3 Radiolabeled Peptides in Nuclear Oncology: Influence of Peptide Structure and Labeling Strategy on Pharmacology

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3.1 Introduction

3.1.1 Peptides in Biology and Medicine

Peptides are necessary elements in more fundamental biological processes than any other class of molecules. The most ubiquitous mode for controlling and modulating cellular function, intercellular com-

munication, immune response, and information-transduction pathways is through peptide-protein noncovalent interactions. For example, peptides function as hormones, neurotransmitters, neuromodulators, growth and growth inhibition factors, and cytokines. Although there are numerous exceptions, such as insulin, oxytocin, and calcitonin, most peptide-ligands are not used directly as drugs, and often the most useful ligands for therapy would be analogs that act as agonists or antagonists of the native ligands. The development of peptides or peptide-mimetics that can target the receptors modulating the biological activities is a top priority in biology, chemistry, and medicine.

Peptides also play important roles in growth and other cellular functions not only in normal tissues but also in tumors. Most tumors express receptors for different peptides, frequently in high density, and many of these receptors mediate growth-regulating effects in vitro. Certain types of tumors also respond to the growth-inhibition or growth-promoting signals of peptides in vivo, an effect which has become an important clinical approach to treat tumors in man. An ubiquitous example is the use of somatostatin (SS) analogs (Fig. 1,

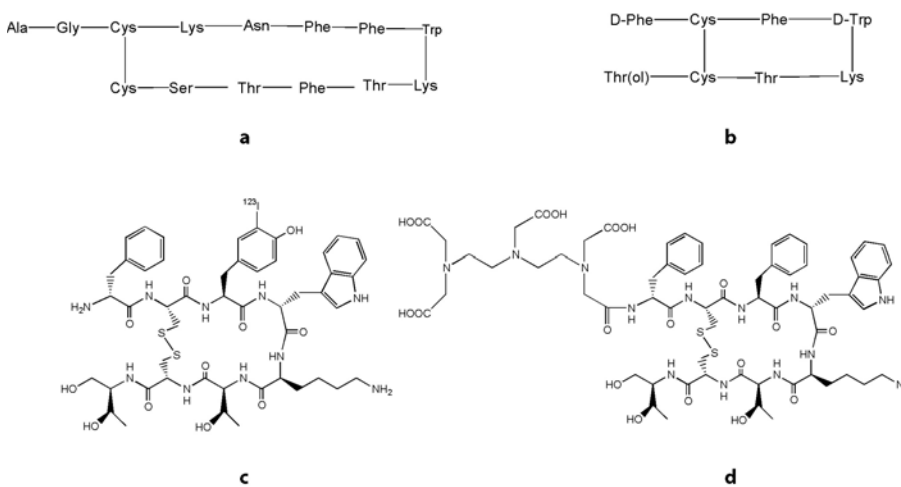

Fig. 1 a–d. Structural formula for: (**a**) SS-14; (**b**) octreotide; (**c**) [^{123}I]-[3-iodo-Tyr^3]-octreotide; (**d**) DTPA-octreotide

see Sect. 3.2.3), whose receptors are overexpressed in many neoplastic tissues. Five human SS receptor subtypes have been identified and cloned (Reisine and Bell 1995). A new development to visualize tumors through peptide receptor targeting began about fifteen years ago when radiolabeled SS analogs were introduced into nuclear medicine for in vivo imaging of human tumors using a gamma camera. Nuclear medicine is mainly a diagnostic discipline; its strength has been the ability to provide images of (patho)physiological functions rather than morphological information. The development in this field advances in the direction of in vivo tissue characterization through imaging of biochemical markers. The use of radiolabeled analogs of regulatory peptides in nuclear oncology is an important step in this direction. The peptides as targeting agents offer several advantages over proteins as, for instance, antibodies. Because of their high molecular weight, antibodies have often shown limited uptake at the target site and slow blood clearance, which results in modest target-to-background ratios (Fischman et al. 1993; Liu and Edwards 1999). In contrast, peptides are readily synthesized via solid phase synthesis, parallel and combinatorial approaches as well as phage-display. They are cheaper and can withstand harsher conditions for modification and labeling. They are less likely to produce immunogenic response, and blood clearance, tissue penetration, and tumor uptake are faster.

However, there are several prerequisites for peptides used as radiopharmaceuticals. Primarily, the corresponding receptors have to be expressed on the target in suitable amounts, overexpression or unique expression being desirable. The peptide ligand should retain the same high affinity to the receptor as the natural compound. For radiotherapeutic applications, internalization appears to be an absolute precondition because of higher residence time. Last, but not least, a main concern of radiolabeled peptides is their metabolic instability, concerning not only the peptide part, but also the stability of the metal-chelator complex or the radiohalogen bond. Other aspects have to be considered when developing radiometal conjugated peptides, i.e., a high rate of complexation, the practicability of radiolabeling, the availability of the radionuclide and some other biochemical properties of the metal and metal-chelator complex that will be discussed later in this chapter.

3.1.2 Radiopeptides and Potential Targets of Radiopeptides in Diagnosis and Therapy

Radiopeptides are composed of a biologically active peptide coupled to a chelator for labeling with radiometals or coupled to prosthetic groups for halogenation. In some cases, direct labeling of peptides is an option if, e.g., the peptide contains Tyr, which can be iodinated, or disulfide bridges, which, upon reductive opening, react with thiophilic ^{99m}Tc(V).

The molecular biology studies preceding successful receptor targeting with radiopeptides include in vitro identification and analysis

Table 1. Expression of receptors on human tumors

Ligand	Receptors	Tumor type
Somatostatin	Somatostatin receptor subtypes sst1–5	Neuroendocrine tumors, SCLC, MTC, tumors of the nervous system, lymphoma (non-Hodgkin's lymphoma, Hodgkin's disease)
VIP/PACAP	$VPAC_1$, $VPAC_2$, PAC_1 receptors	Various adenocarcinomas (stomach, colon, pancreas, lung, etc.)
CCK/gastrin	CCK_1, CCK_2 receptors	MTC, SCLC, stromal ovarian cancer, astrocytoma
LHRH	LHRH receptors	Breast, prostate cancer
α-MSH	MSH receptors	Melanoma
Bombesin/GRP	BB_1, BB_2, BB_3 and BB_4 receptors	SCLC, MTC, glioblastoma, colonic cancer, prostate cancer
Neurotensin	NTR1, NTR2, and NTR3 receptors	Ewing sarcoma, meningioma, MTC, astrocytoma, SCLC, exocrine pancreatic cancer
Opioid	Opioid receptors	SCLC, neuroblastoma, breast cancer
Substance P	NK1 receptors	Glioblastoma, astrocytoma, MTC, breast, peri- and intratumoral blood vessels
GLP-1	Glp-1 receptors	Insulinomas
Oxytocin	Oxytocin receptors	Endometrium, breast cancer
Neuropeptide Y	NPY receptors subtypes Y_1–Y_6	Breast, brain cancer

of receptors with biochemical, biomolecular, and immunological techniques (Reubi 1995). For example, radioligand binding analysis and bioassays with cells or membrane preparations are the means to characterize high-affinity binding sites and the pharmacological profile of a given peptide. Anatomical information about the distribution of receptors in tissues is obtained by quantitative receptor autoradiography, which measures radioligand binding on tissue sections and thus enables localization of receptors at the microscopic level. Only a minority of the large number of potentially useful regulatory peptides and peptide families has been more or less thoroughly investigated so far and future work will probably reveal a multitude of clinically useful peptide-based radioligands. Table 1 gives an overview of some typical receptors for regulatory peptides, which are (over)expressed on various human cancers, and it lists the peptides studied for receptor targeting.

