A Ticket to Ride: Peptide Radiopharmaceuticals

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Over the past three decades, biospecific imaging agents have evolved from large proteins (i.e., antibodies) to antibody fragments (i.e., $F(ab')_2$ and Fab fragments) to smaller "molecular recognition units" such as Fv fragments, antigen binding domain fragments and small biologically active peptides. The smaller size of these molecules confers desirable pharmacokinetic properties, such as higher target-to-background ratios and faster blood clearance, that are favorable for imaging. Molecular engineering techniques now permit the peptide to carry the radionuclide-binding group in its structure while maintaining high-affinity binding to the receptor site. An important component to this system is the ability to radiolabel these agents with high specific activity using short-lived radionuclides, particularly ^{99m}Tc. Recently, the application of small radiolabeled biologically active peptides for external imaging of a variety of biological processes has received considerable interest. These applications have ranged from the current widespread use of somatostatin analogs for imaging numerous types of tumors to the development of radiolabeled chemotactic peptides for infection imaging. In this review, we will describe many of the parameters for the rational development of peptide-based imaging agents, including: classes of peptides for imaging, methods for radiolabeling peptides, current biologically active peptide-based radiopharmaceuticals and future prospects for this new technology.

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The high affinity and specificity of antibodies should make them highly desirable for diagnostic imaging. Clinical studies with these agents, however, have often demonstrated limited binding at the target site, relatively slow blood clearance and modest target-to-background ratios. The high molecular weight of intact antibodies and even Fab fragments limits localization diffusion, which usually requires long intervals between administration and recording diagnostically useful images. In general, the pharmacokinetics of lesion localization are poorly matched to the physical half-life of the most available and least expensive radionuclide for imaging, ^{99m}Tc. An additional problem is the nonspecific accumulation of any IgG at sites of inflammation. This observation led to the use of radiolabeled nonspecific polyclonal IgG for the detection of inflammation, where it has proven to be equal or superior to specific monoclonal antibodies for localizing focal sites of infection.

In the evolution of biological diversity, amino acids have played a pivotal role as building blocks for a vast array of molecular signaling, signal transduction and recognition/transformation units (Fig. 1). Amino acids can act as neurotransmitters (i.e., GABA, glycine, glutamate) and provide a pool of precursors for the adrenergic, dopaminergic and serotonergic neurotransmitters. When assembled into small peptides, amino acids have generated a variety of hormones, releasing factors, neurotransmitters and neuromodulators. As larger constructs, amino acids comprise molecular recognition systems (immunoglobulins and receptors) and, when assembled into enzymes, can catalyze nearly the entire lexicon of organic reactions.

Some of the unfavorable imaging properties of intact antibodies and conventional fragments might be overcome with smaller specifically designed binding-domain fragments such as single-chain antigen binding proteins and peptide analogs of hypervariable sequences. In addition, nature has provided a vast array of small biologically active peptides with binding affinities that are comparable to or greater than antibodies. These compounds provide multiple new avenues for radiopharmaceutical development. In the following sections we will discuss classes of peptides for imaging, methods for radiolabeling peptides, current biologically active peptide-based radiopharmaceuticals and future prospects for this new technology.

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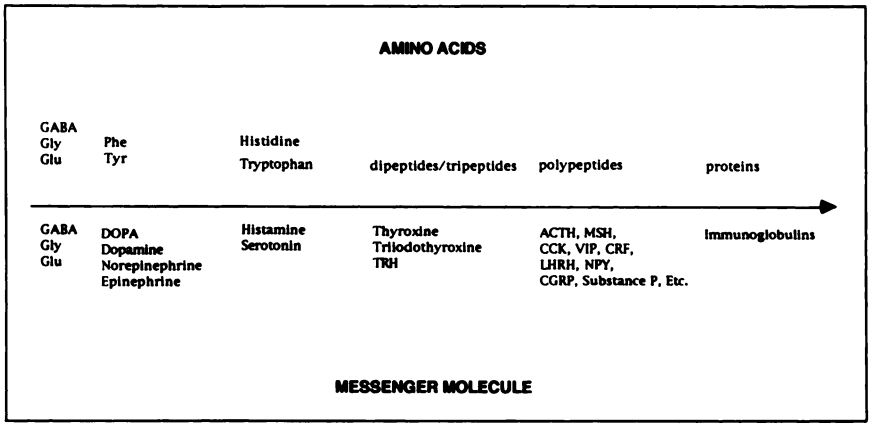

FIGURE 1. Role of amino acids in the evolution of biological diversity.

