Radiometal-Labeled Agents (Non-Technetium) for Diagnostic Imaging

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Received March 19, 1999 (Revised Manuscript Received July 5, 1999)

Contents

I. Introduction

A. Chemistry and Biology of Radiometals

The use of radiometal-labeled small complexes and biomolecules as diagnostic agents is a relatively new area of medical research. In the late 1940s and early 1950s, the use of nuclear technology for medical purposes began, with nuclear reactors, accelerators, and cyclotrons being applied to medical isotope production. In 1959, Brookhaven National Laboratory developed the ^{99}Mo/^{99m}Tc generator,[1] and in 1964 the first ^{99m}Tc radiotracers were developed at the University of Chicago.[2] Research into ^{99m}Tc radiopharmaceuticals was the beginning of the study of coordination chemistry as it relates to diagnostic imaging. Although ^{99m}Tc is the most widely used radionuclide for diagnostic imaging,[3−5] a myriad of other radiometals have been or are being investigated for their uses in nuclear medicine. This review will discuss the non-technetium-labeled radiometal-labeled agents used in gamma scintigraphy and positron emission tomography (PET).

B. Gamma Scintigraphy and PET

Imaging modalities widely used in nuclear medicine include gamma scintigraphy and PET. Gamma scintigraphy requires a radiopharmaceutical containing a nuclide that emits gamma (γ) radiation (Table 1) and a gamma camera or SPECT (single-photon emission computed tomography) camera capable of imaging the patient injected with the gamma-emitting radiopharmaceutical. The energy of the gamma photons is of great importance, since most gamma cameras are designed for specific energy windows, generally in the range of 100−250 keV. Radionuclides that decay with gamma energies lower than this range produce too much scatter, while gamma energies >250 keV are difficult to collimate, and in either case the images may not be of sufficient quality. PET requires a radiopharmaceutical labeled with a positron-emitting radionuclide (β^+) (Table 2) and a PET camera for imaging the patient. Positron decay results in the emission of two 511 keV photons 180° apart. PET scanners contain a circular array of detectors with coincidence circuits designed to specifically detect the 511 keV photons emitted in opposite directions.

Radiometal-labeled agents are used in both imaging modalities, but currently, more radiometals are used in gamma scintigraphy than in PET. For gamma scintigraphy and PET, radiopharmaceuticals labeled with metal radionuclides are injected into patients to diagnose medical problems such as cancer, infection, thrombosis, kidney and liver abnormalities, and cardiological and neurological disorders. The biological distribution of radiopharmaceuticals is

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10.1021/cr980451q CCC: $35.00
Published on Web 08/11/1999

Carolyn Anderson was born and raised in Superior, WI. She received her B.S. degree from the University of Wisconsin—Superior in 1985. In 1984 she received a fellowship to attend the Summer School in Nuclear Chemistry at San Jose State University. She pursued her Ph.D. in Inorganic Chemistry with Professor Gregory R. Choppin, studying the electrochemistry and spectroscopy of uranium complexes in room-temperature molten salts. On completion of her Ph.D. in 1990, she moved to Washington University School of Medicine (WUSM) in St. Louis, MO, to carry out postdoctoral research with Professor Michael J. Welch in the development of radiopharmaceuticals for PET imaging. In 1993 she was promoted to her present position as Assistant Professor of Radiology at WUSM. In 1996 she obtained a joint appointment in the Department of Molecular Biology and Pharmacology at WUSM. She is active in the American Chemical Society and the Society of Nuclear Medicine and has been a lecturer at the Summer Schools in Nuclear Chemistry at San Jose State University and Brookhaven National Laboratory. Her current interests include the development of radiometal-labeled tumor receptor-based radiopharmaceuticals for PET imaging and targeted radiotherapy of cancer.

Michael Welch was born in Stoke-on-Trent, England. He received his B.A., M.S. and Ph.D. degrees in 1961, 1964, and 1965 at Cambridge University in London, England. His Ph.D. research was on the chemistry of excited tritium atoms. After completion of his Ph.D., he accepted a research associate position at Brookhaven National Laboratory to work on carbon atom chemistry with Dr. Alfred P. Wolf. In 1967, he became Assistant Professor of Radiation Chemistry in Radiology at Washington University School of Medicine in St. Louis, MO, to carry out research on the medical uses of cyclotron-produced radionuclides. He was promoted to Associate Professor in 1970 and then to Professor of Radiation Chemistry in Radiology in 1974 and Professor of Chemistry at Washington University in 1976. In 1990 he became the Co-Director of the Division of Radiological Sciences. His other appointments at WUSM include Professor of Molecular Biology and Pharmacology (1990) and Professor of Biomedical Engineering (1996). Professor Welch has received numerous awards, most notably the American Chemical Society Award for Nuclear Chemistry (1990), the American Chemical Society Midwest Award (1991), and The Society of Nuclear Chemistry de Hevesy Nuclear Pioneer Award (1992). His current research interests are in the medical application of radionuclides, the development of accelerator targetry, and the development of new radiolabeling techniques.

generally governed by two factors: (1) perfusion (or blood flow) and (2) specific biochemical processes such as receptor/antigen binding. Radiometal agents are also used to monitor various types of cancer therapy and to determine dosimetry on large doses of metal radiopharmaceuticals used for targeted radiotherapy.

C. Desirable Properties of Radiometals

Tables 1 and 2 show the wide variety of non-technetium gamma- and positron-emitting radiometals, their decay characteristics, and methods of production. In designing radiometal-based radiopharmaceuticals, important factors to consider include the half-life of the radiometal, the mode of decay, and the cost and availability of the isotope. For diagnostic imaging, the half-life of the radionuclide must be long enough to carry out the desired chemistry to synthesize the radiopharmaceutical and long enough to allow accumulation in the target tissue in the patient while allowing clearance through the nontarget organs. Ideally, the half-life should be as short as possible to reach these two goals and limit the radiation dose to the patient. Radiometals for radiopharmaceuticals used in PET and gamma scintigraphy range in half-life from about 10 min (^{62}Cu) to several days (^{67}Ga). The desired half-life is dependent upon the time required for the radiopharmaceutical to localize in the target tissue. For example, heart or brain perfusion-based radiopharmaceuticals require shorter half-lives, since they reach the target quickly, whereas tumor-targeted radiolabeled monoclonal antibodies often take longer to reach the target for optimal target-to-background ratios to be obtained.

Another important factor in choosing radionuclides for diagnostic imaging is their cost and availability. Radionuclide generators are considered ideal, since they consist of a longer-lived parent isotope that decays to a shorter-lived daughter radionuclide. The daughter can be easily separated from the parent by either ion exchange chromatography (the more common method) or solvent extraction. If the parent isotope is of relatively low cost, then even small

Table 1. Gamma- and Beta-Emitting Radionuclides

isotope	$t_{1/2}$ (h)	production methods	decay mode	E_γ (keV)	E_{β^-} (keV)	ref
^{67}Cu	62.01	accelerator, ^{67}Zn(n,p)	β^- (100%)	91, 93, 185	577, 484, 395	
^{67}Ga	78.26	cyclotron	EC (100%)	91, 93, 185, 296 388		176
^{90}Y	64.06	^{90}Sr/^{90}Y generator	β^- (72%)		2288	176
^{111}In	67.9	cyclotron, ^{111}Cd(p,n)^{111}In	EC (100%)	245, 172		176
^{99m}Tc	6.0	^{99}Mo/^{99m}Tc generator	IT (100%)	141		176
^{201}Tl	72 h	cyclotron	EC (100%)	135, 167		176
		^{203}Tl(p,3n)^{201}Pb(p,n)^{201}Tl	Hg X-rays			

Table 2. Positron-Emitting Radionuclides

isotope	$t_{1/2}$(h)	methods of production	decay mode	E_{β^+} (keV)	ref
^{55}Co	17.5	cyclotron, ^{54}Fe(d,n)^{55}Co	β^+ (77%) EC (23%)	1513, 1037	176,177
^{60}Cu	0.4	cyclotron, ^{60}Ni(p,n)^{60}Cu	β^+ (93%) EC (7%)	3920, 3000 2000	13,16,176
^{61}Cu	3.3	cyclotron, ^{61}Ni(p,n)^{61}Cu	β^+ (62%) EC (38%)	1220, 1150 940, 560	13,16,176
^{62}Cu	0.16	^{62}Zn/^{62}Cu generator	β^+ (98%) EC (2%)	2910	16,176
^{64}Cu	12.7	cyclotron, ^{64}Ni(p,n)^{64}Cu	β^+ (19%) EC (41%) β^- (40%)	656	14,16,176
^{66}Ga	9.5	cyclotron, ^{63}Cu(α,nγ)^{66}Ga	β^+ (56%) EC (44%)	4150, 935	176
^{68}Ga	1.1	^{68}Ge/^{68}Ga generator	β^+ (90%) EC (10%)	1880, 770	176,178
^{82}Rb	0.022	^{82}Sr/^{82}Rb generator	β^+ (96%) EC (4%)	3150	176
^{86}Y	14.7	cyclotron, ^{86}Sr(p,n)^{86}Y	β^+ (33%) EC (66%)	2335, 2019 1603, 1248 1043	21,176

hospitals can have a ready supply of the daughter radionuclide as needed. A few radiometals used for radiopharmaceuticals for imaging are produced by a nuclear reactor. Other radiometals are accelerator- or cyclotron-produced, which is a more expensive mode of production, since the cyclotron or accelerator can only produce one isotope at a time.