Once the structure of the natural peptide ligand has been obtained, there are several other steps to follow until a new radiopharmaceutical is developed (Heppeler et al. 2000).

3.2 Design of Peptide-Based Radiopharmaceuticals

As indicated above, a radiopeptide is composed of different parts, most importantly being the bioactive peptide, which may be coupled to a spacer and this again to a chelator or a prosthetic group (Fig. 2).

The spacer may be a simple covalent bond but may also be introduced to improve the pharmacologic properties like binding affinity, ability to internalize into tumor cells, suitable biodistribution, etc.

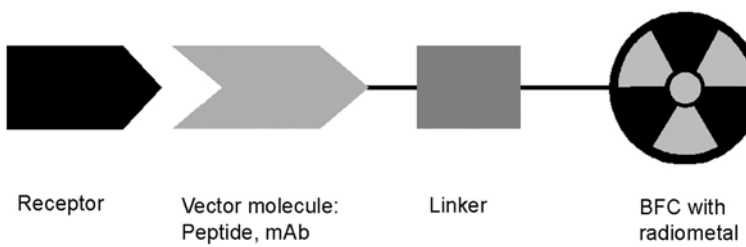

Fig. 2. Design of a receptor-mediated radiopharmaceutical

3.2.1 Important Radionuclides for Imaging and Therapy

The use of metal complexes as diagnostic and therapeutic agents is a relatively new area of medical research. The introduction of radiometals in nuclear medicine started in 1959 when the first ^{99}Mo/^{99m}Tc generator was developed at Brookhaven National Laboratory (Upton, NY). Since then, ^{99m}Tc has been the most widely used radionuclide for diagnostic imaging. Additional important radionuclides useful for imaging are listed in Table 2, including some of the longer-lived positron emitters.

Gamma scintigraphy requires a radiopharmaceutical containing a radionuclide that emits γ-radiation with an energy between 100–250 keV and a gamma camera. Positron emission tomography (PET) requires a radiopharmaceutical labeled with a positron-emitting

Table 2. Nuclear properties of important gamma- and positron-emitting radionuclides for biomolecule labeling

Isotope	Physical half-life (h)	Decay mode	E_γ (keV)	$E_{\beta+}$ (keV) average
^{67}Ga	78.26	EC (100%)	91, 93, 185, 296, 388	
^{99m}Tc	6.0	IT (100%)	141	
^{111}In	67.9	EC (100%)	245, 172	
^{18}F	1.83	β^+ (96.76), EC (3.3%)		649
^{124}I	100.3	β^+ (22.8%), EC (11%)		1,530
^{55}Co	17.5	β^+ (77%), EC (23%)		1,513, 1,037
^{62}Cu	0.16	β^+ (98%), EC (2%)		2,910
^{64}Cu	12.7	β^+ (19%), EC (41%) β^- (40%)		656
^{68}Ga	1.1	β^+ (90%), EC (10%)		1,880, 770
^{86}Y	14.7	β^+ (33%), EC (66%)		2,335, 2,019, 1,603, 1,248, 1,043
^{123}I	13.2	EC (100%)	159	

radionuclide (β^+) and a PET camera. A variety of gamma- and positron-emitters have been used for peptide labeling. Besides the energy of emission, there are some other factors to consider in designing a radionuclide-based peptide radiopharmaceutical, for example, the physical half-life, the type of decay, cost, and availability of the radioisotope.

Unlike radionuclides used for diagnostic imaging, therapeutic radionuclides by definition emit radiations that have a high linear energy transfer (LET) in order to destroy tumor tissue. These radionuclides with potential for therapy fall into three main categories: (a) β-emitting radionuclides; (b) α-emitters; and (c) Auger-electron emitters. Each type of these particles has a different effective range of energy deposition and LET properties.

The physical characteristics and range in tissues of commonly used β- and α-emitters are summarized in Table 3. In cases where

Table 3. Nuclear properties of several therapeutic radionuclides

Isotope	Physical half-life	Decay mode		Range	
		max β^--energy (MeV)	γ (keV) (%)	Mean (mm)	Approx. cell diameters
^{67}Cu	2.58 days	0.577 (20%)	91 (7%) 93 (16%) 185 (48%)	0.27	20
^{90}Y	2.67 days	2.27 (100%)	None	2.8	150
^{131}I	8.04 days	0.606	364 (81%)	0.28	20
^{149}Pm	2.21 days	1.07	286 (3%)	0.71	60
^{166}Dy	3.40 days	0.40	82.5 (13%)	0.18	15
^{177}Lu	6.71 days	0.50 (79%)	208 (11%) 113 (6.4%)	0.24	20
^{186}Re	90.6 h	1.071	137 (8.5%)	0.7	60
^{188}Re	16.98 h	2.116	155 (15%)	2.4	130
^{212}Bi	1 h	1.36 (β, 64%) 6.1 (α, 36%)	727 (7%)	0.09 0.06	2–3 3–4
^{213}Bi	46 min	5.8 (α, 2.2%) 8.4 (α, 97.8%)	440 (27.3%)	0.06 0.08	2–3 3–4
^{225}Ac	10 days	5.83 (α, 100%)	None	0.06	2–3

the γ-ray emission is in the diagnostically useful range, the imaging of the biodistribution of the tracer is also feasible (Kwekkeboom et al. 2001). Radionuclides that decay by β-emission are used most extensively for therapeutic applications in current clinical practice. A unique advantage of β-particle emitters over other therapeutic modalities is that not every cell needs to be targeted to be killed (crossfire effect, high LET). The crossfire effect is efficient for lesions larger in diameter than the average path length. Humm (Humm 1986) has classified β-emitting radionuclides as low-range (mean range <200 µm, i.e., Lu-177), medium-range (mean range 200 mm to <1 mm, i.e., Cu-67, Sm-153), and high range (mean range >1 mm, i.e., Y-90). Radioactive emission of α particles results in high LET over a path length of 3–4 cell diameters. The advantage of this property lies in its capability of producing a high degree of tumoricidal activity while sparing the surrounding normal tissues.

3.2.2 Labeling Methods

3.2.2.1 Direct Labeling

Because of the kinetic lability of hard radiometals like Y^{3+}, Lu^{3+}, and lanthanides in general, but also Cu^{2+}, Co^{2+}, etc. and the competition in human blood and other body fluids by proteins like transferrin, albumin, and anions like PO_4^{3-}, CO_3^{2-}, etc., peptides cannot offer any functional groups which provide enough kinetic stability to ensure intact arrival of the radiometal-peptide conjugate at the target. This is different for the pair Tc and Re, which form kinetically inert metal complexes in several oxidation states. In addition, both metals show a high degree of thiophilicity, which makes sulfur-containing peptidic sequences attractive for the binding of these radiometals. Several groups took advantage of this thiophilicity and labeled disulfide-bridged analogs of SS like octreotide, lanreotide, and vapreotide directly with ^{99m}Tc and ^{188}Re (Fig. 3; see Sect. 3.2.3; Thakur et al. 1997). Unfortunately, none of these peptides is very well characterized; in addition, they are too lipophilic, mainly excreted by the hepatobiliary system, and never made the step from preclinical studies to the clinic. Radiohalogens are being coupled to peptides usually taking advantage of the presence of Tyr. An electro-