CLASSES OF PEPTIDES FOR IMAGING

Single-Chain Antigen-Binding Proteins

Recently, it has been suggested that imaging with radiolabeled Fv fragments, which are about 50% smaller than Fabs, may minimize many of the problems associated with the use of intact antibodies and conventional fragments. Although these reagents can be produced by fragmentation of intact antibodies (*1*), most single-chain binding proteins (sFvs) have been prepared by recombinant DNA techniques (*2–6*). In this approach, the gene sequences coding for the variable regions of the light and heavy chains of an antibody are linked with a specifically designed oligonucleotide sequence and the construct is expressed in *E. coli.* In the final product (25–30 kDa), the carboxyl terminus of the variable region of the light chain is linked to the amino terminus of variable region of the heavy chain by a designed 12–20 residue peptide. These proteins have the same antigen specificity as the parent antibody. Recent studies with these sFvs, have demonstrated rapid tumor penetration (*4,7,8*). For example, with the pancarcinoma antibody (CC49), the time for maximal penetration of sFv is 30 min, while intact IgG requires 48–95 hr to attain similar concentrations. Autoradiographic studies comparing the penetration of intact IgG with $F(ab)_2$, Fab and sFvs revealed that sFvs distribute uniformly in tumors while intact antibodies concentrate in the region of or immediately adjacent to blood vessels. The distributions of $F(ab)_2$ and Fab fragments showed intermediate penetration in a size-related manner (*7,8*). When applied to in vivo imaging, sFvs clear from the circulation rapidly ($t_{1/2}$ alpha ~2–5 min and $t_{1/2}$ beta ~2–5 hr), have low levels of accumulation in background organs and localize rapidly in target tissues (*4,9,10*). Although in some cases absolute levels of accumulation in target tissues are lower than with larger fragments, target-to-background ratios are comparable or greater.

Hypervariable Region Peptide Analogs

Since antigen binding is largely imparted by the hypervariable portions of the variable region of antibodies, synthetic peptide analogs of these regions might have even more favorable imaging properties than sFvs. Unfortunately, although peptides derived from hypervariable region sequences can bind antigens with similar specificity to the native antibody, affinity is usually significantly reduced. However, by using sophisticated methods of molecular design, this problem may be surmountable. For example, it has been demonstrated that conformationally constrained and dimeric peptides derived from hypervariable loop sequences can bind antigen with affinities that are up to 40-fold higher than linear sequences (*11*). The combination of detailed nuclear magnetic resonance spectroscopy and molecular modeling has made it possible to determine the influence of specific amino acid residues on antigen binding (*12*), allowing the specific design and synthesis of peptides with improved binding properties.

One important application of hypervariable region peptides to in vivo imaging has already been reported (*13,14*). In this study, 16–31 residue analogs of the hypervariable sequence of the platelet glycoprotein IIb/IIIa directed monoclonal antibody PAC 1.1 were prepared. All of the peptides contained a tripeptide binding group (RYD or RGD) and a metallothionein-derived sequence (KCTCCA) for ^{99m}Tc labeling. These peptides cleared rapidly and images of fresh thrombi in the jugular veins of rabbits and day-old thrombi in the femoral veins of dogs were obtained within 1–2 hr after injection. In control experiments, a ^{99m}Tc-labeled nonspecific peptide did not image the thrombi.

Natural Biologically Active Peptides

In contrast to antibodies, a low and intermediate molecular weight, naturally occurring peptides may be more suitable starting points for radiopharmaceutical development. Peptides are necessary elements in more fundamental biological processes than any other class of molecule. For example, peptides function as hormones, neurotransmitters, neuromodulators, growth and growth inhibition factors and cytokines. Furthermore, in many cases the affinities of peptides for their receptors are significantly greater than that of monovalent antibody fragments.

The molecular weights of biologically active peptides are

extremely diverse; ranging from 3 to 5 residues in TRH, enkephalins and bacterial chemoattractant peptides to over 200 residues in growth hormone. However, in many cases molecular recognition sites are restricted to specific areas of the sequence. For example in PTH and ACTH, biological activity is conferred by the N-terminal sequence. Likewise, for very small peptides, such as TRH and bacterial chemoattractant peptides, the C-terminus can be extended without significant alterations in receptor binding or biological activity. A particular advantage of this focality of binding domains is the ability to modify sequence regions that do not participate in receptor interaction for radiolabeling or optimization of biodistribution. For the smaller peptides, these modifications can be performed by the direct chemical synthesis of analogs by methods of solid-phase or solution synthesis. For larger peptides (>50 residues), analogs are more efficiently prepared by molecular cloning, followed by chemical modification in some cases.

TECHNIQUES FOR RADIOLABELING PEPTIDES

Numerous approaches are currently available for radiolabeling peptides. In general, these techniques are similar to those used for radiolabeling proteins. However, the impact of radiolabeling on biological behavior is frequently more profound with peptides because a smaller number of sites are available for labeling and the likelihood of modifying amino acid residues that are essential for biological activity is greater. In addition, due to the high potency of many peptides and the low tissue concentrations of their receptors, specific activity is often critical.

Methods for radiolabeling peptides with iodine, technetium, indium, gallium, carbon and fluorine have been developed. In the following sections, several examples of radiolabeling strategies and their potential pitfalls are discussed.

Iodination

The technique most widely applied to peptide labeling, primarily for in vitro applications, is radioiodination. Peptides may be radiolabeled directly via electrophilic substitution at reactive aromatic amino acids. Several reagents, such as iodogen, are available for direct iodination of reactive aromatic amino acid residues (*15*). By optimizing reaction conditions, rapid and nearly quantitative labeling can be achieved. Removal of unreacted radioiodine from the reaction mixture can be accomplished by separation techniques such as ion exchange, reverse and normal phase chromatography and desalting or size-exclusion chromatography.

