Pharmacokinetics and Renal Handling of ^{99m}Tc-Labeled Peptides

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^{99m}Tc-labeled peptides, particularly those of a lipophilic nature, are often excreted through the hepatobiliary system, and the subsequent accumulation in the intestine may obscure receptor-mediated uptake in tumor sites in the pelvis. We have therefore explored the route and rate of excretion of a small series of Tc-labeled peptides to shed some light on the mechanisms that influence the clearance of these agents. **Methods:** Pharmacokinetic parameters, biodistribution, routes of elimination of ^{99m}Tc-complexes of 3 model tetrapeptides—namely, acetyl-N-Gly-Gly-Cys-Gly (AGGCG), acetyl-N-Ser-Ser-Cys-Gly (ASSCG), and acetyl-N-Gly-Gly-Cys-Lys (AGGCL)—were determined in rats in vivo. Renal handling of the complexes was studied in the perfused rat kidney. **Results:** After intravenous injection, a relatively fast disappearance of the complexes from blood was found. Although the parameters of distribution in all 3 chelates were very similar, the elimination rate of ^{99m}Tc-AGGCG was higher than those of ^{99m}Tc-ASSCG and ^{99m}Tc-AGGCL. The Tc complexes under study were distributed mainly to the excretory organs (kidneys and liver), and no specific accumulation in other organs or tissues was found. Most of the radioactivity after intravenous administration of the chelates was rapidly eliminated through the urine, but a significant amount was also excreted through the feces, in the following order among the 3 chelates: ^{99m}Tc-AGGCL < ^{99m}Tc-ASSCG < ^{99m}Tc-AGGCG. Different proportions of glomerular filtration and secretion in renal tubules of the complexes were found in the perfused rat kidney. Elimination by glomerular filtration was dominant only in the case of ^{99m}Tc-AGGCL, whereas the rate of filtration of ^{99m}Tc-AGGCG was very low because of its high protein binding. Various rates of secretion into renal tubules were shown for all 3 agents. This renal excretion pathway was decisive in ^{99m}Tc-AGGCG and lowest in ^{99m}Tc-AGGCL. ^{99m}Tc-ASSCG was eliminated by both mechanisms at similar rates. **Conclusion:** These studies show that increasing the hydrophilic nature or reducing the negative charge of the peptides will reduce their hepatobiliary excretion, whereas the incorporation of suitable peptide sequences permits them to exploit efficient routes of renal excretion, such as tubular secretion, thereby optimizing the pattern of biodistribution of these radiopharmaceuticals.

Key Words: tetrapeptides; ^{99m}Tc; pharmacokinetics; peptide complexes

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Somatostatin receptors are expressed by a variety of tumors (*1,2*), and, consequently, radiolabeled somatostatin analogs have been found to be promising diagnostic agents in cancer scintigraphy (*3,4*). Besides ^{123}I and ^{111}In, ^{99m}Tc is the radionuclide of choice for labeling somatostatin receptor-specific peptides, because of its short half-life, optimal energy of γ photons, and thus high counting rate and high-quality images. For labeling with ^{99m}Tc, a combination of the receptor-specific peptide with small peptide sequences capable of chelating Tc has been proposed (*5*). The resulting agents are formed entirely of an amino acid sequence that exhibits both affinity to the receptor sites and the ability to bind Tc. This concept of labeling biologic macromolecules with Tc by incorporation of chelating tetrapeptides into their structure may be used not only for radiolabeling of somatostatin analogs but also for labeling of other receptor-specific peptides and proteins.

Different tetrapeptides may be used as the chelating portion and the corresponding pharmacokinetics of such hybrid peptides would be dependent on both parts of such agents. Because kidney excretion is the main elimination pathway of radiolabeled somatostatin analogs, the affinity of the chelating part of a hybrid peptide to the renal transport systems could markedly influence the rate of elimination of such peptides and their metabolites. For this reason, the pharmacokinetics and analysis of renal elimination mechanisms in rats of 3 peptides with potential as chelators of hybrid peptides—namely, complexes of ^{99m}Tc with acetyl-N-Gly-Gly-Cys-Gly, acetyl-N-Ser-Ser-Cys-Gly, and acetyl-N-Gly-Gly-Cys-Lys, were evaluated and compared.