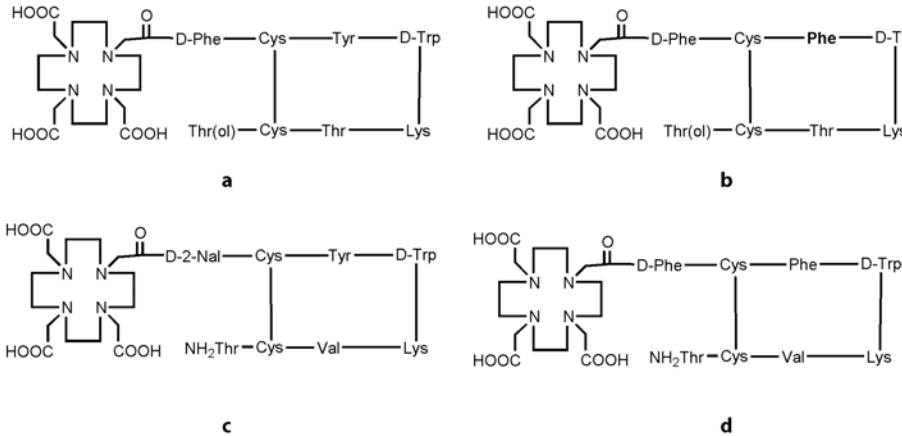

Fig. 3 a–d. Prototypical DOTA-peptides used for radiotherapy: (**a**) DOTA-[Tyr^3]-octreotide (DOTA-TOC); (**b**) DOTA-octreotide (DOTA-OC); (**c**) DOTA-lanreotide (DOTA-LAN); (**d**) DOTA-vapreotide

philic substitution reaction usually gives pure and well-defined radiopeptides.

3.2.2.2 The Bifunctional Chelator Approach

Most often, labeling strategies rely on the utilization of a multidentate ligand capable of chelation of the desired radionuclide. For example, all radiometals except ^{64,67}Cu and ^{55}Co listed in Tables 2 and 3 are hard acids with 3+ as the major oxidation state in solution. As they are kinetically labile, polydentate chelators need to be utilized for an efficient encapsulation and in vivo stabilization.

There are mainly two strategies: the prelabeling and the postlabeling methods. The prelabeling approach involves the formation of the radionuclide-chelator complex prior to conjugation to the peptide. If the radionuclide is introduced into its chelator after the chelator has been attached to the carrier, this is referred to as a postlabeling. The decision on which strategy is to be adopted will be influenced by a variety of considerations. For example, when complex formation can only be achieved under nonaqueous or otherwise harsh conditions

and the biomolecule is sensitive to these conditions, the prelabeling approach is more indicated. Still, this method is complicated and time-consuming because of multiple steps in preparation and purification, and therefore not suitable for routine clinical applications. The postlabeling approach is the most practical method for the development of peptide-based radiopharmaceuticals.

The ideal chelator should satisfy requirements correlating aspects of coordination chemistry with in vivo behavior. Factors to be considered include the kinetic and thermodynamic stability, stereochemistry, charge, lipophilicity and the redox properties of the metal complex. For lanthanides and lanthanide-like radiometals, the bifunctional octadentate chelators satisfy these requirements. Derivatives of diethylenetriaminepentaacetic acid (DTPA) are used for the fast incorporation of radiometals; the first clinically approved peptide-based imaging agent has been the DTPA-derivatized SS analog octreotide labeled with ^{111}In (Fig. 1 d). The coupling to the peptide is achieved either by using DTPA dianhydride or tri-*t*-butyl-DTPA as prochelators (Fig. 4; Achilefu et al. 2000). This potential octadenticity of DTPA may convey additional stability to the radiometal complex as In(DTPA)$^{2-}$ was shown to have coordination number 8 in the solid state and in solution (Maecke et al. 1989). Because of in vivo instability, DTPA is not suitable for any other nuclide than ^{111}In. Attempts to use DTPA-peptide conjugates labeled with ^{90}Y for therapy (Stolz et al. 1996) have not been as successful as the use of macrocyclic bifunctional chelators (BFC) for labeling of peptides (de Jong et al. 1997).

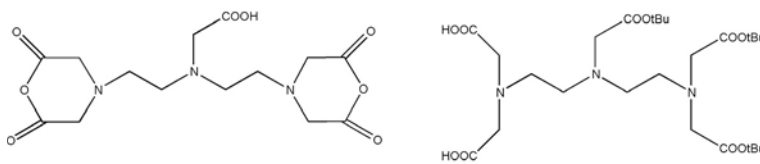

Fig. 4 a, b. Precursors of diethylenetriaminepentaacetic acid (DTPA) for biomolecule coupling: (**a**) DTPA dianhydride (cDTPA); (**b**) tri-*t*-butyl-DTPA (activated via cyclic anhydride formation)

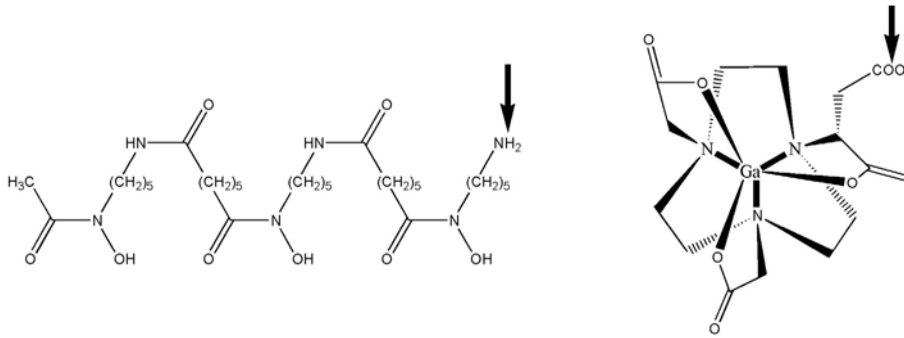

Fig. 5. a Desferrioxamine-B (DFO), a bifunctional chelator for labeling with $^{67/68}$Ga. **b** Ga(NODASA), a (radio)metal complex with three carboxylate groups, protected via the metal, and with potential for biomolecule coupling using the prelabeling approach. The coupling site is indicated by the *arrow*

Radiolabeling with Ga^{III} is of interest because of the access to three radioisotopes for imaging (Table 2). Two approaches were used to label SS analogs with radio-gallium. The use of DFO (desferrioxamine B; Fig. 5 a) allowed fast complexation, whereas the new chelator NODASA (1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid; Fig. 5 b; Andre et al. 1998) has three five-membered chelate ring-forming carboxylate groups protected by Ga(III) allowing a free β-carboxylate group for coupling to a SS analog. This prelabeling strategy allows the covalent coupling of a well-defined radiometal complex of high-specific activity to a biomolecule. The same type of chelator has been derivatized in order to make it also available for the postlabeling approach (Eisenwiener et al. 2002).

The DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)-based BFCs (Fig. 6 a–c) continue to be the most widely studied ligands for linking trivalent metallic radioisotopes to biomolecules (Cutler et al. 2000; Hu et al. 2002; McDevitt et al. 2001). The most common method involves the attachment of the peptide to one of the four acetate groups via a CO-NH bond. This conjugation can be made either via an activated ester of one carboxylate group (Fig. 6 a), or using a monoreactive DOTA prochelator like

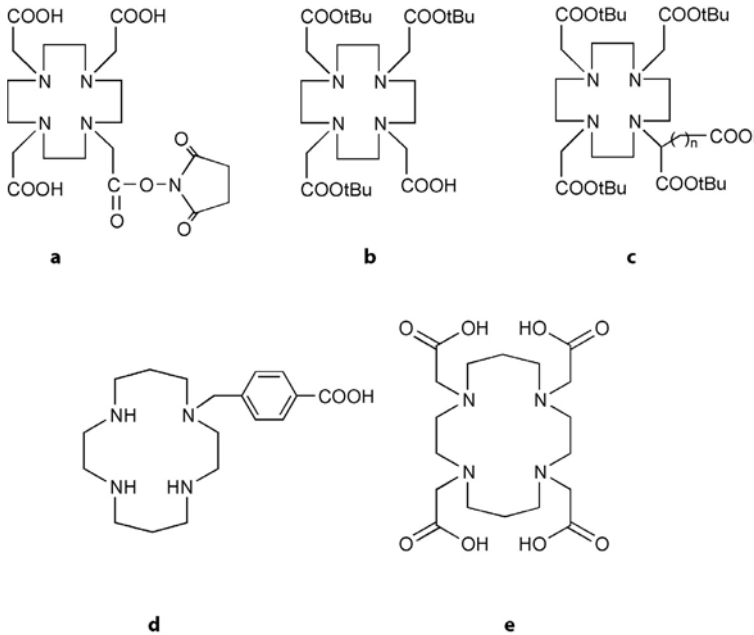

Fig. 6 a–e. Structures of DOTA derivatives: (**a**) *N*-hydroxysuccinimide (NHS) ester of DOTA; (**b**) tri-*t*-butyl-DOTA; (**c**) DOTASA(tBu)$_4$, $n=1$ and DOTAGA(tBu)$_4$, $n=2$; (**d**) CPTA; (**e**) TETA

DOTA(tBu)$_3$ (Fig. 6 b; Heppeler et al. 1999). The prochelator approach is perfectly compatible with peptide synthesis in solid phase or in solution and DOTA(tBu)$_3$ was coupled to SS analogs with 65±5% yields after deprotection and purification. DOTA, used unprotected, was also coupled to the same peptide with about 40% overall yield (Albert et al. 1998).

A disadvantage of this type of conjugation is the loss of one acetate arm for coordination to the radiometal center. To provide eight strong donor atoms for coordination, DOTA has been modified at one of the nitrogen atoms (Fig. 6 c; Eisenwiener et al. 2000). After coupling to the biomolecule and deprotection, a BFC-peptide conjugate is obtained, available for the labeling with different radiometals (^{111}In, ^{90}Y, ^{177}Lu and other lanthanides).

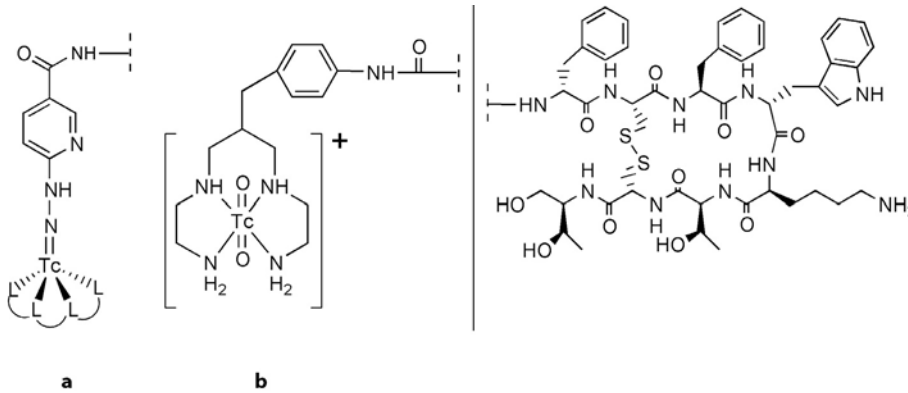

Fig. 7. a Structure of Tc-HYNIC-octreotide; **b** ^{99m}Tc-labeling of octreotide, using the N_4-tetramine chelator

The labeling of peptides with radioisotopes of Cu(II) is of interest mainly because of its two radionuclides ^{64}Cu and ^{67}Cu. The chemistry of copper radiopharmaceuticals has been reviewed extensively and comprehensively (Blower et al. 1996). BFCs for Cu(II) include 4-(1,4,8,11-tetraazacyclotetradec-1-yl)methyl)benzoic acid (CPTA; Fig. 6d), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA; Fig. 6e) and DOTA. All three chelators were coupled to the SS analogs through a carboxylate group.

Several approaches to label SS analogs with BFCs for ^{99m}Tc labeling have been published. HYNIC (2-hydrazinonicotinic acid; Fig. 7a) is of interest as a ^{99m}Tc-binding unit because of the potential monodenticity of this ligand which leaves coordination sites on the Tc atom free to be completed by different coligands which may be beneficial for the fine tuning of the biodistribution. HYNIC was described before to label successfully different biomolecules with ^{99m}Tc (Abrams et al. 1990; Edwards et al. 1997). [^{99m}Tc-N_4-D-Phe^1]-octreotide (Fig. 7b) was shown to bind with high affinity to the somatostatin releasing inhibiting factor (SRIF) receptor and showed high and specific tumor uptake in a SRIF receptor-positive tumor (Maina et al. 2002).

3.2.3 Prototypical Peptides for Imaging and Targeted Radiotherapy

The first diagnostically studied and also radiotherapeutically employed regulatory peptides were analogs of SS. Throughout this chapter the discussion will solely be based on radiolabeled SS analogs. SS binds with high affinity to SS receptors expressed on target tissues exerting a large number of biological effects. Five such receptor subtypes (hsst1–5) have been cloned in recent years. An important aspect with regard to targeting is the finding that these receptors are overexpressed on a variety of human tumors, mainly of neuroendocrine origin (Schaer et al. 1997). The most relevant receptor subtype is hsst2, but all the other receptor subtypes are to some degree also overexpressed on human tumors. There are two biologically active forms of SS consisting of 14 (SS-14) and 28 (SS-28) amino acids that show only very low metabolic stability in human blood with half lives of 2–3 min and can therefore not be used clinically. Modifications leading to octapeptides afforded SS receptor binding ligands with much lower proteolytic degradation rate. One of the clinically approved peptides is octreotide, a short analog of SS, which retained a high binding affinity to hsst2, reduced affinity to hsst3 and hsst5, and absent affinity to hsst1 and hsst4 (see Table 4). The chemical structures of SS-14 and octreotide are shown in Fig. 1 a, b.