Direct labeling has proven to be so reliable and convenient that many peptide analogs have been prepared with Tyr substituted for Phe or added to sequence sites that are not critical for biological activity or receptor binding. Where these modifications are not possible or the oxidizing conditions of direct iodination are considered to be too harsh, an alternative approach is iodination via prelabeled reagents, such as the Bolton-Hunter reagent (N-succinimydyl-3-(4-hydroxyphenyl)proprionate). In this case an activated aromatic compound (a phenolic conjugate) is radioiodinated, purified and linked to the peptide by acylation of a free amino group (*16*).

The problem of in vivo dehalogenation of radioiodinated antibodies led to the investigation of nonphenolic aromatic compounds as radiolabeling reagents, i.e., N-succinimidyl-5-(iodo)-3-pyridinecarboxylates (SIPC) (*17*), N-succinimidyl-3-iodobenzoate (*18*) and N-succinimidyl-4-(iodobenzoate) (*19,20*). In most cases, these reagents are prepared from trialkylstannyl precursors and demonstrate improved in vivo stability. This improvement is thought to be related to their structural differences from iodotyrosines/thyroxine which are substrates for dehalogenases in a number of tissues. The structural requirements for minimizing in vivo dehalogenation (*21,22*) were evaluated by Zalutsky et al. Their findings indicate that ring substituents, alkyl chain length and the nature of the protein linkage contribute to in vivo stability. Zalutsky and colleagues also demonstrated that octreotide, radioiodinated with SIPC, has greater stability than directly iodinated Tyr-3-octreotide (*23*). Although applications of these conjugates to peptide radiolabeling have been limited, they hold great promise for the future development of stable radioiodinated pharmaceuticals.

Two radionuclides of iodine, ^{123}I and ^{131}I, have been used for scintigraphic imaging of peptides (*24,25*), however, the high cost of ^{123}I and the high radiation dose and poor image quality associated with ^{131}I have prompted the investigation of other radionuclides. These include ^{99m}Tc and ^{111}In for single-photon imaging and ^{18}F, ^{11}C and ^{68}Ga for PET.

Technetium

The combination of low cost, availability, excellent imaging properties, favorable dosimetry and high specific activity make ^{99m}Tc a logical choice for peptide labeling. Two approaches, bifunctional chelates and direct labeling methods have been described for radiolabeling proteins and peptides with ^{99m}Tc. The bifunctional methods employed DTPA (*26*), N_2S_2 (*27*), N_3S (*28*), BATOs (*29*), and 6-hydrazinonicotinate (HYNIC) (*30*) groups as chelates.

Direct labeling usually generates free sulfhydryl groups by reduction of disulfide bridges in the protein. In general, this method suffers from two problems: (1) technetium frequently forms unstable complexes with the protein; and (2) there is poor control of the labeling site (*31*). Despite these limitations, direct labeling has been useful for preparing ^{99m}Tc-labeled antibodies. It is unlikely that direct labeling can be readily extended to smaller peptides because most small peptides do not contain disulfides and peptide structures are often altered by random addition of the radiolabel. In many peptides, even slight alterations in the ring structure can result in dramatic alterations in biological activity. In small cyclic peptides like oxytocin and vasopressin, increasing the size of the ring by a single CH_2

group (replacement of a cysteine by a homocysteine residue) markedly decreases biological activity. In contrast, the even smaller structural perturbation induced by replacing the S-S moiety by a C-S group results in super active analogs. Although the biological activity of somatostatin is less sensitive to alterations in the ring structure and active analogs with varying numbers of residues in the ring have been prepared, activity is affected by the conformation of the ring residues. Thus, application of thiol-based direct labeling of peptides is almost certain to be unsuccessful. For example, in an effort to prepare ^{99m}Tc-octreotide, Thakur et al. (*32*) demonstrated that direct labeling via disulfide reduction, reduced receptor affinity by four orders of magnitude, confirming that alterations of the cyclic part of this peptide can dramatically reduce biological activity.

In addition to disulfides, other functional groups in peptides can contribute to ^{99m}Tc binding. However, at present there is no method for predicting the precise geometry of chelation or the effects of radiolabeling on receptor binding or biological activity. Application of this method of direct ^{99m}Tc labeling has been reported for thyrotropin releasing hormone (TRH) (*33*) and more recently for a 19 residue analog of laminin, PA22-2 (*34*). Biological activity of ^{99m}Tc-TRH was considered to be similar to ^{3}H-TRH on the basis of chromatographic data and the time course of accumulation by the pituitary. Similarly, biological activity of PA22-2 was implied by altered distribution in the lungs of mice with emphysema and melanoma compared to controls. However, in neither case were receptor binding studies performed to evaluate the effect of labeling on affinity.