D. Properties of Radiometal-Labeled Imaging Agents

The design of radiometal-based imaging agents requires understanding the correlation of the physical properties of radiometal complexes with their in vivo behavior. Knowing information such as the redox properties, stability, stereochemistry, charge, and lipophilicity of the radiometal complex in some cases can help predict the in vivo behavior. The design of metal complexes generally involves specifying which organ or tissue is to be targeted. For example, it is known that negatively charged compounds tend to clear through the kidneys, many positively charged ions accumulate in the heart, and an overall neutral complex is required for crossing the blood-brain barrier. Lipophilic complexes are usually cleared through the hepatobiliary system and may accumulate in fatty tissues. Stereochemistry is often important when targeting complexes to specific receptors. Although thermodynamic stability of the complex is often an important factor, kinetic stabilty may better predict in vivo stability. Throughout this review, we will discuss these different considerations in the design of radiometal-based diagnostic imaging agents.

E. Organization of the Review

This review is organized into two major sections. Chapter 2 focuses on the chemistry of radiometal-labeled imaging agents, including the different radiometals used in nuclear medicine and their methods of production. Chapter 3 discusses radiopharmaceuticals based on which disease state they target. This allows a comparison of radiopharmaceuticals labeled with different radiometals that have been evaluated for the same disease. The goal of this review is to discuss the chemistry and biological behavior of radiometal radiopharmaceuticals, since these two factors are closely linked, and must both be considered in the design of these agents.

II. Chemistry of Radiometal-Labeled Imaging Agents

A. Radionuclide Production

1. Production of ^{66}Ga, ^{67}Ga, and ^{68}Ga

There are three gallium radionuclides with decay characteristics that are suitable for either gamma scintigraphy or PET imaging. ^{67}Ga is cyclotron-produced, most commonly by the nuclear reaction ^{68}Zn(p,2n)^{67}Ga on enriched ^{68}Zn. The nuclide was first produced for human use in 1953,[6] and several methods have been described for the separation. The most common separation techniques utilize solvent extraction, ion exchange, or both, although coprecipitation with ferric hydroxide has also been used.[7]

Gallium-68 ($t_{1/2}$ = 68 min) is produced from the ^{68}Ge/^{68}Ga generator,[8] and its decay is 89% by positron emission. The long half-life of the parent nuclide ^{68}Ge ($t_{1/2}$ = 280 days) gives the generator a useful life of 1−2 years, allowing PET imaging at facilities without an on-site cyclotron. The generator is commercially available, but to date, ^{68}Ga has been used in only a limited number of clinical studies. Due to the widespread application of ^{68}Ge in transmission sources for PET scanners, there is a shortage of ^{68}Ge for the use of this parent daughter system in producing ^{68}Ga radiopharmaceuticals. Consequently, ^{68}Ge is often considered cost-prohibitive as a radionuclide generator.

Gallium-66 is a cyclotron-produced positron-emitting nuclide which has been used in a limited number of studies requiring a medium half-life positron-emitting nuclide where a longer than 1 h half-life is needed.[9,10] This nuclide can be produced in small biomedical cyclotrons, utilizing the ^{66}Zn(p,n) ^{66}Ga reaction.[11] In this review, radiopharmaceuticals labeled with ^{67}Ga and ^{68}Ga will be discussed.

2. Production of ^{111}In and ^{113m}In

The most widely used radioisotope of indium in radiopharmaceutical studies is ^{111}In. Indium-111 ($t_{1/2}$ = 67.9 h) is cyclotron-produced, generally by the ^{111}Cd(p,n) ^{111}In nuclear reaction. The ^{111}In is separated from the cadmium using techniques similar to that discussed above for the separation of ^{67}Ga from zinc targets.[7] Ion exchange, solvent extraction, and coprecipitation with $Fe(OH)_3$ have all been used. This nuclide decays by electron capture with emission of gamma photons of 173 and 247 keV (89% and 95% abundance respectively) and is widely used in gamma scintigraphy.

Indium-113m is formed using the ^{113}Sn/^{113m}In generator. The parent ^{113}Sn has a half-life of 115 days and the ^{113m}In a half-life of 1.7 h. The parent nuclide is produced by neutron irradiation of a tin target. In the mid- to late-1960s, studies were carried out using the ^{113}Sn/^{113m}In generator.[12] The use of ^{113m}In decreased, however, since the Anger camera was far more efficient for imaging the 140 keV gamma photon from ^{99m}Tc compared to the 393 keV gamma from ^{113m}In.

3. Production of ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, and ^{67}Cu

The radionuclides of copper offer a selection of diagnostic (^{60}Cu, ^{61}Cu, ^{62}Cu, and ^{64}Cu) and therapeutic (^{64}Cu and ^{67}Cu) isotopes (Tables 1 and 2). The positron-emitting diagnostic nuclides have a wide range of half-lifes (10 min to 12.7 h) and are cyclotron- or generator-produced. Copper-64 was initially produced using a reactor by the ^{64}Zn(n,p)^{64}Cu nuclear reaction but more recently has been produced by the ^{64}Ni(p,n)^{64}Cu nuclear reaction using a biomedical cyclotron. A target has been specifically designed for the production of this nuclide,[13,14] and by altering the enriched isotope of nickel used as a target, large quantities of ^{64}Cu, ^{60}Cu, and ^{61}Cu have been produced. Copper-62 is generator produced from the decay of ^{62}Zn, while ^{67}Cu is only produced in useable quantities by spallation reactions at high-energy accelerators.[15] Due to the more widespread availability, ^{64}Cu is preferred for labeling proteins, peptides, and agents with long blood clearance. Copper-60 is preferred for agents with short blood clearance such as hypoxia agents discussed later in this review. A recent exhaustive review by Blower et al. in 1996[16] discusses the status of copper radionuclide production chemistry, radiochemistry, and radiopharmacology.

4. Production of ^{86}Y and ^{90}Y

A review on the general coordination chemistry of yttrium has been presented[17] which focuses on novel compounds which have been structurally described; however, it does not focus on compounds of biological interest. There are two radioisotopes of yttrium that have been utilized in preparing radiopharmaceuticals: ^{90}Y ($t_{1/2}$ = 64.06 h) and ^{86}Y ($t_{1/2}$ = 14.7 h). Yttrium-90 is a pure β^- emitter, has applications for targeted radiotherapy, and is usually produced following the decay of the parent nuclide ^{90}Sr. Strontium-90 is a fission product with a 27.8 year half-life. Yttrium-86 is produced by the ^{86}Sr(p,n) ^{86}Y nuclear reaction and is a positron-emitting nuclide that has been used as an alternate label for ^{90}Y for applications in PET imaging.[18−20] Stronium-86 composes 9.9% of natural strontium, and targets of enriched strontium oxide or carbonate have both been used.[21,22] Typical separation procedures involve dissolution, chromatographic separation, and recycling of the strontium target.

B. Challenges in Trivalent Metal Ion Chemistry

The coordination and analytical and radiopharmaceutical chemistry of gallium and indium has been reviewed.[23−30] Gallium and indium are in group IIIB of the periodic table. Under physiological conditions, the only oxidation state of gallium and indium in aqueous solution is +3, and this is the oxidation state relevant to radiopharmaceutical chemistry. The complexation of Ga(III) and In(III) is dominated by ligands containing oxygen-, nitrogen-, and sulfur-donor atoms. Gallium(III) and In(III) are both considered to be hard metal ions which will readily bind to harder oxygen donor ligands with respect to the hard acid/hard base theory;[31] however, In(III), is somewhat "softer" than Ga(III), preferring neutral nitrogen and negative sulfur donor atoms.[32] Gallium(III) and In(III) have well-established coordination numbers of 3, 4, 5, and 6 depending on the ligand, and In(III) also readily forms seven-coordinate complexes. In a study by Sun and co-workers, 4-, 5-, and 6-coordinate $N_2S_2O_x$ (where x = 0, 1, or 2) ligands were prepared and it was shown that compared to the 4- and 5-coordinate complexes, the 6-coordinate complexes of both Ga(III) and In(III) were thermodynamically more stable and also more stable in vivo.[33] The ionization potential, ionic radius, and coordination number of Ga(III) are very similar to that of Fe(III), because Fe(III) has a half-filled 3d orbital, similar to Ga(III) which has a filled 3d orbital. Indium(III) has a somwhat larger ionic radius than Ga(III) or Fe(III), hence preferring the higher coordination numbers.