The first radiopeptide used for in vivo localization of tumors was [^{123}I]-[3-iodo-Tyr^{3}]-octreotide (Fig. 1 c). Despite some spectacular early imaging results (Lamberts et al. 1990) and an almost optimal pharmacologic profile showing high hsst2 affinity ($IC_{50} = 2.0 \pm 0.7$ nM) and a high rate of internalization into tumor cells, this radioligand finally turned out not to be useful as a diagnostic tool. The reasons are its lipophilicity causing hepatobiliary excretion and therefore a very low diagnostic sensitivity in the abdomen. In contrast, the chelator-modified molecule, DTPA-octreotide (Fig. 1 d), which was designed to be complexed with the diagnostic radiometal $^{111}In^{3+}$, shows a rather low in vitro pharmacologic profile (low binding affinity to hsst2, $IC_{50} = 22 \pm 3.6$ nM and slow internalization rate), but the hydrophilic metal complex conveys high hydrophilicity to the targeting molecule and changes its pharmacokinetics, including predominant kidney excre-

Table 4. Affinity profiles (IC_{50}) for human sst1-sst5 receptors for a series of somatostatin analogs

Compound	sst1	sst2	sst3	sst4	sst5
SS-28	5.2±0.3	2.7±0.3	7.7±0.9	5.6±0.4	4.0±0.3
Octreotide (OC)	>10,000	2.0±0.7	187±0.355	>1,000	22±6
Y-DOTA-octreotide[a], (DOTA-OC)	>10,000	20±2	27±8	>10,000	57±22
Y-DOTA-[Tyr^3]-octreotide, (DOTA-TOC)	>10,000	11±1.7	389±135	>10,000	114±29
Y^{III}-DOTA-[Tyr^3,Thr^8]-octreotide (DOTA-TATE)	>10,000	1.6±0.4	>1000	523±239	187±50
Y-DOTA-[1-Nal^3]-octreotide	>1,000	3.3±0.2	26±1.9	>1,000	10.4±1.6
In-DOTA-[1-Nal^3]-octreotide, (DOTA-NOC^1)	>10,000	2.9±0.1	8±2	227±18	11.2±3.5
Y-DOTA-[2-Nal^3]-octreotide (DOTA-NOC^2)	>10,000	25±1.0	133±68	>10,000	98±12.5
Y-DOTA-lanreotide[b]	>10,000	23±5	290±105	>10,000	16±3.4
In^{III}-DTPA-octreotide	>10,000	22±3.6	183±13	>1,000	237±52
In^{III}-DTPA-[$Tyr]^3$-octreotate	>10,000	1.3±0.2	>10,000	433±16	>1,000
KE108[c]	2.6±0.4	0.9±0.1	1.5±0.2	1.6±0.1	0.65±0.1

[a] Octreotide: D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys (disulfide bond).
[b] Lanreotide: [D-2-Nal^1,Tyr^3,Val^6,$ThrNH_2^8$]octreotide.
[c] KE108; see Fig. 12.

tion. Consequently, it became the first imaging vector based on a radiopeptide; the commercial name is Octreoscan (Mid-South Imaging & Therapeutics, Memphis, TN), registered worldwide. In addition, the advantage of the DTPA-peptide conjugate was the highly practical labeling kinetics which can be performed in any nuclear medicine department or even in a private nuclear medicine practice.

In addition to Octreoscan, a ^{99m}Tc-labeled peptide has also been FDA approved recently and registered in several countries. It is based on a carbocyclic hexapeptide, modified with a N_3S-chelator,

and commercialized under the trade name Neotect (Berlex Laboratories, Montville, NJ; ^{99m}Tc-Depreotide).

^{111}In is not a very useful therapeutic radionuclide; therefore, conjugates were designed, synthesized, and evaluated preclinically which fulfilled the need for the labeling with the β-emitters, e.g., ^{90}Y and ^{177}Lu. DOTA was the preferred chelator, forming kinetically and thermodynamically stable metal complexes. The prototypical DOTA-coupled SS-based octapeptides used for radiotherapy are shown in Fig. 3.

3.3 Preclinical Characterization of Somatostatin-Based Radiopeptides

3.3.1 Binding Affinities and Affinity Profiles

The molecular basis of the use of radiopeptides in patient studies is the presence of receptors. Therefore, an important step in the characterization of a radiopeptide is the determination of the receptor affinity and – if receptor subtypes are present – the receptor affinity profile.

Introducing a chelator may strongly affect the biological and pharmacological properties of a peptide. The binding assay can be performed on intact cells or on membrane preparations, either by direct or competitive binding assays, thereby assessing alterations in affinity and specificity of the radiopeptide compared to the natural peptide (Reubi et al. 2000). As shown in Table 1, there are several receptors (over)expressed on different types of tumors, therefore knowing the affinity pattern for receptor subtypes is very important, since each subtype usually has a different expression profile on different tumor types. This is definitely true especially for SS receptors sstr1–5 (Schaer et al. 1997).

3.3.1.1 Peptide-Structure Affinity Relationship

Table 4 displays the affinity profiles of selected SS analogs (some of them shown in Fig. 3a–c) along with their Y(III)/In(III)-chelator-peptide conjugates in comparison with the natural peptide SS-28 and with octreotide. The binding affinities are expressed as IC_{50} values using ^{125}I-[Leu^8, D-Trp^{22}, Tyr^{25}]-SS-28 as radioligand. The assump-

tion made is that nonradioactive metals behave like their radioactive congeners.

The affinity profiles were determined by using cell lines transfected with SS receptor subtypes hsst1–5. Peptide modifications as well as the influence of chelator structure and radiometal (labeling strategy) were studied.

Small structural modifications in the peptide were shown not only to alter the binding affinity but also the subtype binding profile. For instance, modifications in 3-position of DOTA-octreotide [complexed with $^{nat}Y(III)$] leads to a marked improvement of hsstr2 affinity when replacing Phe^3 (Y^{III}-DOTA-OC) for Tyr^3 (Y^{III}-DOTA-TOC) but the hsstr3 affinity decreases by a factor of 15. Furthermore, replacement of Phe with 1-naphthylalanin (1-Nal,DOTA-NOC^1) increases the hsstr2, 3, and 5 affinity, rendering this peptide a very promising candidate for the imaging and targeted radiotherapy of a broader spectrum of human tumors. How subtle structural changes may affect the binding potency is shown by substituting 1-Nal for 2-Nal. This modification results in a peptide with low affinity to hsstr2, 3, and 5. Modifications at the C-terminus have a distinct influence on the sstr2 affinity; Y^{III}-DOTATATE has a sevenfold higher sstr2 affinity if compared to Y^{III}-DOTATOC, whereas the affinities towards sstr3 and 5 decrease.

Focusing on the peptide part in a peptide-based radiopharmaceutical, it is clear that lipophilicity, peptide size, and susceptibility to degradation by peptidases play a vital role (Lister-James et al. 1996).

3.3.1.2 Influence of the Chelate and (Radio)Metal on the Targeting Properties of Radiometallopeptides

In this chapter we will analyze the important role of the radiometal complex geometry as well as charge, size, fluxionality, etc. on DOTA-$[Tyr]^3$-octreotide pharmacology.

Except for the labeling of biomolecules with ^{99m}Tc and $^{186,188}Re$, DOTA has been used for all other radiometals of relevance in nuclear oncology. The use of one single chelator and one single chelator-peptide conjugate for a variety of radiometals allows lyophilized kit formulations and has the advantage that potential clinical applications will be approved easier by ethical committees. Our gold standard molecule for the in vivo localization of SS-receptor-positive tumors and

Table 5. Radiometal dependence on M-DOTATOC affinities to hsstr2

Compound	IC_{50} (nM)
SS-28	2.7±0.3 (19)
Y^{III}-DOTATOC	11±1.7 (6)
Ga^{III}-DOTATOC	2.5±0.5 (6)
Co^{II}-DOTATOC	0.44±0.11 (5)
Bi^{III}-DOTATOC	37±10 (3)
Ga^{III}-DOTATATE	0.2±0.04
Y^{III}-DOTATATE	1.6±0.4
In^{III}-DOTATATE	2.4±1

their targeted radionuclide therapy is [$DOTA^0$-Tyr^3]-octreotide. This molecule has been labeled with $^{66,67,68}Ga^{III}$, $^{111}In^{III}$, $^{90}Y^{III}$, $^{213}Bi^{III}$, and $^{57}Co^{II}$. Table 5 shows the binding characteristics of several metallopeptides to hsstr2 versus a ^{125}I-labeled SS-28 as radioligand.

