Biodistribution of Two Octreotate Analogs Radiolabeled with Indium and Yttrium in Rats

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Abstract. *Background: In this study, two octreotate derivatives N-[4-carboxy-4-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane-1-yl]butanoyl]-Tyr^3-octreotate (DOTAGA-tate) and N-[[4,10-bis(carboxymethyl)-7-(1(1,3-dicarboxypropyl))-1,4,7,10-tetraaza-cyclododec-1-yl]acetyl]-Tyr^3-octreotate (DOTA-t-GA-tate) were radiolabeled with ^{111}In or ^{88}Y and their biodistribution profiles together with their elimination characteristics in rats were compared. Materials and Methods: Radiolabeling of the peptides with high radiochemical purity was carried out in an acetate buffer with gentisic acid as radioprotective compound. Biodistribution profiles of the radiolabeled peptides were determined in intact male Wistar rats after an intravenous dose of 1 μg/kg. For elimination pathways analysis, studies in intact rats in metabolic cages and perfused rat kidney and liver were carried out. Results: Fast radioactivity clearance from rat tissues (excepting somatostatin receptor-rich organs and the kidney) was determined for all agents under study. Profound radioactivity uptake in organs with a high density of somatostatin receptors (namely the adrenals and pancreas as biomarkers of somatostatin receptor-positive tissue) was slightly higher for radiolabeled DOTAGA-tate when compared with DOTA-t-GA-tate. Significantly higher accumulation in kidney and somewhat lower urinary elimination of ^{111}In-labeled peptides in comparison with that of ^{88}Y-agents were determined. Perfused rat kidney experiments confirmed that glomerular filtration was the main elimination mechanism for the compounds under study; their bile clearances in the perfused rat liver were negligible. Conlusion: ^{111}In(^{88}Y)-DOTAGA-tates exhibited higher distribution into somatostatin receptor-rich organs when compared with the corresponding radiolabeled DOTA-t-GA-tates. Higher uptake of ^{111}In-labeled peptides in the kidney is attributed to its different coordination properties.*

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The successful application of ^{111}In-labeled octreotide (OctreoScan®) in the detection of tumors with a high density of somatostatin receptors has triggered a wide search for somatostatin derivatives with improved characteristics for diagnosis and treatment of neuroendocrine tumors and their metastases (1-8). Namely, if somatostatin receptor-positive tumors and metastases exhibit good uptake of gamma-labeled somatostatin analog, a replacement of a diagnostic radionuclide with a therapeutic one (for example Y-90) could provide an agent useful for peptide receptor radionuclide therapy.

In preclinical studies, two octreotate derivatives [^{88}Y]-*N*-[4-carboxy-4-[4,7,10-*tris*(carboxymethyl)-1,4,7,10-tetraazacyclododecane-1-yl]butanoyl]-*D*-phenylalanyl-*L*-cysteinyl-*L*-tyrosyl-*D*-tryptophyl-*L*-lysyl-*L*-threonyl-*L*-cysteinyl-*L*-threonine, cyclic (2→7) disulfide (Y-DOTAGA-tate) and [^{88}Y]-*N*-[[4,10-*bis*(carboxymethyl)-7-(1(1,3-dicarboxypropyl))-1,4,7,10-tetraaza-cyclododec-1-yl]acetyl]-*D*-phenylalanyl-*L*-cysteinyl-*L*-tyrosyl-*D*-tryptophyl-*L*-lysyl-*L*-threonyl-*L*-cysteinyl-*L*-threonine, cyclic (2→7) disulfide (Y-DOTA-t-GA-tate) showed promising properties as therapeutic agents with a high affinity to somatostatin receptors, namely to SST2 subtype (9), higher than for Y-DOTA-Tyr^3-octreotide (Y-DOTA-tate) which is in clinical use at present (3). Therapeutical and radiotoxicological effects of these agents might have been predicted after scintigraphic examination of a selected patient with the peptide labeled with ^{111}In instead of ^{90}Y administered prior to prospective target therapy. However, small structural modifications of the agent, including the introduction of a different radiolabel in the chelator, may affect both binding properties for the various somatostin receptor subtypes and

