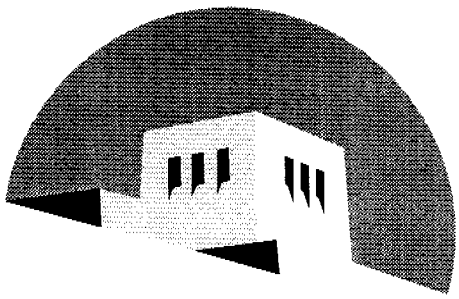

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# Correspondence Continuing Education Courses for Nuclear Pharmacists and Nuclear Medicine Professionals

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# *The Current Status of Radiolabeled Peptides for Imaging and Therapy*

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# THE CURRENT STATUS OF RADIOLABELED PEPTIDES FOR IMAGING

## STATEMENT OF OBJECTIVES

The purpose of this correspondence lesson is to increase the reader's knowledge of current research efforts in the development and utilization of radiolabeled peptides for nuclear medicine imaging. In addition, the reader will review the clinical use of the first radiolabeled peptide approved for use by the U.S.F.D.A., In-111 pentetreotide.

***Upon successful completion of this material, the reader should be able to:***

1. describe the general chemical structure of peptides.
2. compare and contrast the structural differences of peptides with that of monoclonal antibodies.
3. list the radionuclides that have been used for radiolabeling peptides for both pre-clinical and clinical use.
4. summarize the chemical bond formation that allows peptides to be radiolabeled with radionuclides useful for nuclear medicine imaging.
5. compare and contrast the radionuclides that have been used for radiolabeling peptides.
6. describe the biological role of naturally occurring peptides.
7. describe the different classes of peptides useful for nuclear medicine imaging.
8. discuss the development, characteristics, preparation, quality assessment procedure, pharmacology, pharmacokinetics, indications and efficacy of In-111 pentetreotide.
9. discuss the potential role of vasoactive intestinal peptide in nuclear medicine imaging.
10. discuss the potential role of chemotactic peptides in nuclear medicine imaging.
11. discuss the potential role of RGD and RYD peptides in nuclear medicine imaging.
12. discuss the potential role of atrial natriuretic peptide in nuclear medicine imaging.

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# THE CURRENT STATUS OF RADIOLABELED PEPTIDES FOR IMAGING

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## INTRODUCTION

Peptides play an important role in such fundamental bodily functions as homeostasis and cell signaling (1). Recently they have been the focus of much interest as diagnostic radiopharmaceuticals(2). Peptides represent a logical next step in radiopharmaceutical development as they can be made to possess some of the characteristics which make monoclonal antibody (MAb) technology so attractive as targeting agents. The promise of MAb technology, in relation to diagnosis and therapy, has not been fulfilled (3). The large molecular weight of these proteins is significantly responsible for the limitations of this approach. Barriers to diffusion into diseased tissue, as well as the natural long-term blood residence time of these large proteins, results in low target-tissue accumulation compounded with high background activity, thereby limiting their utility. The promise of MAb technology, however, resides in its unique characteristic of generating proteins which recognize particular molecular sequences of other proteins or carbohydrates and which bind to these sequences with relatively high affinity ($10^{-8}$ - $10^{-10}$ M). Peptides may provide an avenue which allows us to exploit the specific binding potential of such compounds without the disadvantages associated with large molecular size.

It appears likely that high affinity binding to such antigens can be accomplished using smaller fragments of MAbs or synthetic peptides. In addition to mimicking MAbs in application, peptides found endogenously also have diagnostic utility, as naturally occuring peptides play important roles in cell signaling and control. As more is learned about the role of peptides in tissue regulation and disease, it is likely that the abnormal expression of peptide receptors or overproduction of peptide messenger may be a critical key in the evolution and control of disease (4-6).

Exploration of such phenomena with radiolabeled probes may result in useful diagnostic information with potential for therapeutic application.