An important result of this experiment is that obviously the metal ion has a marked influence on the affinity of the metallopeptide, e.g., Co^{II}-DOTATOC shows a better sstr2 binding than Ga^{III}-DOTATOC, Y^{III}-DOTATOC, and Bi^{III}-DOTATOC. As demonstrated, the conjugation of the metal-DOTA complex may even result in an improved ligand compared to the natural peptide. It is not yet clear why the metal ion which is remote from the pharmacologic part of the peptide has such a distinct influence.

Preliminary comparative data using two-dimensional ^{1}H-NMR studies of the Ga^{III}-, In^{III}-, Y^{III}-DOTATOC are not conclusive and the peptides resist crystallization. The model peptides In^{III}-, Y^{III}-, and Ga^{III}-DOTA-D-PheNH_2, however, could be crystallized and their X-ray crystal structure determined. The structures differ in different ways. Ga^{III}-DOTA-D-PheNH_2 (Fig. 8 a) has a pseudo-octahedral structure, the macrocycle showing a *cis*-geometry. The equatorial plane is formed by two transannular nitrogens of the tetraaza ring and two oxygens of the respective carboxylate groups. One carboxylate group is free and the carbonyl oxygen of the peptide bond forming the linkage to D-PheNH_2 is not bound to the Ga^{III}. This is in contrast to Y^{III}(In^{III})-DOTA-D-PheNH_2 (Fig. 8 b), which are octacoordinate complexes including the amide carboxy oxygen (Heppeler et al. 1999).

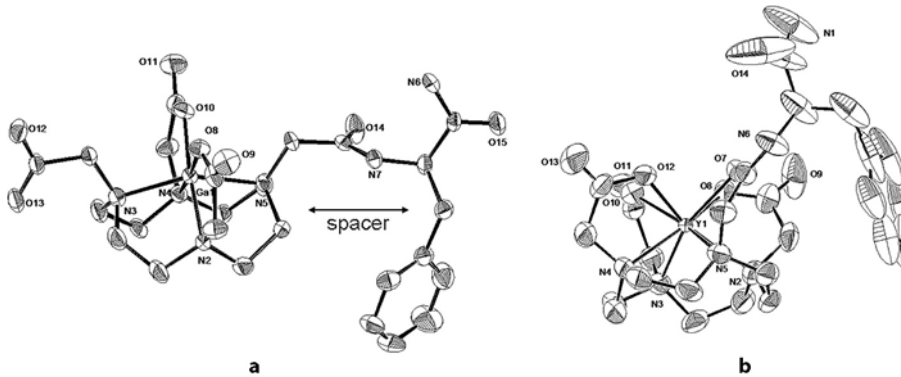

Fig. 8. a ORTEP plot of the crystal structure of [Ga^{III}-DOTA-D-PheNH_2], a pseudo-octahedral structure with a *cis*-geometry of the macrocycle. One carboxylate group is free and the carbonyl oxygen of the peptide bond forming the linkage to D-PheNH_2 is not bound to the Ga^{III}. **b** ORTEP representation of the crystal structure of [Y^{III}-DOTA-D-PheNH_2], an octacoordinate complex including the amide carboxy oxygen (reproduced with permission from Heppeler et al. 1999)

The complex geometry is a somewhat distorted antiprism. ^{1}H-NMR studies showed that also in solution the Ga^{III}-complex is hexacoordinate whereas the Y^{III}-DOTA-D-PheNH_2 and In^{III}-DOTA-D-PheNH_2 complexes are octacoordinate, the latter showing much higher fluxionality (Maecke et al. 2001), which may explain the differences between ^{111}In- and ^{90}Y-DOTA-TOC in the biodistribution study.

The hexacoordination of the Ga^{III}-complex may explain the improved kidney clearance of ^{67}Ga-DOTATOC compared to ^{90}Y-DOTATOC, and the improved pharmacological profile may depend on the free carboxymethyl arm bound to the peptide, allowing for more flexibility due to a spacer function. This hypothesis was tested by developing a conjugate with the optimal chelator for Ga^{III} radioisotopes, NOTA (1,4,7-triaza-1,4,7-triacetic acid). NOTA was modified in order to allow a spacer between the chelator (NODAGA = 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid) and the peptide, separating the signal producing chelate from the biologically active peptide (Fig. 9). This combination has the additional advantage that the extremely stable Ga^{III}-NOTA complexes make any interference

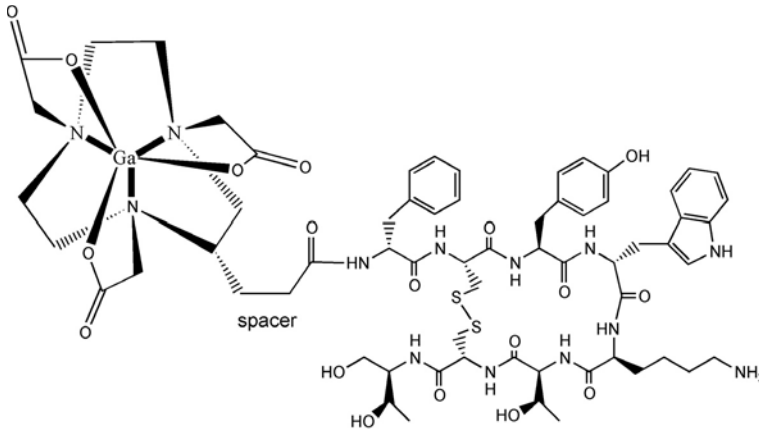

Fig. 9. Structural formula of Ga-NODAGA-TOC

and false interpretation of biological and pharmacological data due to potential transchelation chemistry rather unlikely. Indeed, Ga^{III}-NODAGA-TOC conjugate shows similar pharmacologic and biodistribution parameters like Ga^{III}-DOTATOC (Eisenwiener et al. 2002).

Preliminary data (Heppeler 2000) indicate that Co^{II}-DOTA-D-PheNH_2 has a very similar structure to the Ga^{III} complex and this along with the charge difference may explain the high potency of the corresponding metallopeptide. The low pharmacologic potency and profile of the Bi^{III}-complexed peptide is not understood at all at this time.

3.3.2 Structure Influencing Pharmacokinetics

The in vivo evaluation in animal models of radiopeptides is of major importance, since it is the first "real" indicator of compound pharmacokinetics. As a model, tumor cells for which the peptide is being investigated are transferred from cell culture into nude mice or rats, inducing tumor growth. Upon administration of the labeled peptide in these experimentally grown tumors, the potential of tumor targeting can be evaluated. An important aspect of this is the proof

