: Use of polyglutamic acids to reduce uptake of radiometal-labeled minigastrin in the kidneys. J Nucl Med *46*: 1012-1015, 2005.
9 Van Eerd JE, Vegt E, Wetzels JF, Russel FG, Masereeuw R, Corstens FH, Oyen J and Boerman OC: Gelatin-based plasma expander effectively reduces renal uptake of ^{111}In-octreotide in mice and rats. J Nucl Med *47*: 528-533, 2006.
10 Gotthardt M, Van Eerd-Vismale J, Oyen WJ, De Jong M, Zhang H, Rolleman E, Maecke HR, Behe M and Boerman O: Indication for different mechanisms of kidney uptake of radiolabeled peptides. J Nucl Med *48*: 596-601, 2007.
11 Reubi JC, Schar JC, Waser B, Wenger S, Heppeler A, Schmitt JS and Macke HR: Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur J Nucl Med *27*: 273-282, 2000.
12 Reubi JC, Waser B, Schaer JC and Laissue JA: Somatostatin receptor sst1-sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. Eur J Nucl Med 28: 836-846, 2001.
13 Ginj M, Schmitt JS, Chen J, Waser B, Reubi JC, De Jong M, Schulz S and Maecke HR: Design, synthesis, and biological evaluation of somatostatin-based radiopeptides. Chem Biol *13*: 1081-1090, 2006.
14 Wild D, Schmitt JS, Ginj M, Macke HR, Bernard BF, Krenning E, De Jong M, Wenger S and Reubi JC: DOTA-NOC, a high affinity ligand of somatostatin receptor subtypes 2, 3 and 5 for labelling with various radiometals. Eur J Nucl Med Mol Imaging *30*: 1338-1347, 2003.
15 Brunskill NJ, Nahorski S and Walls J: Characteristics of albumin binding to opossum kidney cells and identification of potential receptors. Pflügers Arch 433: 497-504, 1997.
16 Gekle M, Mildenberger S, Freudinger R, Schwerdt G and Silbernagl S: Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases. Am J Physiol *272*: 668-677, 1997.
17 Gekle M, Mildenberger S, Freudinger R and Silbernagl S: Kinetics of receptor-mediated endocytosis of albumin in cells derived from the proximal tubule of the kidney (opossum kidney cells): influence of Ca^{2+} and cAMP. Pflügers Arch *430*: 374-380, 1995.
18 Gekle M, Mildenberger S, Freudinger R and Silbernagl S: Endosomal alkalinization reduces J_{max} and K_m of albumin receptor mediated endocytosis in OK cells. Am J Physiol *268*: 899-906, 1995.
19 Robben J, Reubi JC, Pollak Y and Voorhout G: Biodistribution of [^{111}In-DTPA-$_D$-Phe^1] octreotide in dogs: uptake in the stomach and intestines but not in the spleen points towards interspecies differences. Nucl Med Biol *30*: 225-232, 2003.

Received February 14, 2008
Revised April 22, 2008
Accepted May 8, 2008