Radiolabeled peptides represent a new class of agents for exploitation of radiopharmaceuticals. Comprised of polymers of amino acids, peptides display a variety of sizes and shapes as well as biologic activity. Peptides are formed through the polymerization of amino acids. Of the 20 amino acids usually found in nature, 19 of them have the general structure $H_2N$-CHR-COOH. They differ only in the chemical structure of the side chain R. The 20th amino acid, proline, is similar but its side chain is bonded to the nitrogen atom to give the imino acid. Except in the case of glycine, the central carbon atom is asymmetric and is always the L-isomer. Unless indicated, all the amino acids discussed herewith will be assumed to be the L-isomers. More than half of the amino acids commonly encountered in peptides and proteins contain functional side chains. These include acidic carboxy-groups (aspartic, glutamic acids); basic residues (lysine, arginine, histidine); hydroxy-amino acids (serine, threonine, tyrosine); sulfur-containing amino acids (cysteine, methionine); and, finally, the hetero-cyclic indole ring of tryptophan. In addition, there are amino acids with aliphatic residues (alanine, valine, leucine and iso-leucine) as well as the aromatic amino acid phenylalanine and, glycine, the simplest amino acid with only a hydrogen atom for a side chain.

Amino acids are linked into peptides and proteins by the peptide bond. The polypeptide backbone consists of a repeated sequence of three atoms of each residue in the chain. These include the amide nitrogen, the alpha-carbon and the carbonyl-carbon.

-NH-$^{a}$CHR-CO-**NH-$^{a}$CHR-CO**-NH-$^{a}$CHR-CO-

The peptide bond is formed when the amino group of one amino acid condenses with the carboxylic acid group of a second amino acid, liberating water (7). The peptide bond confers a particular three-dimensional structure on polypeptides. The hydroxyl group is nearly always trans to the carbonyl oxygen. The peptide bond unit is rigid and planar and no freedom of rotation occurs around the peptide bond due to the partial double-bond nature of the peptide linkage. There is no such character between the alpha-carbon and the amino group or the alpha-carbon and the carboxylic acid group. Therefore, a large degree of freedom is found on either side of the peptide bond, allowing the peptides to take a wide variety of conformations.

Polymerization of amino acids through peptide bond formation results in linear polypeptide chains. All proteins and polypeptides have this basic structure. Proteins differ only in the number of amino acids linked together and the sequence in which the various amino acids occur. The sequence of amino acids in a polypeptide chain generally identify a protein unambiguously. Peptides are often referred to as polymerized amino acids with a relatively small number of residues and a defined sequence. There is no maximum number of residues in a peptide. The term peptide is appropriate to a chain or polymer of amino acids if its physical properties are those expected from the sum of its amino acid residues and there is no fixed three-dimensional conformation.

Polypeptides are longer chains usually of defined sequence and length, and proteins are generally those polypeptides that occur naturally and have a definite three-dimensional structure under physiological conditions. For the sake of our discussion we will use the term peptide throughout, referring to a number of amino acid polymers with biological activity or those that are analogs of biologically-active peptides.

Since each of the 20 amino acids is chemically distinct and each can, in principal, occur at any position in a polypeptide chain, there are 20 to the *n*th power different possible polypeptide chains, *n* amino acids long (e.g., $20^5$ = 3,200,000 different polypeptide chains possible for five amino acids). Hence, the repertoire of potential peptides that can be described is vast and provides an opportunity for exploitation in radiopharmaceutical development, as well as drug development (8).

When assembled into small peptides, amino acids have generated a large number of hormone-releasing factors, neurotransmitters and neuromodulators. Larger polypeptides comprise molecular recognition systems such as the immunoglobulins, receptors and enzymes.

## RADIOLABELING TECHNIQUES

As discussed above, peptides are comprised of the same basic building blocks as proteins, therefore techniques which have been developed for radiolabeling proteins can be applied to peptides. However, one must consider that since peptides possess fewer amino acid residues than proteins there are fewer sites available for labeling. The likelihood of modifying an amino acid residue crucial for biological acitivity is therefore greater. Consequently, optimization of labeling must be determined empirically. In addition, due to the high potency of many peptides and the low tissue concentrations of their target receptors, specific activity becomes a critical element in the preparation of radiolabeled peptides. Efforts to prepare peptides with extremely high specific activity are required to avoid potential flooding of the receptor with cold or unlabeled peptide.