To overcome the problems associated with direct labeling, methods have been developed which utilize high-affinity chelates to bind ^{99m}Tc to specific sites on peptides. These chelates can be used in two ways. In the first method, the bifunctional reagent is prelabeled with ^{99m}Tc and then conjugated to the peptide, similar to the indirect iodination reagents. If more than one reactive group is present, multiple species can result. To achieve high specific activities, the unreacted labeling reagent and unlabeled peptide must be removed from the reaction mixture. Since these species can be similar in size to the labeled peptide, chromatographic purification is often difficult. In the second method, the chelating agent is covalently attached to the peptide prior to radiolabeling. This method usually results in a single radiolabeled product, making chromatographic separation simpler. Both methods have been highly successful for radiolabeling antibodies and can be directly extended to peptides.

Theoretical considerations suggest that indirect methods may be preferable. The chemistry of ^{99m}Tc labeling is better defined and more predictable with this approach, since the bioactivity and receptor-binding characteristics of the conjugate can be determined before and after labeling.

Recently, ^{99m}Tc-labeling of several peptides has been reported using a variety of methods, including HYNIC (*35*) and diaminedithiol (*36*) derivatized chemotactic peptides for infection imaging and N_2S_2 and N_3S analogs of the apolipoprotein fragment, SP-4 (*37*) for imaging atherosclerosis. The HYNIC and diaminedithiol derivatized chemotactic peptides demonstrated biological activity and receptor binding characteristics similar to the unmodified peptide.

Indium and Gallium

Indium-111 has been used extensively for radiolabeling monoclonal antibodies. The chemistry of this group III metal and the half-life of this isotope make it ideal for radiolabeling intact immunoglobulins, where imaging studies are usually performed over intervals of several days. Rapid and stable labeling is usually accomplished using bifunctionalized derivatives of the polyaminocarboylate chelates EDTA and DTPA (*38*). DTPA has been used as a bifunctional chelating agent for radiolabeling α-melanocyte-stimulating hormone (α-MSH) with ^{111}In for imaging melanoma (*39*), formylated-Met-Leu-Phe chemotactic peptides for infection imaging (*40*), laminin fragments for targeting tumor-associated laminin receptors (*41*) and atrial natriuretic peptide (ANP) for imaging ANP receptors in the kidney (*42*). Gallium-68 has been used for radiolabeling chemotactic peptides (*43*) and a somatostatin analog using DTPA and desferrioxamine (*44*), respectively.

CURRENT PEPTIDE RADIOPHARMACEUTICALS

The number of biologically active peptides that have potential as imaging agents is so vast that even a list of the most promising candidates would span many pages. Although applications of these reagents is still in its infancy, many important imaging agents have already been developed. Table 1 is a partial listing of some peptides that have been radiolabeled and used for in vivo imaging. In the following sections, we will review the current status of radionuclide imaging with biologically active peptides. This will be followed by a perspective on future applications.

Somatostatin

The peptide that has attracted greatest interest as an imaging agent is somatostatin. Somatostatin is a small regulatory peptide with a wide spectrum of actions on multiple organs. The most well defined biological activity of somatostatin is its inhibitory effect on the secretion of numerous hormones, including growth hormone, thyrotropin, insulin, glucogon, vasoactive intestinal peptide and secretin, among others. In addition, receptors for somatostatin are expressed on a variety of human tumors and their metastases (*45*). In numerous studies, somatostatin and its analogs have been shown to inhibit tumor growth (*46*).

Although the first report of in vivo imaging with a somatostatin analog appeared in 1976 (*47*), further development was delayed because the native peptide is rapidly degraded by plasma and tissue proteases (*48*) and the role of somatostatin in tumor growth was not identified until years later. Now, somatostatin analogs that are more resistant to biological degradation have been developed. One of these compounds, octreotide, is currently in use for the treat-

TABLE 1
Peptide Imaging Agents

Peptide	No. of amino acid residues	Radionuclide labeling	Principle application	References
Somatostatin	14	^{131}I, ^{123}I, ^{111}In	Tumors	47,50,51,55
Octreotide	8	^{99m}Tc, ^{68}Ga		
Bacterial Chemotactic peptides	3	^{111}In, ^{99m}Tc, ^{68}Ga	Infection	40,35,43
Interleukin 8	79	^{125}I	Infection	80
Endothelin	21	^{125}I	Atherosclerosis	81
Apo-lipoprotein fragments	18	^{125}I, ^{123}I, ^{99m}Tc	Atherosclerosis	37,82
Corticotropin Releasing Factor	41	^{125}I	Tumors	83
Captopril	3	^{18}F	? Hypertension	84
Atrial Natriuretic Factor	28,24	^{123}I	? Hypertension	42
Insulin	51	^{131}I, ^{18}F	Diabetes	85,86

ment of a variety of tumors (*49,50*). The success of octreotide for tumor therapy led to the application of ^{123}I-labeled Tyr-3-octreotide for in vivo tumor imaging (*51*). This radiopharmaceutical retains biological activity and binds specifically to somatostatin receptors. In studies with transplantable pancreatic tumors (CA 20948) in rats, it was demonstrated that in vivo imaging is rapid and can be blocked with unlabeled peptide. These results were verified in patients with tumors that express somatostatin receptors (*52*). After injection of the radiolabeled peptide, about 50% of the radioactivity is cleared from the blood pool within 2 min and simultaneous accumulation in the tumor occurs. In most cases radioactivity is retained in the tumor for 24–48 hr and residual activity is cleared by the liver through the biliary system into the intestinal tract (*53*).