There are two requirements for using Ga(III) and In(III) complexes as radiopharmaceuticals: they should be stable to hydrolysis (formation of complexes with OH^-) and they should be more stable than the Ga(III)− and In(III)−transferrin complexes. In aqueous solution, free hydrated Ga(III) is stable only under acidic conditions, with insoluble $Ga(OH)_3$ forming as the pH is raised. Between pH 3 and about 9.5, insoluble $Ga(OH)_3$ is the primary species, whereas above pH 9.6, the soluble gallate ion ($Ga(OH)_4^-$) forms. In(III) also hydrolyzes easily, forming insoluble hydroxides at pH > 3.4. In the preparation of Ga(III) and In(III) coordination complexes, ligand exchange is often necessary since the precipitation of $Ga(OH)_3$ and $In(OH)_3$ occurs more rapidly than complexation with ligands that bind Ga(III) and In(III) at a slower rate. For example, $GaCl_3$ or $InCl_3$ is generally first complexed with a weakly coordinating ligand such as acetate or citrate, and then this weak coordination complex is used to prepare complexes of higher stability.

The large formation constant of Ga(III)−transferrin (log K_1 = 20.3)[34] and In(III)−transferrin (log K_1

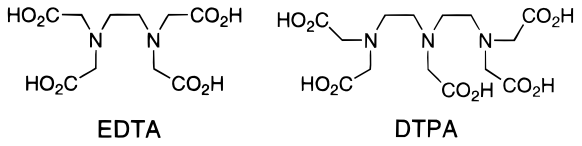


Figure 1. The ligands ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) for complexing Ga(III) and In(III).

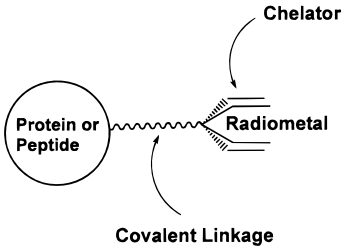


Figure 2. Schematic demonstrating a bifunctional chelator (BFC). The chelator can complex a metal and also contains a functional group which forms a covalent linkage to a biological molecule, such as a protein or peptide.

= 18.74)[35] and the high plasma concentration of this protein (0.25 g/100 mL) thermodynamically favor the in vivo exchange of many Ga(III) and In(III) complexes with transferrin. The majority of Ga(III) and In(III) complexes used as radiopharmaceuticals have very high thermodynamic stability or are kinetically inert to exchange with transferrin in vivo. Ligands that form highly stable complexes are generally multidentate and contain carboxyl, amino, or thiol groups.

In aqueous solution, the most prevalent species present of yttrium is Y(III), and therefore, many of the same chelators that complex In(III) in a stable configuration have been utilized with Y(III). The majority of studies using yttrium radioisotopes involve the labeling of ^{90}Y or ^{86}Y to larger biomolecules through BFCs, generally derivatives of DTPA and DOTA (Figures 1 and 3). It has been shown that ^{90}Y–DTPA is not very stable in vivo,[36] and free ^{90}Y accumulates in the bone.[37] For this reason, the macrocyclic chelator DOTA has replaced DTPA since it forms more kinetically inert complexes.[36,38]

C. Bifunctional Chelators for Attaching Radiometals to Biomolecules

Within the last 10 years, there has been a considerable amount of research in the area of radiometal-labeled receptor-targeted agents. Receptor ligands can be larger biomolecules such as monoclonal antibodies and peptides or smaller organic molecules such as folic acid. The radiometal is connected to these biomolecules via a bifunctional chelating agent (BFC) (Figure 2), which consists of a chelator to complex the radiometal and a functional group for attachment to the biomolecule. Functional groups that form amide, thiourea, urea, Schiff base, or thioether linkages with amine or thiol groups on proteins and peptides have been described.[39–46] The first BFCs described were analogues of EDTA and DTPA.[47–50] Several improvements have been made to the originally developed BFCs, and they are described in a review article by Gansow.[44] Commonly used BFCs for radionuclides of copper, technetium, and rhenium are described in a review by Schubiger et al.[46] Two other thorough reviews by Jurisson et al. and Hnatowich describe BFCs designed for isotopes of indium, yttrium, and rhenium.[3,51]

D. Challenges in Copper Chemistry

The chemistry of copper is restricted to two principal oxidation states (I and II), and the coordination and redox chemistry of copper is well documented. Copper is ubiquitous in nature and its biochemistry and metabolism in humans is well-known. A good review on the biological chemistry of copper is a book by Linder.[52] As described in section II.A.3, there are several different available copper isotopes with a wide-range of half-lives. The availability of longer-lived copper radionuclides, such as ^{64}Cu and ^{67}Cu, has led to the development of copper-labeled biological molecules for tumor targeting using monoclonal antibodies and peptides. Shorter-lived copper radionuclides are currently used to form lipophilic copper complexes for measuring blood flow and hypoxia. We will focus on more recent developments in the area of copper radiopharmaceuticals.

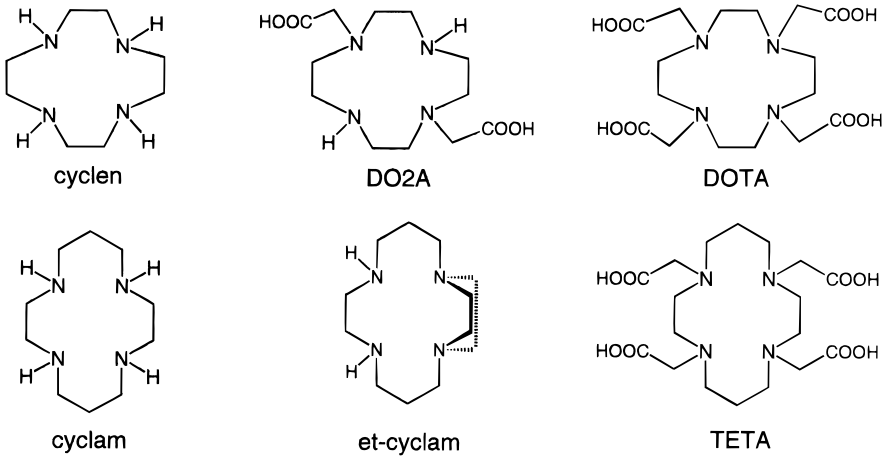


Figure 3. Macrocyclic chelators for complexing Cu(II).

The design of copper chelators for diagnostic imaging agents has been dependent on the desired characteristics of the targeting molecule. For example, ligands that form lipophilic, neutral, copper complexes have been evaluated as blood flow agents. These copper complexes labeled with shorter-lived copper radionuclides were designed to be stable enough to clear the blood and localize in either the heart, brain, kidney, or tumor upon the first pass through the blood to these tissues. Upon reaching the tissue of interest, complexes that release the copper are advantageous, since the copper is then trapped in the tissue without washout. Some examples of these complexes include the Cu(II) thiosemicarbazones first designed by Petering as anticancer agents[53] and then evaluated as radiopharmaceuticals by Green.[54] Thiosemicarbazones labeled with shorter-lived copper radionuclides have also been developed to image hypoxia in the heart[55,56] and in tumors.[57] These agents will be discussed in detail later on in the article.

The second class of copper radiopharmaceuticals are agents that take a longer time to localize in the target. Copper-labeled biomolecules such as monoclonal antibodies (mAbs) and peptides for tumor imaging fall into this category. In order for this class of radiopharmaceutical to be effective, the copper must be very stably bound to the biological molecule, requiring a chelator with high in vivo stability. For Cu(II) complexes to be stable in vivo, it has been demonstrated that kinetic inertness is more important than thermodynamic stability. Moi and co-workers[58] showed that Cu(II) complexes of BFCs of EDTA and DTPA (Figure 1) rapidly dissociated in human serum, and Cu(II) bound to albumin, even though the Cu(II) complexes had high thermodynamic stability constants (log $K_{Cu-EDTA}$ = 18.7; log $K_{Cu-DTPA}$ = 21.4). Copper(II) has been found to have much greater kinetic inertness (and consequently in vivo stability) with macrocyclic chelators than with linear polyamino−polycarboxylate ligands.[59] Kukis and co-workers[60] showed that there is differential biological stability between various macrocyclic chelators, with Cu-labeled DOTA BFCs being more stable in serum than Cu-labeled TETA BFCs. Jones-Wilson and colleagues[61] compared the thermodynamic stability of six Cu(II) macrocyclic chelators differing in carbon backbone and charge, with their in vivo behavior and confirmed the trends shown by the research of the Meares' group (Figure 3). In addition, it was shown that the charge of the Cu(II) complex was very important in the biodistribution, and negatively charged complexes cleared through the body much more quickly than did positively charged agents.