MATERIALS AND METHODS

Chemicals

Three peptides, acetyl-N-Gly-Gly-Cys-Gly (AGGCG), acetyl-N-Ser-Ser-Cys-Gly (ASSCG), and acetyl-N-Gly-Gly-Cys-Lys (AGGCL), were synthesized with a model 431A Applied Biosystems solid-phase synthesizer (PerkinElmer Inc., Wellesley, MA) on a preloaded Wang resin (Sigma-Aldrich, St. Louis, MO) using Fmoc for α-amino acid protection. The peptides were purified by reversed-phase high-performance liquid chromatography (HPLC) using an acetonitrile/trifluoroacetic acid gradient. The identity of the pure peptides was confirmed by mass assessment with matrix-assisted laser desorption mass spectrometry (Lasermat; Finnigan MAT, San Jose, CA).

[^{14}C-methoxy]methoxyinulin (UVVR, Prague, Czech Repub-

lic) was used as a marker of glomerular filtration rate. ^{99m}Tc-mercaptoacetyltriglycine (MAG3) was prepared from a kit (MAG3-Kit; NRI, Rez, Czech Republic). All other chemicals used were of analytic grade.

Radiolabeling of Peptides

A ligand exchange method of labeling was used. A commercial glucoheptonate kit (Glucoheptoscint, Fleurus, Belgium) was reconstituted in 2 mL ^{99m}Tc-pertechnetate eluate (activity, ~300 MBq). Three hundred micrograms peptide was dissolved in 150 μL 0.5 mol sodium phosphate buffer (pH 11), and 150 μL reconstituted glucoheptonate kit was added. The solution was heated at 90°C for 10 min and then cooled to room temperature.

The solution from the labeling procedure was then filtered by a microcentrifuge tube filter (cellulose triacetate) (Whatman International Ltd., Maidstone, UK), 12,000 molecular weight cutoff, and analyzed by instant thin-layer chromatography (ITLC) on silica gel impregnated glass fiber sheets (Gelman Science Inc., Ann Arbor, MI) (in 0.15 mol NaCl) on a radiochromatograph (Berthold, Wildbad, Germany) and by HPLC with radiometric detection. For HPLC analysis, 10 μL labeled peptide was diluted 100 times with 0.01 mol sodium phosphate buffer (pH 6.0). Ten microliters diluted solution was taken for HPLC analysis on a Sepharon SGX 18C (7 μm, 3.3-mm inner diameter × 150 mm) reversed-phase column (Tessek, Prague, Czech Republic). A Pharmacia LKB 2248 HPLC pump with Gradient Master GP 962 (UOCHB, Prague, Czech Republic) and a noncommercial radiodetector were used. HPLC was performed using a mobile phase consisting of methanol/0.01 mol sodium phosphate (pH 6.0) at a flow rate of 0.5 mL/min using gradient elution: 95% buffer in methanol for 3 min; 95%–5% buffer in methanol over 10 min (linear gradient); 5% buffer in methanol for 10 min; 5%–95% buffer over 5 min (linear gradient); and 95% buffer in methanol for 2 min (total analysis time, 25 min).

Pharmacokinetic Study In Vivo

Four male Wistar rats weighing 230–250 g were used for the study. Animals were fasted for 18–24 h before the experiment. Agents under study were administered intravenously into the tail vein of rats after administration of ketamine/xylazine anesthesia. At selected intervals after dosing (2, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min), blood samples were withdrawn from the cannulated carotid artery to determine the plasma concentration profile of the compounds under study. The principal pharmacokinetic parameters (total plasma clearance, distribution volume of central compartment, distribution volume at steady state, mean residence time, and elimination half-life) were calculated using a Gauss-Newton method by a program, MULTI (*6*). Pharmacokinetic parameters were derived from the individual plasma concentration–time curves. A 2-compartment open model with elimination from the central compartment was used to describe the pharmacokinetics of the agents under study.