Although ^{123}I-Tyr-3-octreotide has proven to be useful for tumor imaging, several problems prevent widespread application: (1) pure ^{123}I is not widely available and a complicated procedure is required for radiolabeling; (2) the short half-life of ^{123}I (13 hr) hampers delayed imaging; and (3) accumulation of radioactivity in the liver, gallbladder and GI tract complicate the imaging of tumors in the abdomen. To minimize these problems, ^{111}In-labeled DTPA octreotide was developed. The receptor binding and biological activity of ^{111}In DTPA octreotide are similar to those of octreotide (*54*). About 90% of this radiopharmaceutical is cleared by the kidneys within 24 hr after injection. Compared with ^{123}I-octreotide, hepatic and biliary accumulation of ^{111}In-DTPA-octreotide is much lower; 2% versus 40% at 4 hr after injection (*55*). The differences in clearance of these two forms of octreotide are probably related to differences in hydrophilicity. Indium-111-DTPA-octreotide is definitely a better agent for imaging abdominal tumors, but tumor localization could still be obscured in some situations, particularly at late imaging times (*56*).

Indium-111-DTPA-octreotide is useful for detecting tumors that express somatostatin receptors (Fig. 2) (*57,58*). Table 2 summarizes the range of peripheral tumors that have been imaged with this reagent. Following intravenous injection, radiolabeled octreotide has not been useful for imaging CNS tumors when the blood brain barrier is intact. The lack of positive octreotide imaging in some somatostatin receptor-positive tumors (in vitro binding of somatostatin-14, somatostatin-28) but not octreotide could be related to selective expression of different receptor subtypes (*59*). This problem may be addressed by developing radiolabeled analogs with broader receptor specificity.

Despite the success of radiolabeled octreotide for tumor imaging, enthusiasm must be tempered with caution. Although blockade of tumor uptake has been demonstrated in animal models and positive imaging in patients is well correlated with ex vivo measurements of receptor expression,

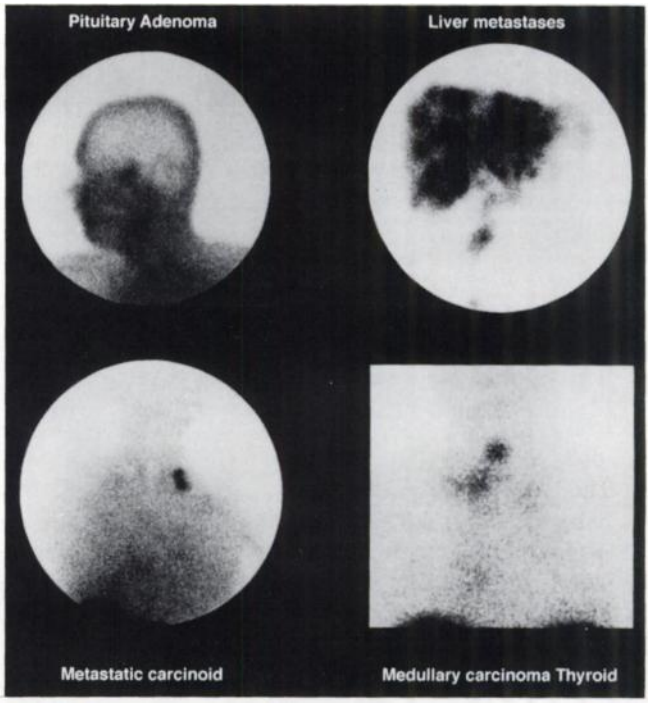

FIGURE 2. Examples of tumors that have been successfully imaged with radiolabeled octreotide.

TABLE 2
Detection Frequencies of Human Tumors with ^{111}In-Octreotide

Tumor type	In vivo ^{111}In-octreotide imaging	In vitro receptor autoradiography
Pituitary adenomas		
GH	4/4 (100%)	45/46 (98%)
TSH	2/2 (100%)	2/2 (100%)
Inactive	6/9 (67%)	6/15 (40%)
Endocrine pancreas tumors	18/21 (86%)	31/35 (89%)
Carcinoids	37/39 (95%)	54/62 (88%)
Paragangliomas	29/31 (94%)	11/12 (93%)
Pheochromocytomas	2/5 (40%)	38/52 (73%)
Medullary thyroid carcinoma	8/12 (67%)	10/26 (38%)
Lung tumors		
SCLC	5/8 (63%)	4/7 (57%)
NonSCLC	3/4 (75%)	0/17 (0%)
Neuroblastoma	5/5 (100%)	15/23 (65%)
Meningiomas	11/11 (100%)	54/55 (98%)
Glial tumors		
Astrocytomas	4/4 (100%)	14/17 (83%)
Glioblastomas	0/2 (0%)	1/45 (2%)
Merkel cell tumors	4/5 (80%)	—
Breast carcinoma	39/52 (75%)	33/72 (46%)
Adenocarcinoma	5/29 (17%)	6/95 (6%)
Lymphoma		
Hodgkins	3/3 (100%)	2/2 (100%)
NonHodgkins	10/11 (91%)	26/30 (87%)