The choice of BFC can dramatically affect the pharmacokinetics, distribution, and metabolism of the radiopharmaceutical, which will ultimately determine the clinical usefulness of the drug. Anderson et al. investigated three copper BFCs conjugated to human serum albumin (HSA) for blood pool imaging.[62] They found that although the thiosemicarbazone BFC was less stable in vivo over longer time periods than the macrocyclic BFC studied, the shorter imaging times required for imaging blood pool showed both agents to perform equally. A study on four copper BFCs (Figure 4) showed that chelate charge and lipophilicity played a role in kidney retention of copper radiolabeled antibodies and that transchelation of the copper radiolabel to proteins such as superoxide dismutase (SOD) appeared to be a significant factor for accumulation in the liver.[63] Further metabolism research has been carried out with a ^{64}Cu-labeled peptide, ^{64}Cu−TETA−octreotide (^{64}Cu−TETA−OC), and evidence of the formation of ^{64}Cu−SOD in the liver was confirmed by size exclusion chromatography and gel electrophoresis.[64] These metabolism experiments raise the question of which chelator is optimal for copper radionuclides when targeting tumors. The authors of this article are currently carrying out research to better understand the relationship between kinetic inertness and in vivo stability of Cu(II) complexes and to devise chemical methods and/or in vitro assays to predict in vivo stability.

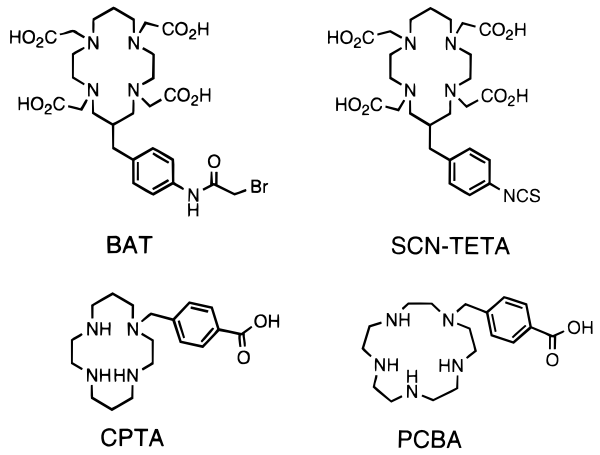


Figure 4. Four BFCs used to complex ^{64}Cu and ^{67}Cu to mAb 1A3.

III. Determining the Optimal Imaging Agent for Specific Diseases

A. Blood Pool and Myocardial Imaging Agents

Because of the convenient half-life of ^{68}Ga and the fact that it is generator-produced and therefore more widely available, considerable interest lies in the development of ^{68}Ga-labeled imaging agents. Gallium-68−citrate as been used to quantify pulmonary vascular permeability using PET.[65] The crystal structure of Ga−citrate was recently reported demonstrating the isolation of a $[Ga(Cit)_2]^{3-}$ anion.[66] ^{68}Ga−citrate is not stable in the blood, and the actual radiopharmaceutical in vivo is ^{68}Ga−transferrin. This agent is taken up in the lungs immediately after injection. Other applications of ^{68}Ga include evaluating vascular permeability in lung disease and lung transplant.[67,68] The use of PET allows quantification capabilities that are not possible with ^{67}Ga and gamma scintigraphy.

During the last 10 years, there have been significant advances in the development of ^{68}Ga-labeled myocardial imaging agents. Lipophilic Ga(III) complexes, both neutral and cationic, have been shown

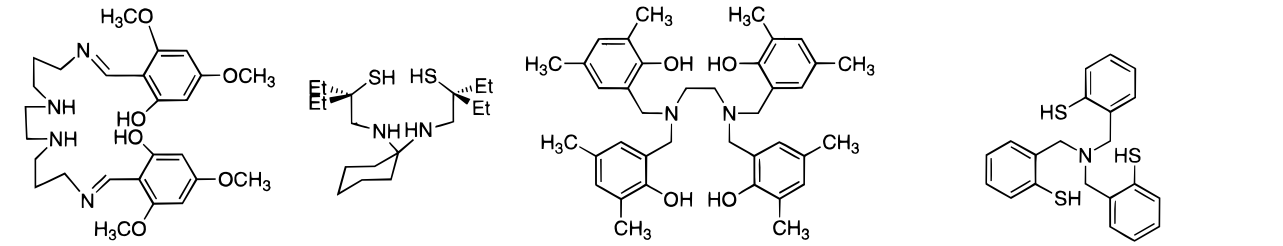


Figure 5. Four chelators that have been labeled with gallium radionuclides for brain and heart imaging. ^{68}Ga-labeled S3N showed the highest brain uptake of any gallium agent to date.

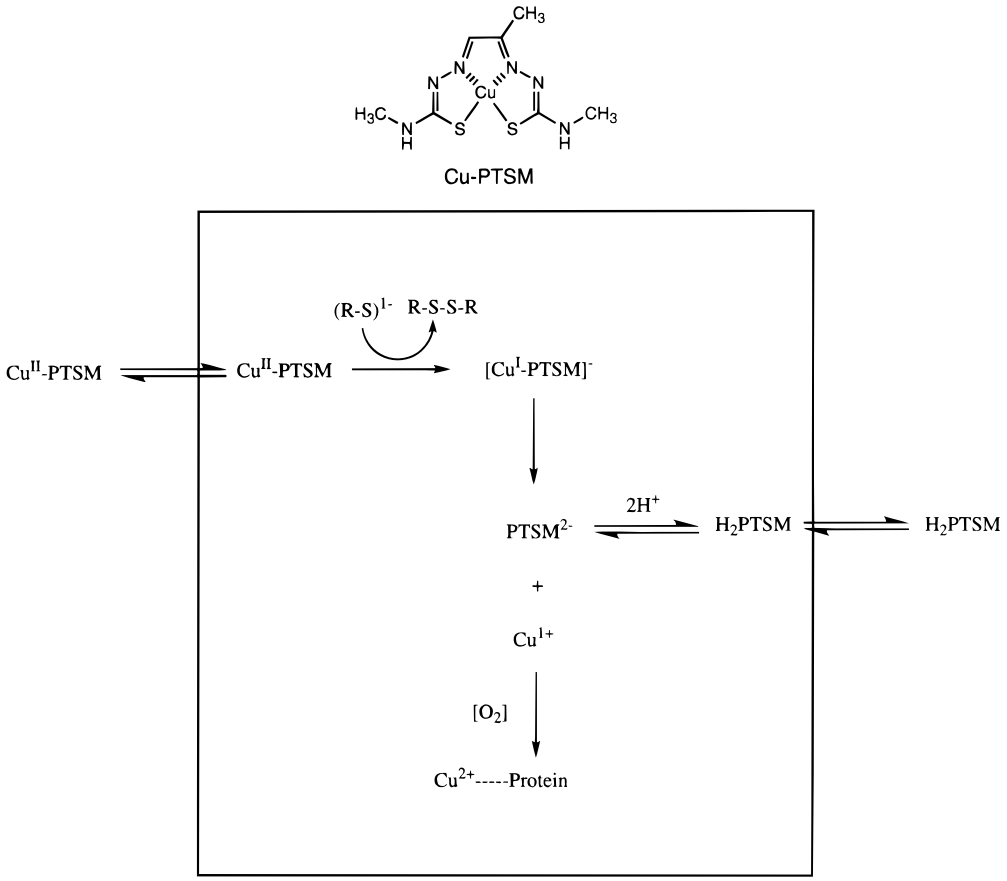


Figure 6. Mechanism of how Cu−PTSM is trapped inside cells. Cu(II)PTSM is reduced to Cu(I)PTSM which dissociates to Cu(I) and "free" ligand. The Cu(I) is then reoxidized back to Cu(II) and binds nonspecifically to intracellular proteins, thereby trapping the Cu(II) inside the cell.

to localize in the heart. Tsang and co-workers prepared a series of hexadentate bis(salicylaldimine) ligands that formed lipophilic cationic Ga(III) complexes and found that one of the complexes, ^{68}Ga−[(4,6-MeO$_2$sal)$_2$BAPEN]$^+$ (Figure 5), exhibited significant myocardial uptake and retention over the neutral salicylaldimine ligands.[69,70]

Other ligands that have been evaluated for myocardial imaging include a ^{68}Ga complex with a tetradentate N_2S_2 ligand (BAT-TECH) (Figure 5).[71,72] This ^{68}Ga complex showed significant uptake in the heart; however, the activity washed out over time, and the blood activity remained constant after 30 min. Another complex evaluated as a heart agent is the ^{68}Ga complex of THM$_2$BED (Figure 5).[73] This complex was taken up in the heart and to a smaller extent in the brain, but it had a high accumulation in the blood and quickly washed out of the heart and brain. Another complex that showed significant heart uptake was a small, neutral, lipophilic complex of ^{68}Ga labeled to a tris(2-mercaptobenzyl) amine (S_3N) ligand (Figure 5).[74] This agent had a heart:blood ratio in Sprague−Dawley rats of 11 at 60 min postinjection. PET images in a dog model clearly delineated the heart, although there was high background from the liver and lungs.