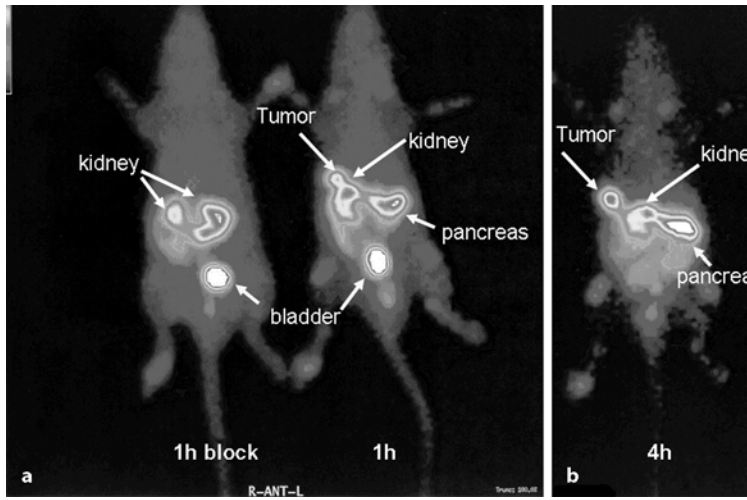

Fig. 10. Four-hour postinjection scintigraphy of two Lewis rats bearing subcutaneous tumors in the left hind leg: (**a**) rat with a coinjection of ^{111}In-DOTATOC and excess of cold peptide; (**b**) rat injected only with ^{111}In-DOTA-TOC

of specificity of tumor targeting due to the possibility of blocking the receptors with excess cold peptide. Such an example is shown in Fig. 10 with two rats bearing subcutaneous tumors in the left hind leg. One animal was only injected with ^{111}In-DOTA-TOC (Fig. 10b) and the tumor is nicely shown, as well as the receptor positive pancreas. In the second rat (Fig. 10a), about 500 times excess of nonradiolabeled peptide is coinjected with ^{111}In-DOTA-TOC, competing for the receptors; no radioactive signal is seen from the tumor and the pancreas.

The aim of biodistribution studies in animal models is the evaluation and optimization of the pharmacokinetics of radiopeptides. The uptake in different organs, especially in the receptor-positive organs and in kidneys have to be optimized. For clinical application, the kidney toxicity is the dose-limiting factor. Peptides are taken up by the tubular cells and radiometal chelates are trapped within the lysosomes, high retention of the radiolabel occurring in the kidneys, eventually causing nephrotoxicity (de Jong et al. 2001).

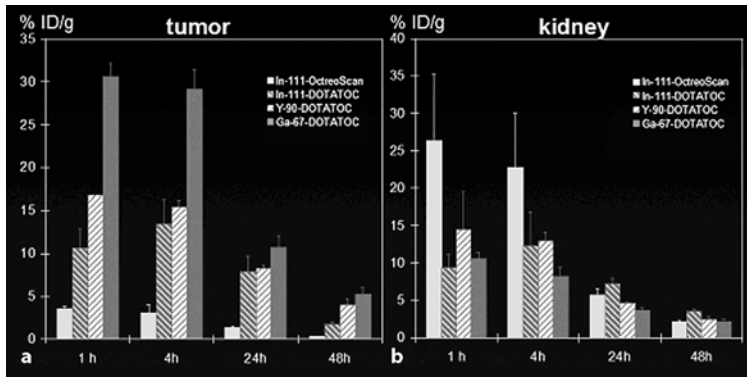

Fig. 11. Tumor (**a**) and kidney (**b**) uptake of ^{111}In-, ^{67}Ga- and ^{90}Y-DOTA-TOC in comparison with Octreoscan

Figure 11 shows a comparison in biodistribution in AR4-2J rat pancreatic-tumor-bearing Lewis rats of four compounds: ^{111}In-Octreoscan (see structure in Fig. 1 d) and DOTA-TOC (see Fig. 3 a) labeled with three different radiometals: ^{111}In, ^{90}Y and ^{67}Ga, respectively (Heppeler et al. 1999). The best tumor-to-kidney ratio corresponds to the gallium-labeled compound, this result being confirmed also in patients. As shown also in Sect. 3.3.1.2, the (radio)metal has a significant influence on the properties (binding affinity, internalization, biodistribution) of the radiopharmaceutical. In addition, this study shows the superiority of the new compounds over the approved ^{111}In-Octreoscan and was the basis to introduce the improved versions into the clinic.

Before therapeutic clinical applications can be performed, radiotherapy studies in animals will be done. For example, Stolz et al. (Stolz et al. 1998) evaluated the therapeutic effect of ^{90}Y-DOTA-TOC on rats bearing CA20948 tumors. Their result showed complete remission of tumors in five out of seven rats. De Jong et al. (de Jong et al. 2001) demonstrated with different SS analogs and different radiometals that the cure rate depends on tumor size, having 100% remission for small tumors.

All these in vivo tests, along with the in vitro assays presented in Sect. 3.3 are helpful for designing and developing new and improved radiopharmaceuticals.

3.3.3 Towards Pan-Somatostatin Ligands

As mentioned above, there are at least five different SS receptor subtypes which may be expressed concomitantly and in various combinations in normal or cancerous tissues (Patel 1999; Reubi et al. 2001). It is therefore of high interest to either develop analogs that bind with high affinity to all five subtypes (pan-SS) for a broader spectrum of tumors or to increase uptake in those tumors which overexpress multiple receptors on their plasma cell membrane. None of the currently used radiopeptides shows such a broad affinity (see Table 4) although some analogs like DOTA-lanreotide (Traub et al. 2001) have been claimed to correspond almost to a pan-SS.

A first example of a universal ligand which can be radioiodinated was recently described by Reubi et al. (Reubi et al. 2002). It is a nonapeptide with reduced size and a metabolically stabilized structure (KE108; Fig. 12; see also Table 4, last entry). It binds with a very high affinity to all five SS receptor subtypes, being equivalent to SS-28 at sstr1 but 2–4 times higher than SS-28 at sstr2–5. In addition, it has agonistic properties at all five subtypes. This peptide was also coupled to DOTA and was shown to still exhibit pan-SS

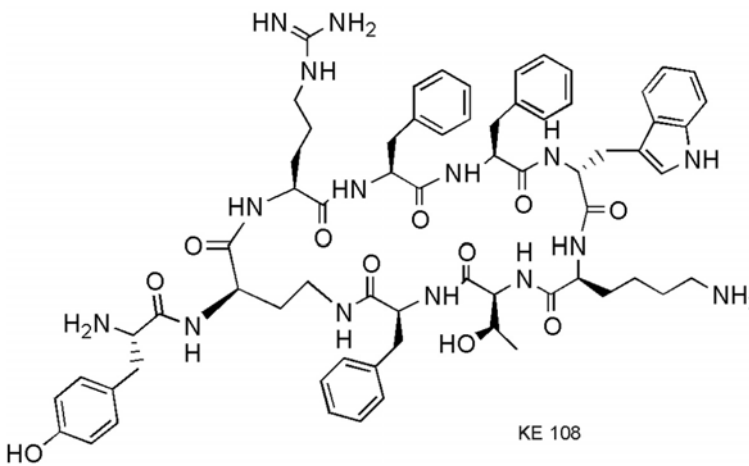

Fig. 12. Structural formula of KE108

character although with slightly reduced affinities to all subtypes (Eisenwiener 2001).

3.4 Patient Studies

As already mentioned, the basis of SS-receptor-targeted radiotherapy is the overexpression of SS receptor subtypes on neuroendocrine tumors.

Herein we present a few patient studies with the gold standard DOTA-[Tyr^3]-octreotide (DOTA-TOC; see Fig. 3a for structure) radiolabeled with different radionuclides for imaging and for therapy, highlighting their superb targeting performance. Fig. 13a and b shows the PET scan of a patient with a neuroendocrine tumor with multiple liver metastases, scanned with ^{68}Ga-DOTA-TOC at 50–80 min after injection. This image reflects the high sensitivity and specificity of the radiogallium-modified peptide, in agreement with the preclinical results found for this radiometallopeptide.