Adapted from references 55 and 58. In vivo and in vitro data were obtained from different groups of patients.

there is limited data on the effect of pretreatment with unlabeled peptides on imaging of human tumors. This is important because a significant amount of localization could be due to nonspecific increases in tissue permeability, particularly at early imaging times. Only blocking studies can determine the fraction of localization that is truly receptor mediated. Recently, it has been reported that pretreatment with therapeutic doses of octreotide decreased the level of accumulation of ^{111}In-DTPA-octreotide in normal tissues but had minimal effect on tumor localization (*60*). These results could be explained in several ways:

1. The circulating concentrations of unlabeled drug were significantly lower than the blocking doses that were used in animal experiments and thus may not have been sufficient for receptor saturation.
2. It is possible that pretreatment with octreotide resulted in upregulation of somatostatin receptors. This effect has been reported for GH_4C_1 pituitary cells (*61*).

These results point out the importance of carefully controlled blocking studies. Such studies are critical if octreotide scintigraphy is to proceed for an imaging technique to a method for the in vivo quantification of somatostatin receptors.

As more and more tumors are shown to express somatostatin receptors, octreotide scintigraphy is rapidly being transformed from a specific to a nonspecific tumor imaging technique. In this regard, it should be compared to other nonspecific imaging agents such as ^{67}Ga-citrate and ^{18}FDG PET. Clearly, radiolabeled octreotide has many advantages over gallium. However, due to the high resolution and sensitivity of PET and the quantitative nature of the measurement, FDG is a more formidable competitor. In fact it has recently been demonstrated that ^{111}In-DTPA-octreotide is considerably less sensitive than ^{18}FDG for detecting a variety of tumors (*62,63*).

Chemotactic Peptides

The development of reagents for the rapid and accurate delineation of focal sites of infection is an important issue in current nuclear medicine practice. Currently, the most commonly used radiopharmaceuticals, ^{67}Ga-citrate and radiolabeled leukocytes, require at least 24 hr between injection and imaging. In addition, radiolabeled cells require complicated labeling procedures that are associated with potential dangers of blood handling. The interval between injection and lesion detection and the inconvenience and potential hazards of in vitro cell labeling could be reduced by radionuclide imaging with small molecules that bind in vivo to both circulating granulocytes and leukocytes already present at the site of inflammation. Due to their high affinity for inflammatory cells and very low molecular weight, analogs of For-MLF are particularly appealing candidates for this application. The parent peptide of this series is a bacterial product that initiates leukocyte chemo-

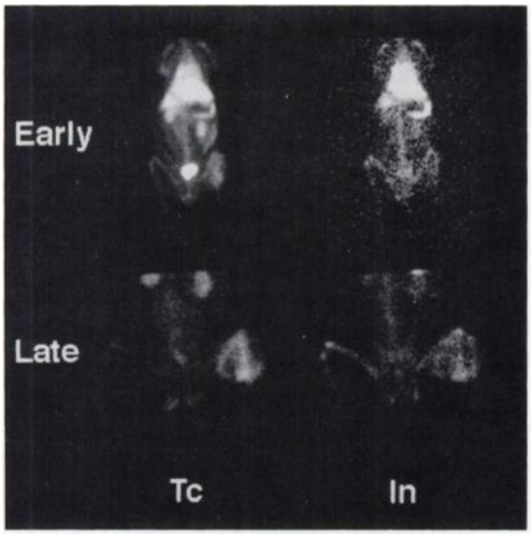

FIGURE 3. Representative dual-photon images of a rabbit with mild infection. The images were acquired at 3 (upper images) and 17 (lower images) hr after co-injection of ^{99m}Tc-labeled chemotactic peptide and ^{111}In-WBCs. Only anterior images are shown. The animals were infected 24 hr before injection of the radiopharmaceuticals.

taxis by binding to high affinity receptors on inflammatory cells (*64–66*). These receptors are present on both PMNs and mononuclear phagocytes. As cells respond to a chemoattractant signal, the affinity of the receptors decreases and additional receptors are expressed, until the site of inflammation is reached (*67–69*). Many synthetic analogs of For-MLF bind to neutrophils and macrophages with equal or greater affinity compared to the native peptide (*64,65,70*).

Like somatostatin receptor imaging, current applications of radiolabeled chemotactic peptides to in vivo imaging is also a case of "re-discovery." Although the first use of a radiolabeled chemotactic peptide for abscess localization was reported in 1982 (*71*), further development was delayed until recently. The delay in application of chemotactic peptides to infection imaging was due to a combination of limitations in radiolabeling technology and a potentially adverse side effect of the compounds. The radiolabeling methods used in the early studies yielded radiopharmaceuticals of relatively low specific activity and pharmacological concentrations of peptide were required for imaging. These doses of peptide were shown to produce profound transient reductions in peripheral leukocyte levels in rabbits and dogs (*72–75*).