For more than a decade, a variety of methods for labeling proteins with radioisotopes of iodine, technetium, indium, gallium, carbon, and fluorine have been developed. These methods can be applied to the labeling of peptides. What follows is a discussion of radiolabeling methods that have been, or could be, applied to peptide radiopharmaceuticals.

**Iodine**

Iodination is probably the most widely utilized method for radiolabeling peptides. Although originally developed for the in-vitro applications of radioabeled peptides, such as in RIA assays, radioiodination can also be applied for imaging purposes. This requires the use of isotopes such as Iodine-123 and Iodine-131, as well as Iodine-124. Peptides may be directly iodinated on reactive aromatic acid residues such as tryrosine (9). To accomplish this, oxidizing agents are used to generate electrophilic iodine species($I^+$) from NaI*. Reagents available for direct iodination include the commonly used agents, Chloramine T, iodine monochloride, N-bromosuccinimide, and Iodogen (10).

Quantitative labeling can be achieved with most peptides through the optimization of reaction conditions and the removal of unwanted radioactive by-products. In general, purification of the radiolabeled peptide requires chromatographic techniques such as ion exchange, reverse-phase or size-exclusion chromatography. In many cases, direct labeling has proved to be such a reliable and straightforward technique that many peptide analogs have been prepared with tyrosine substituted for phenylalanine or added to the peptide sequence in positions which are unlikely to affect biological activity or receptor binding (e.g., [$Tyr^3$]-octreotide).

Alternative approaches to direct labeling also exist and these include using pre-labeled reagents such as the Bolton-Hunter reagent [N-succinimydyl-3-(4-hydroxyphenyl) proprionate]. In this case, an activated aromatic compound, a phenol residue, is radioiodinated, purified, and attached to the peptide sequence by acylation of a free amino group, such as the N-terminal amine of the peptide sequence or the epsilon amino group of a lysine residue (11).

One potential drawback of using iodine as a radiolabel for peptides, is the observation *in vivo* of rapid dehalogenation. This has been documented, particularly in the case of radioiodinated antibodies, and has led to the use and investigation of non-phenolic aromatic compounds as radiolabeling reagents [(i.e., N-succinimidyl-5-Iodo-3-pyridine carboxylates (12), N-succinimidyl-3-Iodobenzoate (13) and N-succinimidyl-4-Iodobenzoate (14,15)]. In general, these reagents are prepared from trialkyl stannyl precursors and demonstrate greater *in vivo* stability than directly labeled polypeptides. The improvement in stability is thought to be due to the structural differences of these compounds from iodo-tyrosines and thyroxine which are substrates for dehalogenases in a number of tissues. It has also been found that the Bolton-Hunter reagent is resistant to dehalogenation (16). While still having a phenol group, the Bolton-Hunter reagent possesses an altered aliphatic chain and lacks the amino group of tyrosine. These structural differences impart greater stability to dehalogenation, over direct labeling of tyrosine residues.

The structural requirements for minimizing *in vivo* dehalogenation has been reported to include (a) the ring substituents (b) alkyl chain length, and (c) the nature of the linkage to the polypeptide (17). For example, it has been shown that an octreotide analog radioiodinated with N-succinimydyl-5-Iodo-3-pyridine carboxylate has greater stability than directly iodinated [$Tyr^3$]-Octreotide (18).

The application of these iodinatable conjugates to peptide radiolabeling should hold promise for future development of stable radiopharmaceuticals with regard to therapy. While Iodine-123 and Iodine-131 have been used for scintigraphic imaging of peptides (19,20) the limited availability and the high cost of Iodine-123, and the high radiation dose and poor imaging qualities associated with the use of Iodine 131 have prompted investigations into other radionuclides.