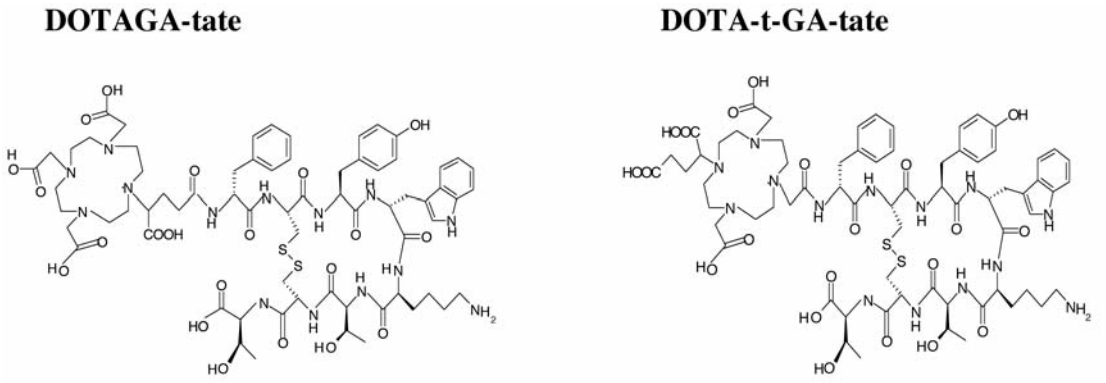

Figure 1. *Structures of the peptides studied.*

the pharmacokinetic profile of the peptide (10, 11). It is therefore crucial to evaluate these possible differences in order to select the best strategy for the patient's treatment. For this reason, the objective of the present study was to compare the distribution and elimination characteristics of DOTAGA-tate and DOTA-t-GA-tate labeled either with radioindium or radioyttrium in rats. The tissues with a high density of somatostatin receptors (the adrenals and pancreas) served as an endogenous marker of the affinity of the agent to somatostatin receptors. For the analysis of elimination mechanisms, perfused rat kidney and liver were employed. In the case of radioyttrium, ^{88}Y instead of ^{90}Y was used as a label as ^{88}Y exhibits a longer decay half-life and emits gamma radiation which can be much easily detected in biological organs and tissues than beta radiation.

Materials and Methods

Radiolabeling of the peptides. For the chelation of radionuclides, two peptides derived from DOTA-Tyr3-octreotate, namely DOTAGA-tate and DOTA-t-GA-tate were used to hold the radiometals with high stability. The peptides used were prepared at the Department of Nuclear Medicine, University Hospital Basel, Switzerland (12). The structures of the peptides used in this study are shown in Figure 1.

Peptides labeled with ^{111}In or ^{88}Y were prepared by adding 5-20 μl of $^{88}YCl_3$ in 0.05 M HCl (Los Alamos National Laboratory, NM, USA) or $^{111}InCl_3$ in 0.04 M HCl (Amersham, UK) (approximate activity 40 MBq) to 200 μl of 0.4 M acetate buffer pH 5 with 7.4 mg gentisic acid and 10 μg of the peptide. After incubation at 90-95˚C for 25 minutes, the quality control of the product was determined by gradient HPLC analysis.

Quality control. Gradient elution was performed on an HPLC Pharmacia-LKB system with a Gradient Master GP 962 (UOCHB Prague, Czech Republic) equipped with a LichroCART 125-4 HPLC Cartridge Purospher RP 18e, 5 μm (Merck, NM, USA) with a UV monitor and a radioactivity monitoring analyzer using 0.1% trifluoroacetic acid as mobile phase A and CH_3CN as phase B. Preparation of the samples for HPLC analysis: to 30 μl of mobile phase A, 10 μl of 10 mM DTPA pH 5 were added with 2 μl of the labeled peptide solution.

Distribution studies. The radiolabeled peptides under study were administered to male Wistar rats weighing 190-245 g intravenously at a dose of 1 μg/kg. At selected time intervals (5 min, 1 h, 2 h, 24 h and 48 h) after dosing, the carotid artery was exposed under ether anesthesia and a blood sample was collected into a glass tube containing heparin. After exsanguination, selected organs were taken out and weighed using an analytical balance. Radioactivity was then measured in a Wallac 1480 Wizard 3 automatic gamma counter, in comparison to three standard radioactivity samples.

Elimination studies. The agents were administered to rats as described above. Following administration, the animals were separately placed into glass metabolic cages, the construction of which allows reliable separation of urine and solid excrement. The animals had free access to a standard diet and water. Two hours after administration, the rats were forced to empty their urinary bladders by handling (immobilization) and urine and feces were collected. The procedure was repeated at 24 h and 48 h time intervals after administration. Radioactivity of the agents in biological samples was measured in a Wallac 1480 Wizard 3 automatic gamma counter, in comparison to three standard radioactivity samples.