As described in section II.D, Cu(II) bis(thiosemicarbazones) demonstrated rapid diffusion into cells followed by trapping of the Cu(I/II) ion, and therefore these agents were labeled with copper radionuclides and evaluated as possible radiopharmaceuticals for myocardial perfusion imaging.[54] Several structural analogues of the bis(thiosemicarbazones) were evaluated as blood flow tracers, and one analogue, Cu(II) pyruvaldehyde bis(N^4-methylthiosemicarbazone) (Cu−PTSM), was chosen for further evaluation (Figure

6).[75] Cu−PTSM was found to have "microsphere-like" kinetics since it is nontissue selective and rapidly extracted from the blood.[76] Human studies were carried out with ^{62}Cu−PTSM in 10 healthy volunteers and 6 patients with coronary artery disease.[77] The results showed that at lower flow rates (<1.5 mL/g/min), flows estimated with ^{62}Cu−PTSM correlated closely with estimates obtained with ^{15}O-labeled H_2O; however, at high flows ^{62}Cu−PTSM did not accurately estimate blood flow, possibly due to the strong binding of the tracer to human serum albumin.

B. Brain Imaging Agents

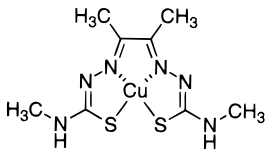
The development of a Ga(III) agent that crosses the blood-brain barrier has been an elusive goal over the past 30 years. There are very few reports of radiogallium complexes that accumulate in the normal brain. The ^{68}Ga-labeled pyridinone derivatives developed by Zhang and co-workers showed uptake in rabbit brain by planar imaging that appeared to accumulate over several hours, but there was no uptake in the mice or rat brains measured by biodistribution.[78] ^{68}Ga−THM_2BED showed slight uptake in the brain at very early times postinjection but showed rapid washout.[73]

More recently, Cutler and co-workers have shown that ^{68}Ga−S_3N (Figure 5) crossed the blood-brain barrier in several animal models.[74] In Sprague−Dawley rats, the ^{68}Ga−S_3N complex does not exhibit "first-pass" uptake into the brain (i.e., the highest uptake immediately after injection) but rather shows slower uptake in the brain followed by slow washout, with a brain:blood ratio of 0.11 at 2 min postinjection, increasing to 3.8 by 60 min. This agent exhibits the most promise for brain imaging of any ^{68}Ga complex evaluated to date.

C. Hypoxia Imaging Agents

The bis(thiosemicarbazone) complex, Cu(II)−diacetyl-bis(N^4-methylthiosemicarbazone) (^{62}Cu-ATSM) (Figure 7), has been shown to be selectively trapped in hypoxic tissue, in both myocardium[79] and tumors.[57] The copper(II), neutral, square-planar complex exhibits high membrane permeability and low redox potential. The analogous complex, Cu−PTSM (Figure 6), is a proven blood flow tracer described in section III.A. By the simple addition of a methyl group to PTSM (pyruvaldehyde to diacetyl), the selectivity for hypoxia was increased dramatically.

Cu(ATSM) has a lower redox potential (−297 mV) compared to that of Cu(PTSM) (−208mV). This difference in the redox values was postulated to be the primary reason for the selective trapping of Cu(ATSM) in highly reductive hypoxic tissue but not in less reducing normal tissue.[55] To better understand what chemical and physical properties of agents are responsible for uptake in hypoxic tissue, Dearling and co-workers investigated a number of different Cu(II) thiosemicarbazones for their uptake in Chinese hamster ovary cells under normoxic and hypoxic conditions.[80] Four Cu(II) compounds were shown to have a significant difference between uptake in normoxic vs hypoxic cells. To follow up on this study, the reduction potential of these Cu(II) bis(thiosemicarbozones) was measured and correlated to hypoxia selectivity.[81] It was found that hypoxia selectivity was dependent on the redox potential of the Cu(II) complex, suggesting that redox behavior may provide a basis for designing redox-selective complexes.



Cu-ATSM

Figure 7. Structure of Cu(II)ATSM

D. Tumor Imaging Agents

1. Radiometal-Labeled Small Molecule Imaging Agents

^{67}Ga-labeled citrate was first used in tumor imaging nearly 30 years ago,[82] and a few years later researchers determined that the ^{67}Ga was actually binding transferrin in vivo.[83] Today, ^{67}Ga−citrate/transferrin remains a widely used radiopharmaceutical for the clinical diagnosis of certain types of neoplasms, such as Hodgkin's disease, lung cancer, non-Hodgkin's lymphoma, malignant melanoma, and leukemia. The mechanism of ^{67}Ga−citrate/transferrin uptake into tumors has long been disputed. The most recent theory is that the ^{67}Ga−transferrin complex binds to the transferrin receptor present on tumor cells and is then incorporated into the cell by receptor-mediated endocytosis.[84] This theory was further examined by determining the uptake of ^{67}Ga−citrate in two cell lines: one that had no transferrin receptors and one where the transferrin receptor was overexpressed.[85] It was found that both transferrin-dependent and -independent mechanisms were responsible for the uptake of ^{67}Ga in these transfected cell lines.

Bis(thiosemicarbazones) were discovered to possess antitumor properties in the 1960s.[86] Eight years later it was found that the antitumor activity of the Cu(II) complexes of these ligands was significantly enhanced over the activity of the ligands alone.[87] These neutral, lipophilic complexes are rapidly taken up by cells, and the Cu(II) is reduced to Cu(I) by intracellular thiols (probably glutathione) (Figure 6).[53,87] The Cu(I) complexes are unstable, and the copper dissociates and binds to intracellular proteins. Copper-64-labeled PTSM was evaluated in two tumor-bearing animal models and found to show proportional uptake to ^{125}I-labeled antipyrene, which is a known blood flow agent.[88] The high lipophilicity which results in high liver uptake and slow hepatobiliary clearance makes intravenously administered Cu−PTSM a less than ideal flow tracer for routine tumor blood flow imaging.

2. Radiolabeled Monoclonal Antibodies for Tumor Imaging

The radiolabeling of antibodies for the detection of cancer in the early 1970s[89−91] marked the beginning of the use of radiolabeled biological molecules for targeting antigens and receptors that are upregu-

lated in tumors. Initially, radiolabeled antibodies were labeled with iodine radionuclides, but currently, the use of radiometal−BFC−antibody conjugates is becoming more prevalent.

Monoclonal antibodies (mAbs) have been produced which bind to antigens present on a large number of tumor types. mAbs have been labeled with radiometals for diagnosis and therapy of cancer, and this has been a subject in many reviews published in the last 10 years.[4,46,92−100] Intact mAbs are large proteins with a MW of 160 kDa, and because of their large size, they have very slow biological clearance and are excreted through the hepatobiliary system. To circumvent these drawbacks, mAb fragments have been produced that have molecular weights ranging from 10 to 100 kDa. Metal radionuclides that have been labeled to mAbs (both intact and fragments) for diagnostic imaging include ^{111}In, ^{67}Ga, ^{99m}Tc, and ^{64}Cu. Currently, three mAb agents, ^{111}In-DTPA-B72.3 (OncoScint), ^{111}In-DTPA-7E11.C5.3 (ProctaScint), and a ^{99m}Tc direct-labeled Fab fragment of IMMU-4 (CEA-SCAN), are approved for clinical use in the United States.

3. Pretargeting Agents for Tumor Imaging and Therapy

Pretargeting involves administration of a mAb (that binds to antigens found on tumor cells) which is covalently linked to a molecule having a high affinity noncovalent binding site for a small rapidly excreted effector molecule. The unlabeled mAb−binder conjugate is given first and is often followed by a clearing agent which will remove the mAb from the circulation but leave it remaining in the tumor. This clearing agent significantly improves the tumor/blood ratio. The small effector molecule is radiolabeled with a radiometal, and this is injected soon after the clearing agent. This pretargeting strategy allows the radiolabeled small molecule to bind to the tumor, and residual radioactivity is then rapidly excreted.

The high-affinity noncovalent binding of biotin to avidin (10^{15} M^{-1}) makes this system attractive for mAb pretargeting methods. Both mAb−biotin and mAb−avidin conjugates have been investigated for pretargeting of radiolabeled avidin and biotin, respectively. Another multistep targeting system is the use of bispecific mAbs. The tumor is pretargeted with a bispecific mAb that is reactive with a tumor-associated antigen and a radiometal-labeled complex. The use of this approach has been demonstrated in several animal studies[101−105] and in clinical studies.[106,107] For both pretargeting techniques, three key features of a desired radiolabeled effector molecule have been suggested: (1) it must be small, hydrophilic, and rapidly diffusible, (2) it must undergo rapid renal elimination, and (3) it must have minimal uptake in normal tissues.[108] The mAb NR-LU-10 (NeoRx Corp.) reacts with a 40 kDa glycoprotein antigen found on adenocarcinomas such as breast, ovarian, colon, and small cell lung cancer. Tumor targeting of ^{99m}Tc- and ^{186}Re-labeled NR-LU-10 has been demonstrated in animal models bearing human colorectal carcinoma xenografts[109] and in patients.[110−112] A streptavidin conjugate of NR-LU-10 (SA-NR-LU-10), which binds four molecules of radiolabeled biotin, was prepared and evaluated in nude mice bearing breast and small cell lung carcinoma xenografts[113,114] and patients.[111] A general schematic of this pretargeting strategy is presented in Figure 8. SA-NR-LU-10 exhibited tumor uptake and blood clearance equivalent to unmodified intact mAb. Treatment with the clearing agent biotin−galactose HSA removed 90−95% of circulating SA-NR-LU-10. The effector molecules, ^{111}In- and ^{90}Y-labeled DOTA−biotin, for RIS and RIT, respectively, showed rapid blood clearance and low normal organ uptake, with urinary excretion of >80% of the injected dose in 2 h. In a nude mouse model, sequential administration of these agents resulted in stable, high efficiency delivery of >20% ID/g of ^{90}Y and ^{111}In to the tumor, with whole body excretion and nontarget organ uptake similar to that of ^{111}In/^{90}Y−DOTA−biotin alone.