Since previous structure-activity studies have established that the C-terminus of For-MLF can be extensively modified without significantly altering bioactivity or receptor binding, this is an ideal site for radiolabeling. Recently, we demonstrated that C-terminal DTPA derivatized chemotactic peptide analogs can be prepared by standard methods of solid phase peptide synthesis and radiolabeled with ^{111}In (*40*). In these studies, four DTPA derivatized chemotactic peptide analogs were synthesized, evaluated for in vitro bioactivity and receptor binding, and radiolabeled with ^{111}In. Biodistribution was determined in normal rats and localization at sites of *E. coli* infection was determined by scintillation camera imaging from 5 min to 2 hr after injection. All of the peptides maintained biological activity (EC_{50} for O_2-production by human PMNs: 3–150 n*M*) and the ability to bind to the oligopeptide chemoattractant receptor on human PMNs (EC_{50} for binding: 7.5–50 n*M*); biological activity and receptor binding were highly correlated ($r = 0.99$). In vivo, biodistributions of the peptides were similar, with low levels of accumulation in the heart, lung, liver, spleen and GI tract. With all the peptides, excellent images of infection sites were obtained within 2 hr after injection.

Although these studies indicated that ^{111}In-labeled chemotactic peptide analogs are effective agents for the external imaging of focal sites of infection, the rapid infection localization and short biological half-life of these agents makes ^{111}In a suboptimal radionuclide for labeling. Clearly, ^{99m}Tc would be a more appropriate nuclide. A new reagent for radiolabeling proteins with ^{99m}Tc via hydrazino nicoti-

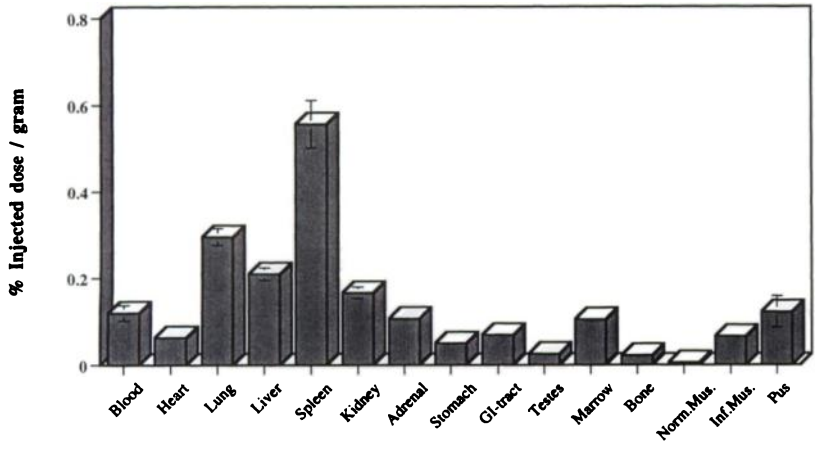

FIGURE 4. Biodistribution of a ^{99m}Tc-labeled chemotactic peptide in rabbits. Each point is the mean ± s.e.m. for six animals.

TABLE 3
Species Specificity of Chemotactic Peptides

Species	Infection imaging	Lung uptake	Liver and spleen uptake	Reduction in leukocyte levels
Rats	+	–	+	–
Rabbits	+	+	+	+
Dogs	+	–	+	+
Monkeys	NT	–	+	+

NT = not tested.

namide (HYNIC) derivatized epsilon amino groups of lysine residues (*30*) was recently reported. This method of preparing high specific activity ^{99m}Tc-labeled compounds was applied to the chemotactic peptide analogs, where specific activities >20,000 mCi/μmole were prepared (*35*). These high specific activity peptides retained their biological activity and binding affinity, and demonstrated imaging characteristics in rats that were similar to the ^{111}In-labeled peptides.

Since the current standard for infection imaging is radiolabeled white cells, a comparison of ^{111}In-labeled leukocytes to ^{99m}Tc-labeled peptides was performed in infected rabbits. The animals were imaged at 3, 6 and 17 hr after injection. Sites of infection were well visualized with both radiopharmaceuticals and the biodistributions were surprisingly similar (*76*). Figure 3 shows representative dual-photon images of an animal with mild infection. In the early images, both agents showed a high level of accumulation in the lungs, bone marrow, liver and spleen (the characteristic pattern for labeled WBCs). In the late images, pulmonary activity decreased for both radiopharmaceuticals. From region of interest (ROI) analysis, the T/B ratios were 3.63 ± 0.37, 5.66 ± 1.97 and 10.47 ± 2.78 for the ^{99m}Tc-peptide and 2.41 ± 1.15, 1.76 ± 0.23 and 5.45 ± 2.37 for ^{111}In-WBCs at 3, 6 and 17 hr after injection. The average ratio of T/B ratios [(T/B)Peptide/(T/B)WBC] was 2.20 ± 1.06. T/B ratios calculated from direct tissue sampling were significantly ($p < 0.01$) higher for ^{99m}Tc peptide (33.6:1) compared to ^{111}In-WBCs (8.1:1). This difference was primarily due to greater accumulation of the ^{99m}Tc-labeled peptide in infected muscle rather than differences in accumulation in normal muscle. The biodistribution of the ^{99m}Tc peptide in rabbits is shown in Figure 4. In contrast to earlier studies with chemotactic peptides that resulted in transient leukopenia, the high specific activity peptide did not reduce the peripheral WBC count in these rabbits. Similar experiments in dogs yielded comparable results (unpublished results).