Perfusion of rat kidney. Rat kidney perfusion experiments *in situ* were essentially those described previously (13). Briefly, the perfusion medium was Krebs-Henseleit buffer (pH 7.40) with glucose (5.6 mmol/l), 5.5% bovine serum albumin (fraction V, Sigma), 5%-6% washed rat erythrocytes, and different amino acids. After cannulation of the ureter, the renal veins and artery, the kidney was perfused *via* the renal artery at a constant arterial pressure of 14.5 kPa at 37˚C. After an equilibration period of 30-35 min, the agent was added in the dose of 0.2 μg per perfusion (an approximate dose administered to the animal in distribution and elimination studies) and the perfusion continued for 60 min. Urine samples were collected every 10 minutes and midpoint samples of the perfusate were also obtained. Inulin was used as the standard for the measurement of the glomerular filtration rate. Elimination parameters of labeled peptides in the perfused rat kidney were characterized by the values of total renal clearance (CL), free

fraction of the peptide in the perfusate (f_u), and by the renal clearance of the free peptide ($CL \times f_u$).

Perfusion of rat liver. A perfused rat liver *in situ* preparation was employed (14). After midline incision of the animal, the bile duct, the portal vein, and the inferior vena cava were cannulated and ligated. Krebs-Henseleit buffer (pH 7.4) with glucose in a single-pass mode was used to release the blood from the liver. The liver perfusion medium consisted of a heparinized Krebs-Henseleit buffer (pH 7.4) with 10 mM glucose, 4% bovine serum albumin, and 10% (v/v) bovine erythrocytes oxygenated with a 95% O_2 and 5% CO_2. After an equilibration period (15 min), the flow of the perfusate was kept at 25 ml/min and the peptide under study in the dose of 0.2 μg per perfusion was added to a 150 ml reservoir. Samples of the input and outflow perfusate were taken up at 10 min intervals in the middle of the 10 min periods of bile collection. The experiments were performed for 90 minutes after the peptide loading. Sodium salt of sulfobromophthalein (BSP; Sigma) was added at 30 nmol/ml perfusate as a marker of liver function. The concentration of BSP in the bile and perfusate were determined spectrophotometrically at 580 nm after addition of 0.1 M NaOH. Elimination rates of labeled peptides in the perfused rat liver were characterized by the values of bile clearance and by the liver-to-perfusate ratio at the end of experiments.

Protein binding determination. Binding of the agents to plasma proteins was determined by equilibrium dialysis at 37˚C (15).

Statistics. Unless otherwise stated each value represents the mean±SD of four animals or experiments. Non-paired Student's *t*-test was used for statistical analyses. Differences with $p<0.05$ were considered as significant.

Results

To determine the radiochemical purity of the labeled peptides, gradient HPLC analysis with radiometric detection was employed. As shown in Figure 2, more than 98% of activity was bound to the peptides and only traces of radiometals in non-chelated form were found. The distribution of radiolabeled peptides under study in selected organs and tissues of rats as the perceentage dose per organ is compared in Figure 3. Radioactivity in tissues of rats is compared in Table I. The values are expressed as a percentage of the administered dose per 1% body weight. An advantage of this unit (when compared with % dose per gram) is the very simple inter-species comparison of results (the relative weight of organs and tissues is very similar in different species). In addition, one can very easily read the results; the homogenous distribution in the body without elimination of the agent results in a value of 1% dose per 1% body weight, higher values represent specific accumulation in the corresponding tissue. As expected, the highest values and long-term retention in pancreas and adrenals were found. Overall, all agents under study were rapidly cleared from the blood and most organs, with the exception of excretory ones and organs with a high density of somatostatin receptors