In another study, Nakamoto and colleagues carried out three-step targeting with biotinylated mAb MLS128, streptavidin, and ^{111}In−DTPA−biotin for targeting LS180 human colon cancer xenografts in nude mice.[115] Their results showed a maximum tumor uptake of ^{111}In−DTPA−biotin at 2 h postinjection 1 day after administration of SA of ∼1.4% ID/g. In the studies by Axworthy and colleagues,[113,114] the tumor-bearing mice were put on a biotin-deficient diet shortly before administration of the SA-NR-LU-10. The disappointing results by Nakamoto and colleagues may be attributed to the endogenous biotin present in their mice or perhaps the circulating SA,

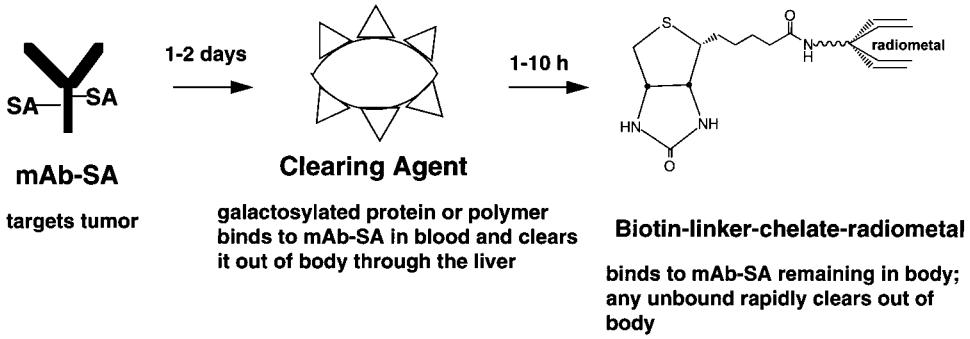


Figure 8. Schematic showing imaging by pretargeting. A mAb conjugated to streptavidin (SA) is injected in vivo. One to two days later, a clearing agent is administered. The clearing agent consists of a galactosylated protein or polymer which binds the mAb-SA in the blood and clears it from the body via the galactose moieties which bind to glycoprotein receptors in the liver.

which will bind ^{111}In−DTPA−biotin and cause slower blood clearance.

The results with two-step pretargeting using bispecific antibodies and ^{111}In-labeled haptens have been very encouraging. In tumor-bearing mice, Kranenborg et al. showed >6%ID/g uptake of ^{111}In−DTPA in renal cell carcinoma (RCC) tumors 3 days after administration of the bispecific anti-RCC × anti-DTPA mAb G250 × DTIn1.[105] Using this same pretargeting strategy, tumor:blood ratios of ∼500 were obtained at 24 h postinjection of ^{111}In−DTPA. When a bivalent hapten was employed, the amount of ^{111}In−di[DTPA]FKYK in the RCC tumor increased to ∼80%ID/g, with tumor:blood ratios of 1400 at 48 h.[116] Clinical studies with ^{111}In-labeled bivalent haptens have also been encouraging. Le Doussal and colleagues compared ^{111}In-labeled *N*-a-(In−DTPA)-tyrosyl-*N*-e-(In-DTPA)-lysine (di-In-DTPA-TL) pretargeted to an anti-CEA, anti-In-DTPA bispecific Fab′−Fab mAb to ^{111}In-labeled F(ab′)$_2$ in six patients with colorectal cancer.[117] The bispecific antibody targeting of the ^{111}In-labeled bivalent hapten showed reduced liver, blood pool, and lower background activity than the ^{111}In-labeled F(ab′)$_2$, which also enabled more clear delineation of the tumors. In more recent studies, this same pretargeting system was used to stage patients with nonsmall-cell lung cancer[106] and medullary thyroid carcinoma,[107] and in both cases excellent contrast was observed.

4. Radiolabeled Receptor Ligands for Tumor Imaging

a. Somatostatin Analogues. One of the first clinically approved peptide-based tumor receptor imaging agents is a radiolabeled analogue of the hormone somatostatin. Somatostatin is a 14-amino acid peptide involved in the regulation and release of a number of hormones, including growth hormone, thyroid-stimulating hormone, and prolactin. Somatostatin receptors (SSR) occur in a number of different normal organ systems such as the central nervous system, the gastrointestinal tract, and the exocrine and endocrine pancreas.[118−120] A large number of human tumors are also somatostatin receptor-positive.[121] Somatostatin has a very short biological half-life, and analogues have been developed, such as octreotide, which show much longer residence times.[122] Octreotide (OC), an eight amino acid SS analogue, has been labeled with ^{111}In using a BFC of DTPA[123,124] and is approved for human use in the United States and Europe as a diagnostic imaging agent for neuroendocrine tumors.[125] Somatostatin analogues, including OC, tyrosine-3-octreotide (Y3−OC), octreotate (TATE), tyrosine-3-octreotate (Y3−TATE), lanreotide (LAN), and RC-160, have been labeled with a wide variety of metal radionuclides, including ^{64}Cu,^{68}Ga, ^{111}In, and $^{86/90}$Y, for diagnostic imaging and radiotherapy (Figure 9). In the following section, several of these will be discussed.

Gallium-68 and ^{67}Ga have been labeled to octreotide using the BFC desferrioxamine-B (DFO).[126,127] DFO, a well-known chelator with high affinity for Fe(III), is used for treating iron overload[128,129] and forms a stable, neutral complex with Ga(III) by coordination through three hydroxamate groups. $^{67/68}$Ga−DFO−OC was stable in vivo and showed high affinity for the SSR both in vitro and in vivo. The biological clearance of $^{67/68}$Ga−DFO−OC was rapid, and the conjugate was excreted through the kidneys and into the urine, similar to ^{111}In−DTPA−OC. DOTA−Y3−OC has also been labeled with ^{67}Ga, and it was shown that ^{67}Ga−DOTA−Y3−OC had signficantly higher tumor uptake and more rapid clearance through the kidneys in tumor-bearing mice than either ^{111}In- or ^{90}Y-labeled DOTA−Y3−OC.[130]

OC has been conjugated to the macrocyclic BFC TETA for labeling with ^{64}Cu.[131] Because of the lability of copper, macrocyclic chelators are necessary to form complexes that are stable *in vivo*. ^{64}Cu−TETA−OC had high affinity for the SSR both in vitro and in vivo and cleared primarily through the kidneys, with very low liver accumulation. Copper-64−TETA−OC is currently being evaluated as a PET imaging agent for neuroendocrine tumors.[132] Preliminary results showed that ^{64}Cu−TETA−OC was able to detect even more SSR-positive lesions than the currently used, clinically approved agent, ^{111}In−DTPA−OC (Pentetreotide) and gamma scintigraphy. ^{64}Cu−TETA−OC significantly inhibited the growth of SSR-positive tumors in rats[133] and therefore has potential for both diagnostic imaging and targeted radiotherapy.

Other ^{111}In-labeled somatostatin analogues have been compared to ^{111}In−DTPA−OC as agents for gamma scintigraphy. Indium-111−RC-160 was compared to ^{111}In−DTPA−OC in four patients and was found to have a slower whole-body clearance, although there appeared to be no difference in sensitivity in detecting tumors between the two agents.[134] Indium-111-labeled DOTA−LAN has been evaluated as an agent for gamma scintigraphy,[135] and this agent was found to bind to SSR subtypes 2−5, whereas OC only binds to subtypes 2 and 5.[136] In a clinical imaging study, Virgolini and colleagues found improved tumor uptake with ^{111}In−DOTA−LAN compared to ^{111}In−DTPA−OC in eight patients.[135]

OC analogues have been labeled with ^{86}Y (PET imaging),[19] and OC and LAN analogues have been labeled with ^{90}Y (radiotherapy) through DTPA and DOTA chelators.[20,136−139] The DTPA chelator was shown to be a suboptimal chelator for ^{90}Y due to the in vivo instability of ^{90}Y−DTPA, resulting in high bone uptake.[19,137] Yttrium-90−DOTA was found to be very stable in vivo, and $^{86/90}$Y−DOTA−Y3−OC demonstrated high target uptake and rapid renal clearance.[20,138] Clinical trials for targeted radiotherapy using ^{90}Y-labeled Y3−DOTA−OC and ^{90}Y−LAN are ongoing, and thus far the results are positive.[140,141]