Biodistribution Study

The complexes under study were administered to 4 male Wistar rats intravenously into the tail vein in a volume of 0.2 mL each. The animals were then housed singly in cages. At selected times after dosing, the carotid artery was exposed under ether anesthesia and a blood sample was collected. After exsanguination, selected organs were removed to determine the distribution of radioactivity.

Elimination Study In Vivo

For an elimination study in vivo, the ^{99m}Tc-complexes were administered to rats as described for the biodistribution study. After administration, the animals were placed singly in glass metabolic cages, the construction of which allowed reliable separation of urine from feces. The rats had free access to standard diet and water. Two hours after administration, the rats were forced by handling (immobilization) to empty their urinary bladder, and urine and feces were collected. The animals were placed in the cages again, and urine and feces were taken repeatedly at 24- and 48-h intervals using the same withdrawal procedure.

Rat Kidney Perfusion

Male Wistar rats weighing 280–320 g were used for perfusion experiments. The perfusion medium was Krebs-Henseleit solution (pH 7.4) containing (mmol/L) Na^+ (143.1), K^+ (5.0), Ca^{2+} (2.5), Mg^{2+} (1.0), Cl^- (126.9), HCO_3^- (25.0), $H_2PO_4^-$ (1.2), SO_4^{2-} (1.0), and glucose (5.6), 5.5% bovine serum albumin (fraction V; Sigma, St. Louis, MO), 5%–6% washed rat erythrocytes, and the following amino acids and metabolic substrates (mmol/L): methionine (0.5), alanine (2.0), glycine (2.0), serine (2.0), arginine (1.0), proline (2.0), isoleucine (1.0), aspartic acid (3.0), glutamine (2.0), oxalic acid (1.0), pyruvate (2.0), and lactate (2.0).

Animals were anesthetized by an intravenous injection of sodium pentobarbital (45mg/kg) into the tail vein. The abdominal cavity was exposed by a ventral incision, and the right ureter was cannulated with a polyethylene cannula. After administration of heparin (500 IU) (Leciva, Prague, Czech Republic) into the inferior vena cava, a metal needle was inserted into the right renal artery through the aorta as quickly as possible, and perfusion was started. The inferior vena cava was cannulated caudal to the branching of the renal veins, and then the perfusion continued in the recirculation regimen in situ. The kidneys were perfused at a constant arterial pressure of 14.5 kPa (after correction for cannula resistance), and the temperature was maintained at 37°C. After an initial equilibration period of 25–30 min, the tested agent and ^{14}C-methoxyinulin were added to the perfusion circuit, and clearance measurements were started after a period of 10 min. Urine samples were collected every 10 min in preweighed vials, and midpoint samples of perfusate were obtained. Perfusate lost from urine formation was compensated by an addition of appropriate aliquots of Krebs-Henseleit saline immediately after each urine collection period to maintain a constant volume of perfusate. The volume of urine was determined gravimetrically.

Rates of elimination of the peptide complexes in the perfused rat kidney were characterized by total renal clearance (CL_{Tr} = urinary activity × volume of urine/activity in perfusate), by the filtered amount of the compounds (CL_{GF} = glomerular filtration rate × free fraction in the perfusate), and net tubular secretion ($CL_S = CL_{TR} - CL_{GF}$). Inulin renal clearance was used as a measure of the glomerular filtration rate. ^{99m}Tc-MAG3 clearance of a control group was used to compare renal clearances of the tetrapeptide complexes under study with that of a reference peptide drug with a very high renal extraction rate.

All animal experiments were approved by the Ethical Committee of the Pharmaceutical Faculty of Charles University.