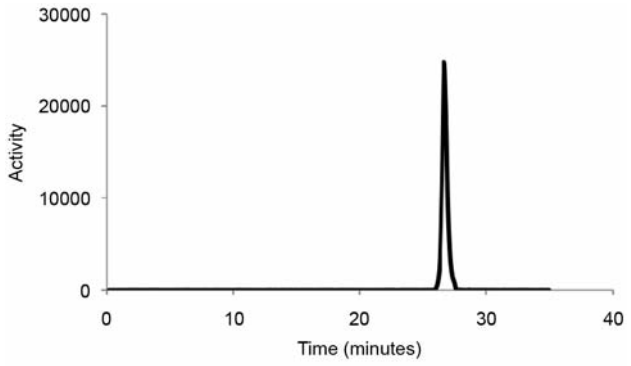

Figure 2. *An example of the HPLC profile of ^{88}Y-DOTAGA-tate.*

(namely the adrenals and pancreas). The kidneys had the greatest uptake of any organ for all agents under study as shown in Figure 3. Radioactivity in the kidney in the shortest time interval (5 minutes after administration) represents principally radioactivity of the agent in the urine as the kidney is the main elimination organ for the peptides under study. In longer time intervals (1 h-48 h after dosing), surprisingly significantly higher kidney accumulation of radioactivity after administration of ^{111}In-labeled peptides in comparison with those labeled with ^{88}Y for both DOTAGA-tate and DOTA-t-GA-tate was determined. In other organs not shown in Figure 3 (namely the heart, spleen, testes, thyroid and brain), less than 1% of administered radioactivity was found during the whole time course and the activity decreased with time.

As shown in Table II, all radiolabeled peptides under study were eliminated mostly by urine during the first 2 hours after administration. By feces, about 15-20% of radioactivity was excreted up to 48 hours. Urine excretion was higher for the peptides labeled with ^{88}Y when compared with ^{111}In-labeled peptides.

The results of the perfused rat kidney experiments are summarized in Table III. Even if the renal clearance of all agents under study was lower than the glomerular filtration rate, the data for total renal clearance corrected for plasma protein binding showed that all compounds were eliminated mostly by glomerular filtration.

Bile clearance of the agents in the perfused rat liver, presented in Table IV, was very low and no substantial differences in interdrug comparison were found.

Discussion

Radiolabeled receptor-specific peptides form an emerging class of radiopharmaceuticals useful in cancer diagnosis and treatment as many tumors overexpress some specific membrane receptors. It is known that octreotide analogs labeled with beta-particle emitters such as ^{90}Y are