Somatostatin analogues have been prepared based on the structure of OC where tyrosine (Y) is substituted for phenylalanine (F) in the 3-position and/or the C-terminal alcohol is replaced with a carboxylate. These substitutions have been made for ^{111}In−DTPA, ^{111}In−DOTA, and ^{64}Cu−TETA conjugates and have resulted in significantly higher SSR-positive tissue uptake compared to ^{111}In−DTPA−OC[142] and ^{64}Cu−TETA−OC.[143] The higher uptake of the Y3−TATE analogues over the Y3−OC and OC analogues is not entirely explained by differences in SSR binding

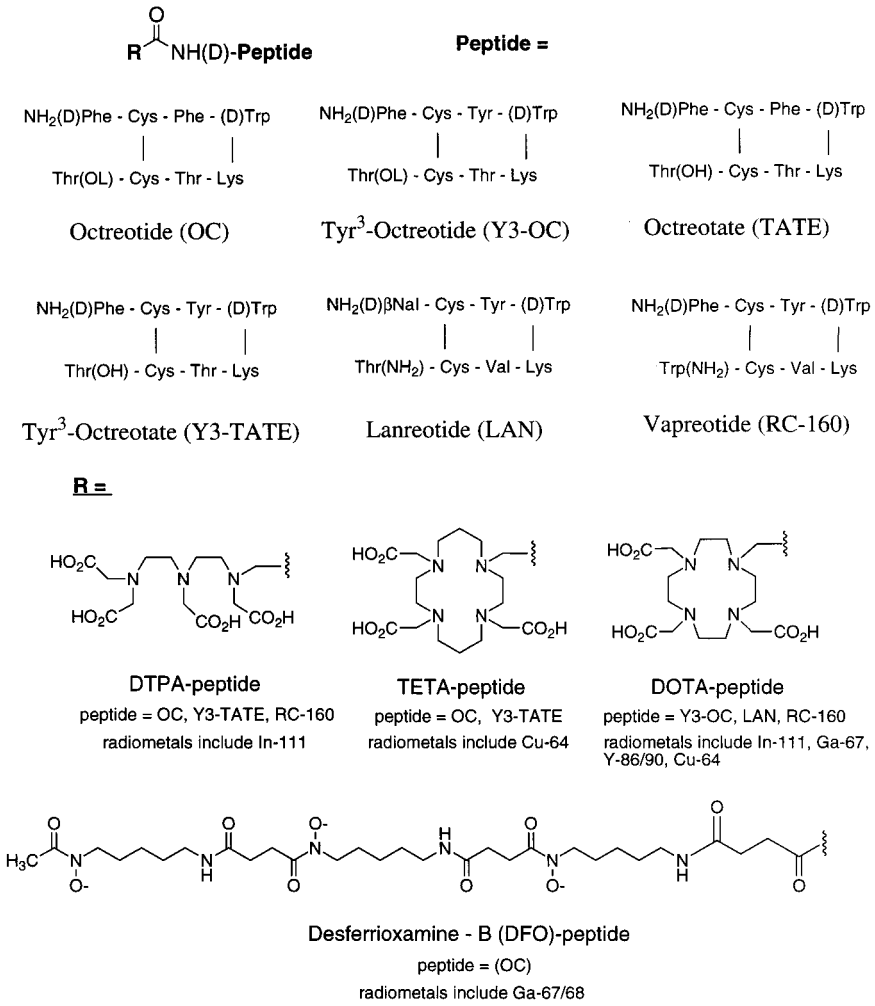


Figure 9. Somatostatin analogues and BFCs which have been conjugated to the various peptides.

affinity. In fact, DTPA−Y3−TATE has very similar binding affinity to DTPA−Y3−OC and DTPA−OC.[142] Data published by de Jong et al.,[142] along with studies by our group at Washington University with ^{64}Cu-labeled TETA−OC, TETA-Y3−OC, TETA−TATE, and TETA−Y3−TATE,[144] showed that the kinetics of uptake of the TATE analogues by SSR-positive cells is much faster than the OC analogues. The more rapid kinetics of uptake and internalization of the TATE analogues appears to be the reason for the increase in uptake in SSR-positive tissues.

b. Folate Receptor Ligands. Folic acid is an essential dietary vitamin used by all eucaryotic cells for DNA synthesis and one-carbon metabolism. Folic acid enters cells through facilitated transport by a membrane transport protein, and certain cells also possess a membrane-associated folate-receptor, folate binding protein, that allows folate uptake via receptor-mediated endocytosis. A number of tumor cell types including breast, ovarian, cervical, and colorectoral are known to overexpress folate binding protein. When folate is conjugated to another molecular moiety, the folate portion of the conjugate is not recognized by the transport system but may still be recognized by the folate binding protein.[145] Therefore, a bioconjugate of a radiometal−chelate moiety attached to folate should be selectively concentrated by cells (such as certain types of tumor cells) that express the membrane folate receptor.

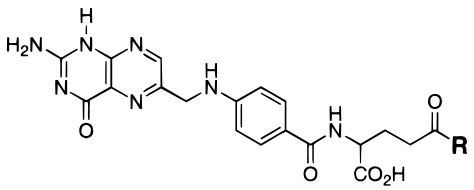


Figure 10. Folate bioconjugates of DTPA and DFO which have been labeled with ^{67}Ga and ^{111}In.

The chelator DFO was used to chelate Ga(III) to the vitamin folic acid (Figure 10).[146] A mixture of two isomers, DFO−folate (α) and DFO−folate (γ), was formed, since DFO was conjugated to two different carboxyl groups on folate. The γ isomer is the only one recognized by the folate receptor. ^{67}Ga−DFO−folate (γ) showed specific binding to the folate receptor, both in vitro and in vivo in a tumor-bearing mouse model on a folate-free diet.[147] Tumor uptake at 4 h postinjection was >5% ID/g with tumor:blood ratios of ∼400.

DTPA−folate was synthesized using two methods: a simple method that produced both α- and γ-conjugates, which requires HPLC isolation of the γ-isomer, and a regiospecific functionalization of the γ-carboxylate on folic acid, which produces only the active γ-conjugate.[148] The second method proved to be much more amenable to large-scale production.

Similar to ^{67}Ga−DFO−folate, ^{111}In−DTPA−folate also showed impressively high tumor uptake and high tumor:blood ratios.[149] With both of these compounds, the highest nontarget organ uptake was in the kidneys, where there are folate receptors in the proximal tubules.[149]

c. Other Tumor Receptor Ligands. Research on somatostatin analogues as tumor imaging and therapy agents has opened up a burgeoning new field of study on a variety of radiolabeled peptide tumor receptor ligands. The CCK−B receptor, which binds both gastrin and cholecystokinin with high affinity, is overexpressed in tumors such as medullary thyroid carcinoma (MTC), small-cell lung cancer, astrocytomas, stromal ovarian tumors, and some gastroenteropancreatic tumors.[150] Reubi et al. then evaluated a series of DTPA-conjugated peptides and found DTPA−DY*Nle*GW*Nle*DF−NH_2 (MP2286) and DTPA−D-AspY*Nle*GW*Nle*DF−NH_2 (MP2288) each to have IC_{50} values of 1.5 nM.[151] The stomach is considered a target organ for CCK−B receptor ligands, and the stomachs of three rats showed specific binding of ^{111}In−DTPA−MP2288 which was blocked in the presence of cold peptide.[151] Indium-111-labeled DOTA-MP2288 (or DOTA-CCK-8) was also found to be internalized in two pancreatic tumor cell lines, suggesting the possibility of using this analogue for tumor imaging.[152]

Another peptide hormone that has been labeled with radiometals is bombesin. Bombesin has a high affinity for the gastrin-releasing peptide receptor, and these receptors have been found to be upregulated in tumors such as prostate cancer,[153] small-cell lung cancer,[154] and pancreatic cancer.[155] Bombesin has been conjugated to 16aneS4, a thiamacrocyclic chelator for labeling with ^{105}Rh(III), using a aliphatic carbon chain tether attached to the amine terminus.[156] Rhodium-105 is a reactor-produced radionuclide with favorable decay characteristics for therapy ($t_{1/2}$ = 1.4 d; E_β = 0.57 MeV). These ^{105}Rh analogues showed high binding affinity for the bombesin receptors with IC_{50} values in the low nanomolar range. Indium-111-labeled DTPA^0, Pro^1, Tyr^4−bombesin also showed a high affinity for the bombesin receptor, with specific uptake in bombesin receptor-positive prolactinoma 7315b tumors implanted in Lewis rats, as demonstrated by scintigraphy.[157]

E. Imaging Agents for Inflammation

The identification of infection and inflammation in patients is the first step in the successful clinical management of infected sites. Although *inflammation* and *infection* are two different processes, they elicit a similar pathophysiologic response from the host. Nuclear medicine imaging cannot differentiate between these two processes.[158] Early in the inflammatory process, local perfusion is increased, followed by an increase in protein space in the immediate vicinity of the lesion. Imaging with radiopharmaceuticals can visualize these early changes in the course of inflammation.