It is interesting that although ^{99m}Tc-labeled chemotactic peptides are excellent infection imaging agents in all species tested, important differences in in vivo bioactivity and biodistribution were observed (Table 3, Fig. 5). For example, although significant concentrations of radioactivity were detected in the liver and spleen of all species, high levels of pulmonary activity were seen only in rabbits. Also, the rat is the only species that we studied that is insensitive to the effect of peptide on peripheral leukocyte levels.

FUTURE APPLICATIONS

Application of radiolabeled biologically active peptides for imaging is in its infancy. These molecules offer major advantages for imaging including: pharmacokinetic properties resulting in rapid localization and blood clearance; ability to synthesize analogs with specific biological, labeling and biodistribution properties; and available approaches for high specific activity labeling with short-lived radionuclides. This combination of early specific imaging with high photon flux radiopharmaceuticals can lead to images with better resolution, and hence improved diagnostic accuracy. In addition to the agents described above, another group of candidate peptides for imaging include the growth factors (*77*). Receptors for these peptides are highly expressed in tumors and following injury. This group of agents includes: TGF-beta (tumor growth factor) to identify receptors associated with neural injury (particularly associated with demyelination); FGF (fibroblast growth factor), to accelerate vessel growth into areas of injury (*78*); PDGF (platelet derived growth factor) to identify sites

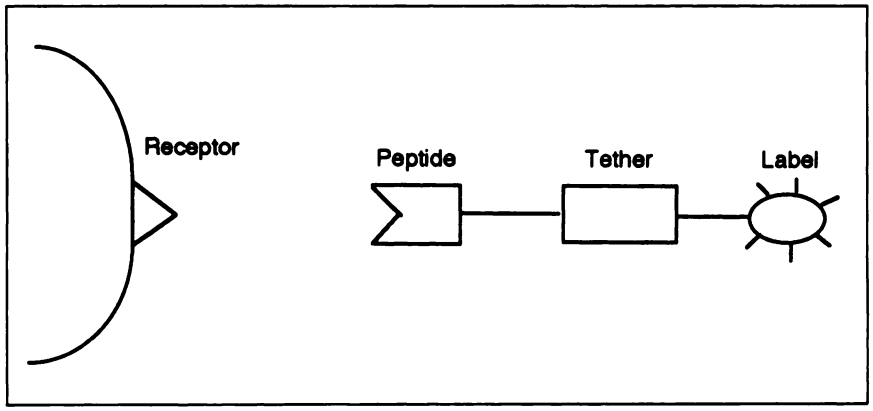

FIGURE 5. Schematic diagram of a peptide radiopharmaceutical in which receptor binding, radiolabeling and biodistribution can be individually altered to optimize clinical utility.

of vascular injury and possibly areas of resetnosis; and EGF (epidermal growth factor) as a marker for gliioma. In addition to the growth factors, other substances such as Np-Y (neuropeptide Y) or CCK (cholecystokinin) could be labeled to examine the satiety center of the brain. A recent survey of the literature identified over 300 receptors and their agonists. Each of these are potential candidates for examination with a peptide-based radiopharmaceutical.

As Figure 5 illustrates, it may be possible to specifically design radiopharmaceuticals with independent manipulation of segments of the reagent that control binding, radiolabeling and biodistribution. With this approach, radiolabeling groups and peptide units can be independently coupled to a relatively low molecular weight "tether." By using specific protecting group strategies, the peptide can be coupled via amino acid residues that are not essential for binding. Also by coupling multiple radiolabeling moieties (such as hydrazino nicotinamide groups) to a single site on the tether, extremely high specific activities can be achieved. Furthermore, the size, charge and polarity of the tether can be varied to optimize biodistribution for specific applications. In some cases, it might be possible to dramatically increase binding affinity by clustering multiple peptide units at a single site. For example, in the case of chemotactic peptides, tetrameric analogs have been shown to have extremely high affinities (*79*).

Another method for improving affinity involves the use of multiple binding determinants. For example, using monoclonal antibody technology, antibodies to specific external domains on peptide receptors can be prepared. Some of these antibodies might bind to the receptor at locations that are different from where the native ligand binds. By preparing a conjugate that contains both the native peptide and a binding site sequence of such an antibody, cooperative high affinity binding might be achieved.

Clearly, the methods for assembling peptides into effective imaging agents is as vast as the number of peptides with important imaging applications. With the close cooperation of biologists, biochemists, radiochemists and clinicians, peptide-based imaging agents promise to be among the cornerstones for radiopharmaceutical development in the twenty-first century and beyond.

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