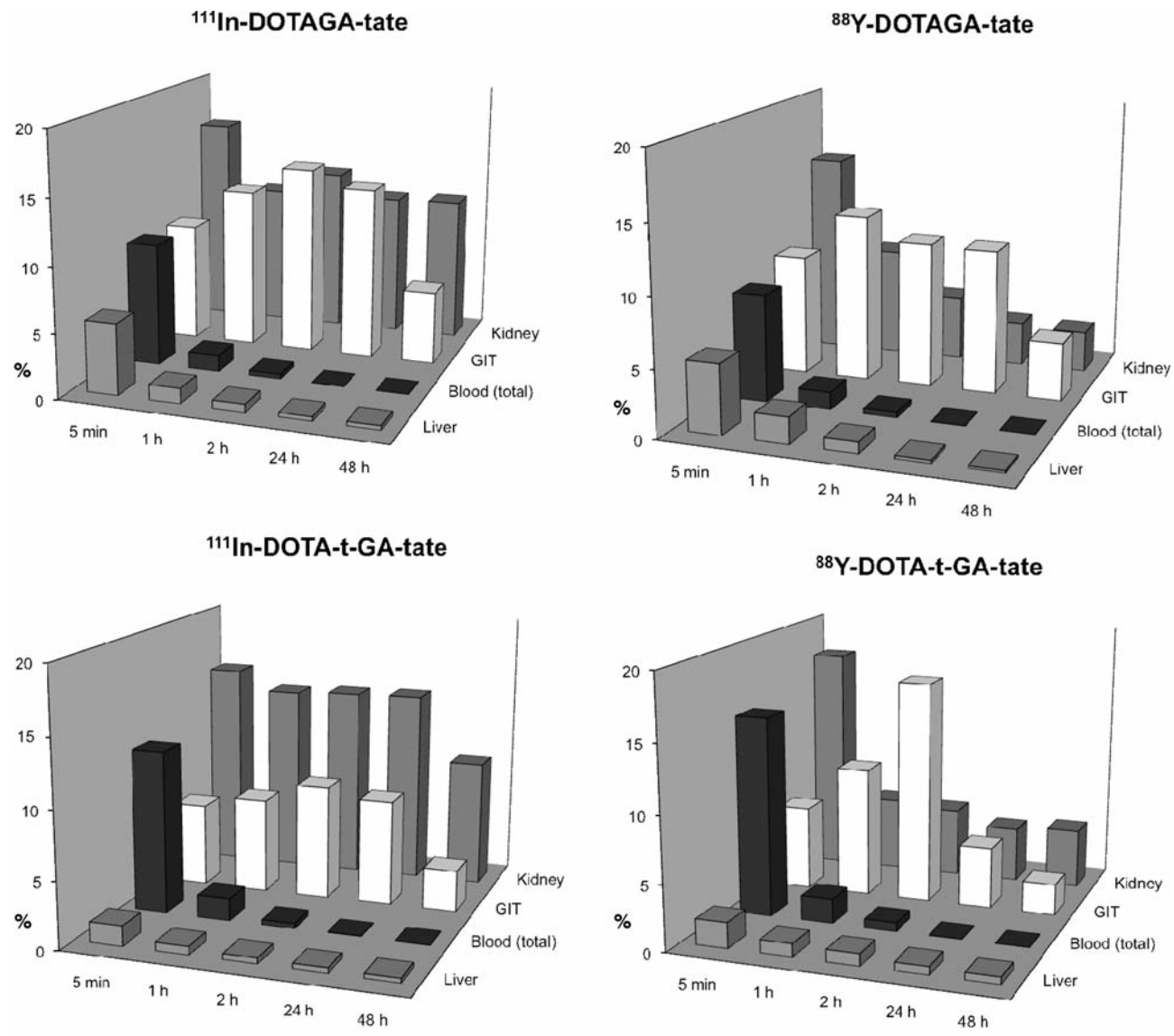

Figure 3. *The distribution of radiolabeled peptides in selected organs and tissues of rats in % dose per organ (distribution for yttrium labeled peptides derived from ref. 9).*

promising candidates for peptide receptor radionuclide therapy of neuroendocrine tumors (16). High and long-term radioactivity uptake in the adrenals and pancreas (biomarkers of the peptide affinity to SSTR2-positive tumors) confirms the excellent *in vivo* affinity of the peptides under study to SSTR-rich tissues. However, persistent retention of radioactivity in the kidneys due to proximal tubular cell reabsorption after glomerular filtration, and thus radiation-induced nephritis, is a well-known problem in peptide-targeted radiotherapy (17, 18). Consequently, there has been a great interest in the mechanism with which the peptides are absorbed into the renal proximal tubular cells. It was believed the mechanism may be competition for negative charges on the tubule cell membrane receptors (19, 20). But some results have indicated that the agents are taken up by receptor-mediated endocytosis by the multifunctional megalin/cubilin tandem receptor and partly also by fluid phase endocytosis (21-25). Even if the infusion of amino acid solutions and their near structures (polyglutamic acid or gelofusine) (26-28) or other structures such as amifostine (29) may partially reduce renal toxicity after peptide radionuclide therapy, renal damage is still the major obstacle to the implementation of such therapy.

Table I. *Radioactivity in selected tissues of rats in % dose per 1% body weight (mean value±SD of four animals).*