A review on the mechanisms of uptake of a variety of metal-containing radiopharmaceuticals in infectious foci has been presented by Oyen and colleagues.[159] One of the most commonly used radiopharmaceuticals for imaging infection is ^{67}Ga−citrate.[160] ^{67}Ga−citrate accumulates in infectious sites due to the binding of ^{67}Ga to the increased protein concentration at the site of inflammation. The mechanism is similar to that of ^{67}Ga−citrate binding to tumors, except in the case of infection, ^{67}Ga is transchelated from transferrin in the blood to lactoferrin present in activated leucocytes and bacterial siderophores.[161,162] Imaging with ^{67}Ga−citrate for infections is usually done 24−72 h following injection.

Another widely used agent is ^{111}In-labeled white blood cells (WBCs).[163] Initial research on the radiolabeling of autologous polymorphonuclear leukocytes was carried out by McAfee and Thakur.[164] One of the compounds they examined was the nonpolar, lipid-soluble complex 8-hydroxyquinoline (oxine) (Figure 11). Indium forms a neutral, lipid-soluble complex with oxine which penetrates cellular membranes and

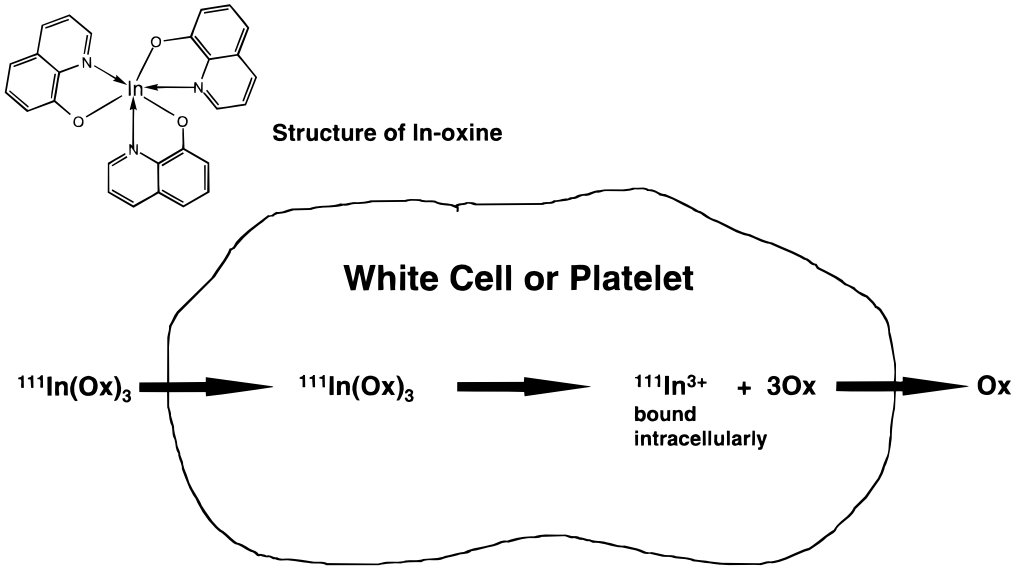


Figure 11. Structure of In−oxine and mechanism of the trapping of ^{111}In inside white blood cells or platelets using ^{111}In−oxine.

can be used to label leukocytes with retention of biological activity.[165] After diffusing intracellularly, the ^{111}In−oxine complex dissociates and the ^{111}In is bound to nuclear and cytoplasmic proteins.[165] Due to the high stability of In(III) with transferrin, the labeling of WBCs is done in the absence of plasma.

Disadvantages of using ^{111}In-labeled WBCs to image inflammation include the exposure of nuclear medicine personnel to blood-borne pathogens and the time-consuming preparation procedures. An alternative to WBC labeling is labeling ^{111}In to nonspecific immunoglobulins such as IgG using the chelator DTPA. It has been hypothesized that ^{111}In−DTPA−IgG accumulates by binding to Fc-γ receptors present on inflammatory cells.[166] This was further confirmed by Fischman et al.[167] An instant kit called MacroScint (RW Johnson Pharmaceutical Research Institute) contains DTPA−IgG for labeling with $^{111}InCl_3$, and this radiopharmaceutical was found to be a very sensitive tool for detection of infectious bone and joint disease.[168]

A disadvantage of ^{111}In-labeled IgG is the lengthy time between injection of the radiopharmaceutical and imaging (generally 1−3 days). The use of faster clearing molecules such as peptides would circumvent this problem. *N*-formyl−methionyl−leucyl−phenylalanine (fMLF) is a bacterial product that acts as a potent leukocyte chemoattractant peptide or chemotactic peptide. This peptide binds to high-affinity receptors on the WBC membrane[169,170] and has been labeled with ^{111}In using DTPA as the BFC. The first report of a diagnostically useful radiolabeled chemotactic peptide was in 1991 by Fischman and coauthors.[171] Indium-111−DTPA−fMLF showed high-affinity binding to human polymorphonuclear neutrophils in vitro. In rats infected with *E. coli*, ^{111}In−DTPA−fMLF was cleared very rapidly from the blood and the site of infection was imagable within 5 min postinjection.[171]

F. Thrombus Imaging Agents

Platelet scintigraphy is used in a variety of clinical situations, including intravascular thrombosis, antiplatelet medication, inflammation, atheroma, and graft thrombogenicity, but it has proved useful in only a few of these cases.[172] The detection of thrombosis is the most important of these applications. The first agent for gamma scintigraphy of platelet deposition in vascular disease was ^{111}In-labeled platelets using ^{111}In−oxine,[173] using a procedure similar to that of labeling WBCs.[164] This method is still used today. The method developed for ^{111}In-labeled platelets was also applied to ^{68}Ga.[174] Other methods used to image thrombosis include the use of ^{111}In-labeled fibrin fragments, specifically fibrin binding domain (FBD).[175] An initial study in 30 patients with deep venous thrombosis showed that ^{111}In−FBD agreed well with other diagnostic tests.[175]

IV. Summary

There have been significant advances in the development of new radiometal isotopes, the chemistry of new radiometal complexes, and the correlation of the chemical structure with the biological behavior. Radiometal complexes, either as small molecules or bioconjugates, are currently being utilized in the diagnosis of a wide variety of disease states including heart disease, brain disorders, and cancer using either gamma scintigraphy or PET. More specific disease states such as heart or tumor hypoxia and the receptor status of certain tumor types are also able to be imaged using radiometal complexes or radiometal−chelate−biomolecule conjugates.

The most exciting aspect of the research in radiometal radiopharmaceuticals is its multidisciplinary nature which coordinates the efforts of chemists with physicists, biologists, and clinicians. The purpose of this review is to not only demonstrate the advances in the chemistry of designing new radiometal-containing radiopharmaceuticals for diagnostic imaging, but also show how the chemistry relates to isotope production, biological evaluation, and clinical studies.

V. Glossary

$(4,6\text{-MeO}_2\text{sal})_2$-BAPEN	bis(4,6-dimethoxysalicylaldimino)-*N*,*N*′-bis(3-aminopropyl)ethylenediamine
$(\text{ROsal})_3$tame	1,1,1-tris(alkoxysalicylaldiminomethyl)-ethane
$(5\text{-MeOsal})_3$-tame	1,1,1-tris(5-methoxysalicylaldiminomethyl)ethane
ATSM	diacetyl-bis(N^4-methylthiosemicarbazone)
BAT	6-[*p*-(bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
BAT-TECH	tetraethyl-cyclohexyl bisaminoethanethiol
BFC	bifunctional chelator
CCK	cholecystokinin
CPTA	4-[(1,4,8,11-tetraazacyclotetradec-1-yl)-methyl]benzoic acid
DFO	desferrioxamine-B
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	diethylenetetraaminepentaacetic acid
EC	electron capture (radioactive decay)
EDTA	ethylenediaminetetraacetic acid
h	hours
HSA	human serum albumin
keV	kiloelectronvolts (10^3)
LAN	lanreotide
MeV	megaelectronvolts (10^6)
MTC	medullary thyroid carcinoma
NOTA	1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid
OC	octreotide
PCBA	1-[(1,4,7,10,13-pentaazacyclopentaadec-1-yl)methyl]benzoic acid
PET	positron emission tomography
PTSM	pyruvaldehyde-bis(N^4-methylthiosemicarbazone)
RCC	renal cell carcinoma
RIS	radioimmunoscintigraphy
RIT	radioimmunotherapy
SCN-TETA	6-[*p*-(isothiocyanato)benzyl]-1,4,8,11-tetraazacyclotetradecane
SPECT	single-photon emission computed tomography
SSR	somatostatin receptor
TATE	octreotate

TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
WBC	white blood cell
Y3-OC	tyrosine-3-octreotide
Y3-TATE	tyrosine-3-octreotate

VI. Acknowledgments

The authors thank the Department of Energy (Grant DE-FG02-87ER60512 (M.J.W.)) and the National Cancer Institute (Grants CA42925 (M.J.W.) and CA 64475 (C.J.A.)) for financial support.

VII. References

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