Protein Binding Determination

Binding of the chelates to the proteins in the perfusion medium was determined by ultrafiltration across a semipermeable membrane (Priesvit, Chemosvit, Slovak Republic) for 20 min at 2000*g* (*7*). Two samples of the perfusate were taken during each rat kidney perfusion. The first sample was taken during the first 10-min collection interval after addition of the radiotracer and the second was taken during the last experimental interval. Two measurements

of protein binding were performed from each perfusate sample. Thus, the mean from 4 sets of experimental data from each perfusion were used for calculation of protein binding.

Radioactivity Measurements

The ^{99m}Tc activity in samples of plasma, urine, and perfusate was measured by a γ counter (Wallac 1480 Wizard 3; Wallac, Turku, Finland). The ^{14}C activity was measured by liquid scintillation counting in Bray's scintillation cocktail (Spolana, Neratovice, Czech Republic) with a β spectrometer (RackBeta 1219; LKB Wallac, Turku, Finland).

Statistical Analysis

Pharmacokinetic and biodistribution parameters from different groups of animals were compared by the Student t test. Differences between groups were considered statistically significant at the $P < 0.05$ level.

RESULTS

ITLC analysis of the radiolabeled peptides showed a labeling efficiency >95% in all cases. HPLC analyses showed a single peak for each peptide, with retention times of 21.3 min for ^{99m}Tc-AGGCG, 1.9 min for ^{99m}Tc-ASSCG, and 24.0 min for ^{99m}Tc-AGGCL.

Examples of plasma concentration–time curves of the tetrapeptide complexes under study after intravenous administration in randomly selected rats are shown in Figure 1. The principal pharmacokinetic parameters of ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL found in rats after intravenous administration are summarized in Table 1. The results document a relatively fast distribution into the tissues and a rapid elimination of the chelates from the blood of the experimental animals.

Results of the elimination study in rats are summarized in Table 2, where the data are given as percentages of radioactivity excreted in urine and feces after intravenous administration of the complexes in selected intervals.

Distribution of radioactivity in whole blood, kidney, and liver at 5 min, 1 h, and 2 h after administration of agents under study is presented in Table 3. In other organs and tissues except those of the gastrointestinal tract, <1% dose per gram or <1% dose per 1% body weight was found.

Renal clearances of ^{99m}Tc-complexes AGGCG, ASSCG, and AGGCL in the perfused rat kidney are presented in Table 4. These clearance values are related to renal clearance of ^{14}C-methoxyinulin as a measure of the glomerular filtration rate and are compared with ^{99m}Tc-MAG3 renal clearance as a reference peptide complex showing a high renal clearance value. No significant changes in renal clearances of the complexes during 60 min of the perfusion were found. Free fractions of the complexes in the perfusion medium indicate a variable degree of binding of the complexes to proteins of the perfusate, as indicated in Table 4.