Time	5 min	1 h	2 h	24 h	48 h
^{88}Y-DOTA-t-GA-tate[a]					
Blood	2.33±0.2	0.29±0.04	0.1±0.01	0.004±0.002	0.004±0.002
Pancreas	11.83±2.23	13.65±1.6	13.7±1.04	3.88±0.26	3.62±0.23
Liver	0.68±0.05	0.37±0.03	0.34±0.01	0.22±0.03	0.19±0.02
Adrenals	18.52±3.16	22.38±4.32	22.72±4.13	14.19±4.61	10.88±2.93
Kidney	23.36±6.84	7.29±1.64	7.28±1***	5.84±1.41*	6.53±1.21**
Skin	1.11±0.12	0.28±0.05	0.12±0.01	0.03±0	0.03±0.01
Muscle	0.43±0.07	0.07±0.01	0.02±0	0.007±0.003	0.014±0.007
^{88}Y-DOTAGA-tate[a]					
Blood	1.22±0.11	0.2±0.02	0.06±0.01	0.004±0.003	0.002±0.002
Pancreas	13.41±3.38	30.39±7.68	29.88±4.1	7.18±0.27	5.71±0.86
Liver	1.73±0.28	0.63±0.06	0.28±0.08	0.08±0.02	0.06±0.01
Adrenals	24.43±3.29	36.78±3.29	37.51±6.56	21.36±1.37	18.87±2.53
Kidney	19.5±3.27	10.26±1.22*	6.03±1.1***	4.33±1.16***	4 ±0.59**
Skin	0.86±0.07	0.36±0.05	0.21±0.02	0.03±0.02	0.04±0.01
Muscle	0.34±0.03	0.06±0.01	0.03±0.01	0.007±0.003	0.005±0.004
^{111}In-DOTA-t-GA-tate					
Blood	1.87±0.23	0.27±0.1	0.06±0.02	0.007±0.003	0.003±0.002
Pancreas	10.21±1.21	24.96±5.4	19.27±4.03	7.71±1.47	6.25±0.42
Liver	0.53±0.07	0.22±0.07	0.14±0.02	0.13±0	0.12±0.01
Adrenals	13.91±2.77	27.78±3.82	23.18±3.26	17.53±2.15	15.07±1.1
Kidney	21.47±0.55	18.14±8.17	17.59±2.93***	19.12±4.92*	13.12±2.51**
Skin	0.81±0.12	0.26±0.06	0.09±0.03	0.02±0.01	0.02±0.01
Muscle	0.35±0.04	0.06±0.02	0.02±0.01	0.004±0.003	0.004±0
^{111}In-DOTAGA-tate					
Blood	1.47±0.21	0.2±0.05	0.06±0	0.004±0.001	0.002±0
Pancreas	12.62±2.02	25.36±4.45	26.76±1.34	9.61±0.09	6.74±0.19
Liver	1.73±0.37	0.41±0.1	0.22±0.01	0.11±0.03	0.12±0.01
Adrenals	22.11±4.78	42.84±5.29	41.88±7.66	29.51±3.72	26.14±3.42
Kidney	21.82±2.33	14.64±2.74*	16.6±2.61***	15.42±3.49***	15.52±3.09**
Skin	0.79±0.14	0.29±0.05	0.16±0.02	0.05±0.01	0.05 ±0.01
Muscle	0.35±0.04	0.06±0	0.02±0	0.008±0.005	0.009±0.001

Significance of differences between the same peptide labeled either with ^{88}Y or ^{111}In: *level 0.05, **0.01, ***0.001. [a]Results for Yttrium labeled peptides derived from ref. 9.

For this reason, both receptor affinity (therapeutic potential) and kidney uptake (risk factor) should be carefully evaluated in a new radiolabeled receptor-specific peptide development. A strategy may be based on a selection of those patients in which a high positive tumor uptake was determined and an acceptable renal cumulative dose was calculated from a distribution profile of the same peptide labeled with diagnostic radionuclide prior to the peptide radionuclide therapy. This approach should be valid only on the condition that a substitution of therapeutic radionuclide with a diagnostic one does not alter the fate of the radiolabeled peptide in the body.

Results presented have shown in rats that even if the distribution profiles of the individual peptides studied (DOTAGA-tate or DOTA-t-GA-tate), labeled either with ^{111}In or ^{88}Y, were generally similar, considerable differences in their renal handling were found. All peptides under study were eliminated in the kidney mostly by glomerular filtration. Renal clearances of the agents corrected for protein binding were very close to the glomerular filtration rate in the perfused rat kidney experiments. When the effect of the label is taken into account, significantly higher kidney uptakes of ^{111}In-labeled peptides were found in comparison with that of ^{88}Y both in distribution studies and in the kidney-to-perfusate ratio.

One possible explanation for the marked difference between renal accumulation of DOTAGA-tate or DOTA-t-GA-tate labeled either with ^{111}In or ^{88}Y could be the different chemical structures of the peptides labeled with the different metals. Liu and co-workers, who studied the

Table II. *Cumulative excretion of radioactivity in rats (in % dose) after administration of the peptides at a dose of 1 μg/kg (mean value±SD of four animals).*

Peptide name	Time				
	2 h	24 h		48 h	
	Urine	Urine	Feces	Urine	Feces
^{88}Y-DOTA-t-GA-tate	56.2±13.2	66.0±13.1	12.3±9.6	68.7±13.1	15.5±10.4
^{88}Y-DOTAGA-tate	48.5±9.7	62.0±10.9	16.3±4.1	66.3±11.6	20.6±4.6
^{111}In-DOTA-t-GA-tate	44.1±7.0	51.7±6.0	13.1±4.7	54.4±5.5	16.5±4.1
^{111}In-DOTAGA-tate	41.7±10.4	48.5±10.2	12.9±3.7	52.5±10.5	17.2±4.5