Labeled with the β-emitter, ^{90}Y-DOTA-TOC proved to be very efficient in targeted radiotherapy of some neuroendocrine tumors. Below are two examples which show complete remission even in these radioresistent tumors. The first scan (Fig. 14) shows a patient with a Merkel cell tumor before (left) and after (right) ^{90}Y-DOTATOC therapy. The uptake of ^{111}In-DOTATOC was not visible after four i.v. injections of 50 mCi/m^2 ^{90}Y-DOTATOC; this was judged a complete remission (CR).

Figure 15 shows the image of another patient with an endocrine pancreas tumor and multiple metastases in the liver. After four i.v. injections, the liver metastases disappeared and the outcome was partial remission (PR) overall; the analysis of more than 400 patients treated with this modality in Basel showed 34% objective response (CR and PR) according to WHO criteria. In addition, stabilization of progressive disease was shown in approximately 50%.

The benefit of targeted radiotherapy with radiolabeled SS analogs is documented and has been proven. Still, targeted peptide receptor-mediated radiotherapy is in its infancy and questions remain how one could enhance the efficacy of the therapy. Renal toxicity is the main dose-limiting factor found so far. Infusion of cationic amino

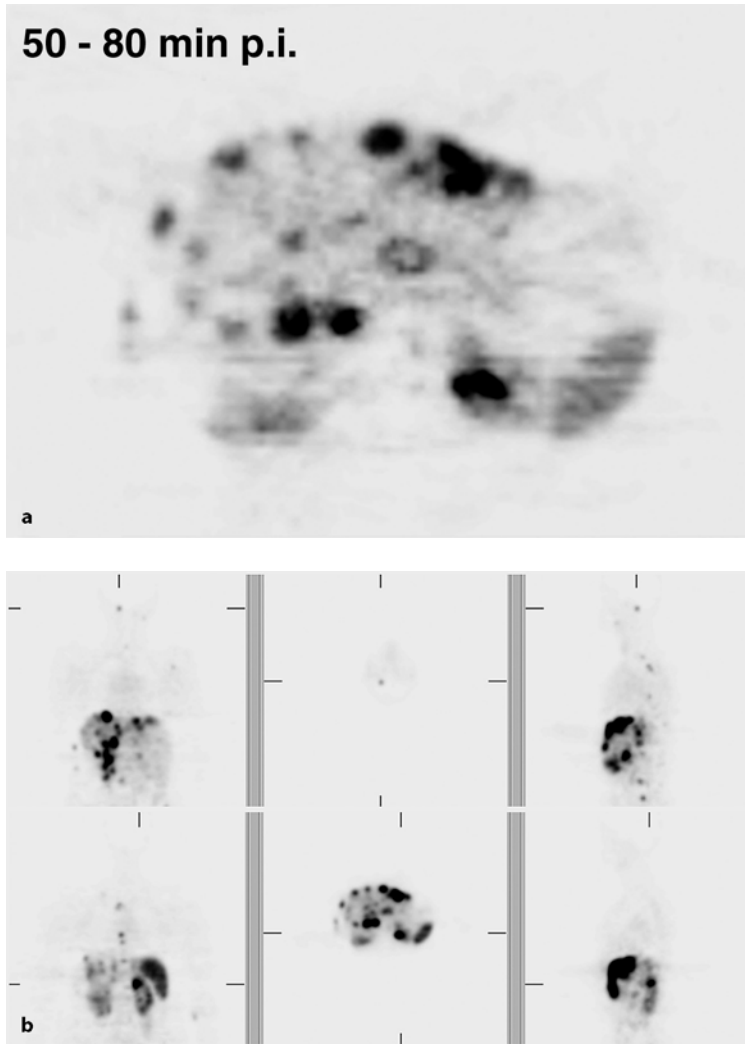

Fig. 13. a UPT: neuroendocrine tumor, ^{68}Ga-DOTATOC PET; multiple liver metastases: minimum ∅ 5 mm, SUV 24.7. **b** UPT: neuroendocrine tumor, ^{68}Ga-DOTATOC PET; previously unknown metastases

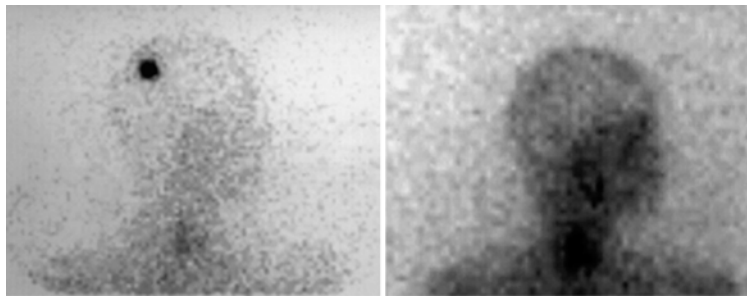

Fig. 14. ^{111}In-DOTATOC scan of a 43-year-old female patient with a Merkel cell tumor before (*left*) and after (*right*) ^{90}Y-DOTATOC treatment

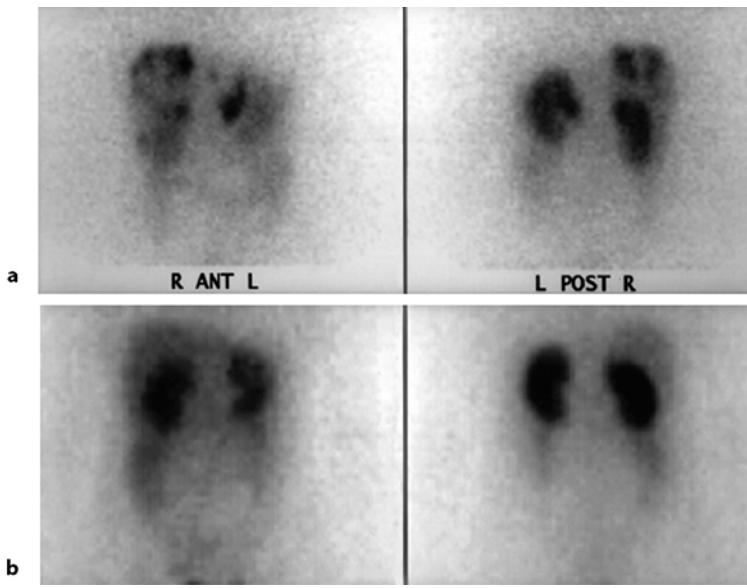

Fig. 15. ^{111}In-DOTATOC scan of the abdomen of a 43-year-old female patient with a recurrence of endocrine pancreatic tumor and multiple liver metastases before (**a**) and after (**b**) ^{90}Y-DOTATOC treatment

acids reduce the uptake of the peptides in the tubular cells of the kidneys. Eventually, structural modifications of the peptides will lead to new radiopeptides with much lower renal uptake. This improvement most likely will come from innovative developments and chelator design from medicinal inorganic chemists.

3.5 Summary and Conclusions

Radiometallo-labeled analogs of SS have shown great benefit in the in vivo localization and targeted radiotherapy of human tumors. The progress and innovation in this clinical application came from the change in strategy, leaving the most widely used radiohalogens for a coordination chemistry approach. The use of chelators appended to the biologically active peptide which convey high thermodynamic and kinetic stability to the radiopeptides did not only improve the pharmacokinetics and pharmacodynamics of the molecules, but surprisingly the biological potency as well.

The most urgent problem to be solved in the field is to improve the kidney clearance of the radiopeptides. The kidney turned out to be the dose-limiting organ in this type of targeted radiotherapy. Coordination chemical strategies have already paved the way to a successful clinical application; it is most likely that chelator modification will further help to improve the renal handling of radiometallopeptides.

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