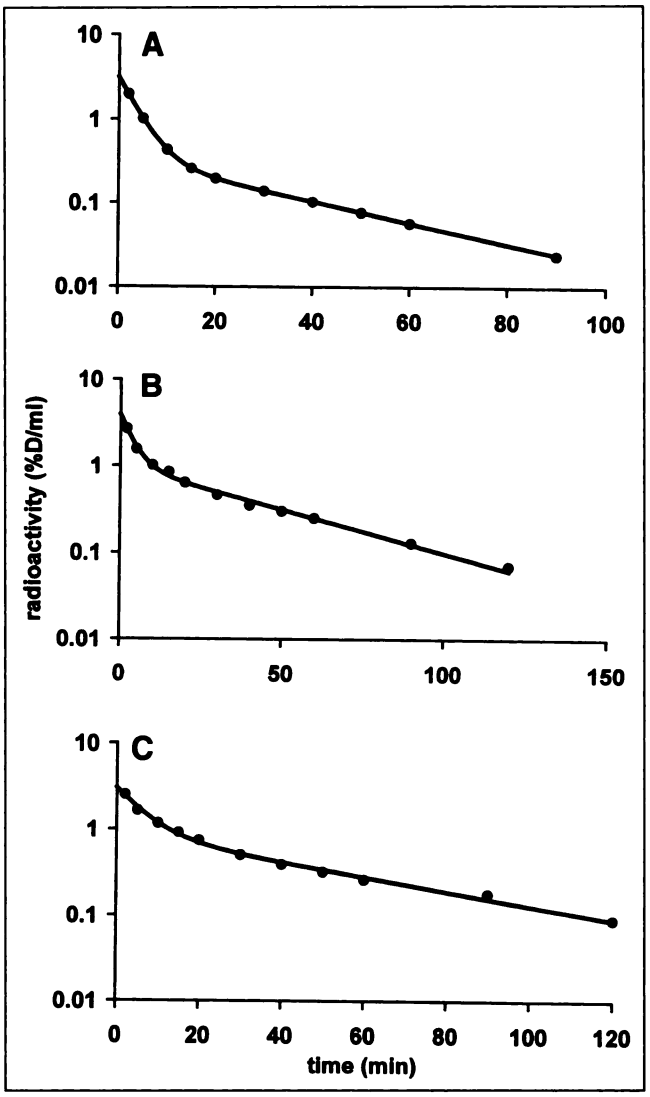

FIGURE 1. Representative plasma concentration–time curves from rats after intravenous injection of ^{99m}Tc-AGGCG (A), ^{99m}Tc-ASSCG (B), and ^{99m}Tc-AGGCL (C). Experimental values and best-fitted curves are presented. %D/mL = percentage dose administered/mL plasma.

DISCUSSION

The tetrapeptides used in this study were chosen to provide a range of physicochemical properties within a closely defined coordination site. Gly-Gly-Cys is the simplest peptide sequence able to provide a triamidomonothiol coordination site for ^{99m}Tc. The N-terminus of the peptide was blocked to provide some protection against serum exopeptidases, and a further Gly- was appended at the C-terminus to indicate the beginning of the extension of the peptide that incorporates the receptor binding site. The resulting AGGCG sequence has previously formed the technetium chelating site in a hybrid peptide based on α-melanocyte stimulating hormone (*8*). In ASSCG, the glycine residues are replaced by serine, which has a hydroxylated side chain and correspondingly gives a more hydrophilic peptide. In AGGCL, the replacement of 1 glycine by a lysine provides an additional positive charge to the compound. Thus, AGGCG and ASSCG would be expected to have a net double negative charge, whereas AGGCL would have a single negative charge.

TABLE 1
Principal Pharmacokinetic Parameters of Chelates in Rats After Intravenous Administration

Parameter	^{99m}Tc-AGGCG	^{99m}Tc-ASSCG	^{99m}Tc-AGGCL
CL (mL/min)	4.71 ± 0.48*	2.21 ± 0.30†	1.70 ± 0.06‡
V_1 (mL)	30.5 ± 3.2	31.1 ± 6.6	32.9 ± 7.0
V_{SS} (mL)	76.1 ± 15.7	83.8 ± 18.3	62.6 ± 7.7
MRT (min)	16.5 ± 4.9*	37.8 ± 4.7	36.9 ± 4.3‡
$t_{1/2}$ (min)	24.9 ± 11.8	32.9 ± 6.0	31.3 ± 4.9

*Significantly different from ^{99m}Tc-ASSCG ($P < 0.05$).
†Significantly different from ^{99m}Tc-AGGCL ($P < 0.05$).
‡Significantly different from ^{99m}Tc-AGGCG ($P < 0.05$).
CL = total plasma clearance; V_1 = distribution volume of central compartment; V_{SS} = distribution volume at steady state; MRT = mean residence time; $t_{1/2}$ = half-life of elimination.
Data are presented as mean ± SD (n = 4).