Table III. *Excretion parameters and renal retention of radiolabeled DOTAGA-tates and DOTA-t-GA-tates in the perfused rat kidney (mean value±S.D. of four animals).*

Parameter	^{88}Y-DOTAGA-tate	^{111}In-DOTAGA-tate	^{88}Y-DOTA-t-GA-tate	^{111}In-DOTA-t-GA-tate
CL_R (ml/min/g)	0.719±0.177	0.654±0.128	0.747±0.093	0.693±0.190
GFR (ml/min/g)	0.917±0.215	0.873±0.165	0.949±0.112	0.935±0.225
F_u	0.91±0.03	0.83±0.08	0.86±0.05	0.85±0.03
GFR × F_u (ml/min/g)	0.836±0.174	0.725±0.076	0.816±0.105	0.798±0.198
Kidney/perfusate ratio	7.1±1.5*	12.4±2.5*	4.4±0.7	5.6±2.3

CLR, Renal clearance; GFR, glomerular filtration rate; F_u, unbound fraction in the perfusate; kidney/perfusate ratio of radioactivity (in 1 g of tissue/1 ml of perfusate) was determined at the end of the perfusion. *Significant difference found at 0.05.

Table IV. *Bile clearance of the peptides in the perfused rat liver (n=3; mean value±S.D.).*

Parameter	^{88}Y-DOTAGA-tate	^{111}In-DOTAGA-tate	^{88}Y-DOTA-t-GA-tate	^{111}In-DOTA-t-GA-tate
Bile clearance (ml/min)	0.0009±0.0004	0.0008±0.0002	0.0008±0.0003	0.0009±0.0002
Liver-to-perfusate ratio	1.73±0.36	1.32±0.13	0.24±0.02	0.23±0.04

physical and structural properties of indium and yttrium complexes of small DOTA-monoamides (DOTA-BA and DOTA-MBA), found higher hydrophilicity for ^{111}In than for their corresponding ^{90}Y analogs, suggesting different coordination spheres in ^{111}In and ^{90}Y complexes of the same DOTA conjugate (30). Yttrium has a larger ionic radius than indium and its coordination number is normally 8 or 9, contrary to indium with a coordination number 6 or 7. Another possibility for the different kidney uptake of DOTAGA-tate and DOTA-t-GA-tate labeled with indium or yttrium is the effect of different concentrations of the radiotracer (which was in fact unknown, as both radionuclides were carrier-free). It has been shown that the uptake of radiolabeled somatostatin analogs in the tumor and SSTR-rich organs is dependent on the amount of peptide (31, 32). No such information concerning the kidney uptake of the peptides is available. In our study, however, the amount of injected peptide was the same in each case. We assume that differences in the indium and yttrium coordination geometry were responsible for the aforementioned distinctions in the renal uptake. However, the actual reason for different renal uptake of ^{111}In- and ^{88}Y-labeled peptides is not clear and further experiments are necessary. In organs and tissues other than the kidney, no systematic differences between the distribution profiles of the peptides labeled with ^{111}In and ^{88}Y were found.

Data on elimination studies have shown that no significant differences in the radioactivity eliminated by feces were found on inter-peptide comparison. On the other hand, when the effect of the radiometal on cumulative radioactivity excretion by urine is taken into account, the amount of ^{111}In-labeled peptides excreted was lower than that for radioyttrium. These findings were in agreement with higher uptakes of ^{111}In-labeled peptides in

the kidneys. Taking the total amount of the peptide determined in the kidney and urine, the values were similar in agreement, with nearly the same rate of renal elimination of individual peptides determined in the kidney perfusion experiments.

As expected from the results published previously (9), the binding affinities of DOTAGA-tate complexes to SSTRs *in vivo* (determined on the basis of the peptide uptake in the organs with high density of SSTRs – namely the adrenals and pancreas) were significantly higher than those for DOTA-t-GA-tate. This conclusion is in agreement with the higher binding affinity of the former peptide to SSTR2 determined *in vitro* (9).

Our observations of different kidney uptake of receptor-specific peptides in dependence of the metal used for labeling represent a complication in an effort to estimate the radiation dose of a therapeutic agent (labeled with radioyttrium) from the distribution profile of a diagnostic one (the same peptide labeled with radioindium).

Our results showed that not only the peptide structure but also choice of radiometal may alter to some extent the biological behavior of peptides directed to cancer visualization or targeted radiotherapy.

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