All peptides labeled with high efficiency. The HPLC retention times of ^{99m}Tc-AGGCG and ^{99m}Tc-AGGCL were similar (21.3 and 24.0 min, respectively), suggesting a similar lipophilicity, whereas that of ^{99m}Tc-ASSCG was much lower (1.9 min), indicating a much more hydrophilic nature.

The pharmacokinetic parameters of the ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL determined in rats documented a relatively rapid disappearance of the compounds from the blood after intravenous administration. Although values of the total plasma clearance and mean residence time for ^{99m}Tc-AGGCG were different from those for ^{99m}Tc-ASSCG and ^{99m}Tc-AGGCL, the values of distribution volume found at steady state and the distribution volume of the central compartment were very similar for all 3 compounds. Thus, the distributions of the complexes under study were very similar, but there were some differences in elimination rates. The biologic half-times of about 30 min for all chelates can be considered to be quite short. It seems likely that the pharmacokinetic behavior of this limited number of tetrapeptide ^{99m}Tc-complexes would be representative of this class of compounds because they are often characterized by rapid pharmacokinetics and rapid tissue penetration (*9*). A determining factor for the pharmacokinetics of tetrapeptides complexed with ^{99m}Tc seems to be the rate of their excretion.

Results of biodistribution studies showed a rapid clearance of the tracers under study from blood and other organs except the bowel. Clearly 2 elimination processes—namely, urinary and biliary excretion—contribute to the tetrapeptide disappearance from blood and other organs and tissues. Elimination experiments showed that these processes occurred in proportions that differed according to the ^{99m}Tc-complex used. Urinary and fecal excretion of ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL was very fast. Nearly all radioactivity excreted in the urine was eliminated within the first 2 h after dosing, and by 48 h >90% of the radioactivity had been excreted in either urine or feces. Similar behavior has been shown for other tetrapeptide complexes with ^{99m}Tc—for example, in ^{99m}Tc-cysteinyltriglycine, which is excreted in mice through the kidney and the liver and the intestine (*10*); and ^{99m}Tc-alanyltriglycine, which showed a high uptake in the kidney and varying degrees of uptake in the liver in mice, baboons, and man (*11*). Together with rapid tissue penetration and excretion of the intact complexes, metabolism by peptidases (mainly in liver and kidney) could also influence the pharmacokinetics, but the extent of this factor is still unclear.

The renal clearance of ^{99m}Tc-AGGCG in the perfused rat kidney was much higher than the glomerular filtration rate, indicating a degree of secretion in renal tubules. Although the renal clearance values for ^{99m}Tc-ASSCG and ^{99m}Tc-AGGCL were slightly lower than the values of glomerular filtration rate as indicated by inulin clearance, after correction for binding to proteins in the perfusion medium, a small contribution of the glomerular filtration to the total excretion in the kidneys can be shown. Only ^{99m}Tc-AGGCL is excreted predominantly by glomerular filtration, with just a small contribution from renal secretion. Even the highest renal clearance of the ^{99m}Tc-complexes under study—i.e., that of ^{99m}Tc-AGGCG—was relatively low compared with oligopeptide complexes such as ^{99m}Tc-MAG3, which show a very high extraction into the kidney. In the perfused rat

TABLE 2
Elimination of Radioactivity into Urine and Feces After Administration of ^{99m}Tc-Tetrapeptides to Rats

	^{99m}Tc-AGGCG		^{99m}Tc-ASSCG		^{99m}Tc-AGGCL	
Interval (h)	Urine	Feces	Urine	Feces	Urine	Feces
0–2	42.7 ± 2.5*	—	58.5 ± 3.5†	—	72.3 ± 2.2‡	—
2–24	3.5 ± 2.3	32.2 ± 10.1	2.7 ± 0.7	27.8 ± 8.1†	3.2 ± 1.7	12.7 ± 2.1‡
24–48	1.4 ± 0.8	13.5 ± 10.4	0.8 ± 0.5	1.6 ± 0.7	1.7 ± 0.7	1.9 ± 1.8
Total	47.6 ± 5.0*	45.7 ± 6.2*	62.0 ± 3.8†	29.4 ± 8.4†	77.2 ± 3.1‡	14.6 ± 2.4‡

*Significantly different from ^{99m}Tc-ASSCG ($P < 0.05$).
†Significantly different from ^{99m}Tc-AGGCL ($P < 0.05$).
‡Significantly different from ^{99m}Tc-AGGCG ($P < 0.05$).
Data are expressed as percentage of administered dose (mean ± SD, n = 4).

TABLE 3
Distribution of ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL to Blood, Liver, and Kidneys of Rats

	^{99m}Tc-AGGCG			^{99m}Tc-ASSCG			^{99m}Tc-AGGCL		
Organ	5 min	1 h	2 h	5 min	1 h	2 h	5 min	1 h	2 h
Blood*	12.1 ± 3.1	0.3 ± 0.1†	0.1 ± 0.1†	11.5 ± 1.1‡	1.1 ± 0.2	0.3 ± 0.1	14.8 ± 1.2	1.4 ± 0.2§	0.3 ± 0.1§
Liver	8.6 ± 1.1†	0.2 ± 0.1†	0.1 ± 0.1†	10.8 ± 1.4‡	2.0 ± 0.3‡	0.7 ± 0.1‡	5.7 ± 0.7§	1.2 ± 0.1§	0.3 ± 0.1§
Kidney	7.2 ± 1.6†	2.3 ± 1.8	1.6 ± 0.7	13.3 ± 2.1	2.2 ± 0.4	1.1 ± 0.2	12.6 ± 0.8§	1.9 ± 1.2	1.0 ± 0.3

*Total blood volume calculated as 6.5% of body weight.
†Significantly different from ^{99m}Tc-ASSCG ($P < 0.05$).
‡Significantly different from ^{99m}Tc-AGGCL ($P < 0.05$).
§Significantly different from ^{99m}Tc-AGGCG ($P < 0.05$).
Data are expressed as percentage of administered activity per organ (mean ± SD, n = 4).

kidney, ^{99m}Tc-MAG3 renal clearance was almost 3-fold greater than the glomuler filtration rate.

The rapid renal elimination of ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL found in the perfused rat kidney supports the significance of renal excretion as the important route of excretion in the body, as has been found for other small peptides complexed with Tc. Vanbilloen et al. (*12*) showed rapid clearance from the blood, mainly by the renal system, for complexes derived from MAG3 in which the mercaptoacetyl group was replaced by an aminoacyl group. This group of complexes showed higher renal excretion than did the chelates in this study, with urinary excretion of some compounds reaching values comparable with that of ^{99m}Tc-MAG3.

Whereas small linear peptides are hydrolysed at the luminal membrane in the proximal tubules and then reabsorbed (*13*) or, as in the case of di- and tripeptides, can be reabsorbed in intact form by peptide transporters PEPT 1 and PEPT (*14*), tetrapeptides complexed with ^{99m}Tc show different behavior in the kidney, characterized by rapid renal excretion including secretion into the renal tubules. Secretion of drugs and other chemicals with a suitable structure in the renal tubules relies on carrier-mediated transport. A specific transport system for anionic compounds is responsible for transport of many organic acids (*15*). This system seems to be responsible for secretion of some ^{99m}Tc-oligopeptide complexes such as ^{99m}Tc-MAG3 because the renal excretion can be depressed by competition for the carriers with compounds such as probenecid. However, the affinity of this transport system for ^{99m}Tc-MAG3 is lower than that for iodohippurate, evidenced by the greater probenecid-induced clearance reduction seen in the case of ^{99m}Tc-MAG3 compared with that seen for iodohippurate (*16*). The relatively lower affinity of MAG3 for this anionic transport system is supported by the observation of competitive inhibition of the tubular transport of ^{99m}Tc-MAG3 by p-aminohippurate in humans (*17*). It can be supposed that this transport system is also responsible for secretion of the ^{99m}Tc-peptide complexes under study. Differences in the rate of net secretion in renal tubules among ^{99m}Tc-AGGCG, ^{99m}T-ASSCG, and ^{99m}Tc-AGGCL could be explained either by their different affinities for the secretion carrier system or by different rates of tubular resorption.

In the case of ^{99m}Tc-AGGCG, about half the administered radioactivity was excreted by hepatobiliary and renal excre-

TABLE 4
Renal Clearance Values of ^{99m}Tc-AGGCG, ^{99m}Tc-ASSCG, and ^{99m}Tc-AGGCL in Perfused Rat Kidney and Comparison with Reference Substances

Parameter	^{99m}Tc-AGGCG	^{99m}Tc-ASSCG	^{99m}Tc-AGGCL
CL_{TR} (mL/min/g)	1.134 ± 0.277*	0.640 ± 0.113	0.517 ± 0.081‡
CL_{IN} (mL/min/g)	0.647 ± 0.169	0.666 ± 0.084	0.566 ± 0.086
CL_{GF} (mL/min/g)	0.123 ± 0.032*	0.406 ± 0.077	0.430 ± 0.066‡
CL_S (mL/min/g)	1.011 ± 0.249*	0.234 ± 0.037†	0.087 ± 0.017‡
CL_{TR}/CL_{IN}	1.76 ± 0.26*	0.96 ± 0.02†	0.91 ± 0.02‡
CL_{TR}/CL_{MAG}	0.60 ± 0.15*	0.34 ± 0.06	0.27 ± 0.04‡
F_U	0.19 ± 0.05*	0.61 ± 0.09†	0.76 ± 0.03‡

*Significantly different from ^{99m}Tc-ASSCG ($P < 0.05$).
†Significantly different from ^{99m}Tc-AGGCL ($P < 0.05$).
‡Significantly different from ^{99m}Tc-AGGCG ($P < 0.05$).
CL_{TR} = total renal clearance; CL_{IN} = inulin clearance; CL_{GF} = filtration clearance; CL_S = secretion clearance; CL_{MAG} = ^{99m}Tc-mercaptoacetyltriglycene renal clearance; F_U = free fraction in perfusate.
Data are presented as mean ± SD (n = 4).

tion. ^{99m}Tc-ASSCG is a considerably more hydrophilic peptide, as indicated by its much earlier retention time in the HPLC analysis. This increase in hydrophilicity, caused by the presence of the hydroxyl groups on the amino-acid side chains, results in a significant shift in excretion toward the renal pathway, about twice as much activity being excreted in the urine as in the feces. Substitution of a single glycine residue in ^{99m}Tc-AGGCG by a lysine results in a peptide with a similar retention time on HPLC, and therefore a similar hydrophilic nature, but lowers the charge on the Tc complex by 1 unit at physiologic pH. The effect of this charge difference is a further shift toward the renal route of excretion. ^{99m}Tc-AGGCL shows >5 times as much in the urine as in the feces, a change even greater than that produced by an increase in hydrophilicity.

CONCLUSION

An "ideal" receptor-targeted radiopharmaceutical shows, in addition to its desired receptor uptake, rapid blood clearance and predominantly renal excretion combined with low renal retention to provide a clear background against which sites of receptor expression may be imaged. Although the overall pattern of distribution of the tetrapeptides under study may be similar, there are also significant differences and trends that can be exploited in efforts to achieve this goal. It seems likely that decreases in the net charge of the tetrapeptide complexes or a combination of changes in charge and hydrophilicity will produce complexes with still more favorable biodistribution profiles. Thus, these results give a more profound insight into the pharmacokinetics of ^{99m}Tc-complexes with tetrapeptides and will help in the design of hybrid receptor-specific peptides that exhibit the optimal pattern of biodistribution.

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