**Technetium-99m**

Technetium-99m is an ideal radionuclide for peptide labeling due to its low cost, widespread availability, excellent imaging properties, and favorable dosimetry. In addition, Tc-99m can also be obtained at high specific activity. There are a number of reports in the literature which show a variety of approaches to radiolabeling proteins with Tc-99m. These include both *direct* and *indirect* methods.

In *direct* labeling methods, technetium is reduced in the presence of the protein or peptide and binds to amino acid residues or functional groups. This approach assumes the protein or peptide will provide a stable reproducible coordination environment for Tc-99m. In general, $TcO_4^-$ is reduced in the presence of the protein or peptide and unbound Tc-99m is removed by chromatographic methods (21). A more recent approach for labeling immunoglobulins with Tc-99m has been to utilize the thiol groups present in the protein as Tc-99m affine coordinating atoms. The supposition is that disulfide reduction liberates free sulfhydryls which preferentially bind Tc-99m when presented as a weak complex (e.g., Tc-99m glucoheptonate) (22). In general, *direct* labeling is complicated by the tendency of technetium to form unstable complexes, and there is poor control over the

labeling sites (23). Despite its limitations, *direct* labeling has proved popular for the preparation of Tc-99m antibodies.

The *direct* approach is unlikely to be broadly applicable to the labeling of peptides for several reasons. The need for Tc-99m to stabilize its bonding with multiple coordination sites would result in atoms from several amino acids being bound to the metal. This would restrict the three-dimensional flexibility of the peptide and possibly inhibit the optimal receptor-binding conformation. In addition, most small peptides do not contain disulfides and, although the secondary and tertiary structures of proteins and antibodies are stabilized by multiple non-covalent interactions, this is not the case with peptides. In many peptides that contain disulfide bridges slight alterations in ring structure can result in dramatic alterations in biological activity. Small cyclic peptides, such as oxytocin and vasopressin, exhibit a marked decrease in biological activity with increasing ring size. In an effort to prepare Tc-99m octreotide it has been shown that *direct* labeling via disulfide reduction reduces receptor affinity by four orders of magnitude (24) thereby confirming that alterations of the cyclic nature of this peptide dramatically reduces biological activity. Irrespective of these considerations, *direct* labeling of peptides has been reported for several peptides, including thyrotropin-releasing hormone (TRH) (25) and, more recently, for a nineteen-residue analog of laminin (PA22-2) (26). In neither case were receptor binding studies reported indicating the effect of labeling on affinity.

In order to overcome the problems associated with *direct* labeling, methods have been developed which utilize high affinity bifunctional chelates to bind technetium. The *indirect* or bifunctional methods employ metal chelating compounds which are able to be covalently attached to the peptide through a reactive side group and bind technetium with available coordinating groups. These chelates can be used in one of two ways. In the first method, the bifunctional reagent is pre-labeled with Tc-99m and then conjugated to the peptide in a manner similar to the Bolton-Hunter iodinating reagent. The second method utilizes the chelating agent as part of the overall peptide sequence and radiolabeling takes place after peptide purification. Both methods have been highly successful for radiolabeling antibodies and could be directly applied to large peptides. To achieve high specific activity in small peptides, both the unreacted labeling reagent and unlabeled peptide must be removed from the reaction mixture when the first method is used. Since these species can be similar in size to the labeled peptide, chromatographic purification may not be straightforward. In addition, if more than one reactive group is present on the peptide, multiple species can result. Theoretically, the *indirect* method should yield only a single radiolabeled species, and chromatographic separation is usually simpler.

Such bifunctional chelating agents include DTPA (27), $N_2S_2$(28), $N_3S$ (29), BATO (30) and 6-hydrazinonicotinate (31). Radiolabeling is achieved by incubating the chelate-peptide conjugate with a less stable complex of Tc-99m such a Tc-99m glucoheptonate. The more stable complex is favored and the Tc-99m is transferred to the chelating group of the peptide-conjugate. Recently, Tc-99m labeling of several peptides has been reported using a variety of methods, including HYNIC (32) and diaminedithiol (33) derivatized chemotactic peptides for infection imaging. The HYNIC and diaminedithiol derivatized chemotactic peptides demonstrated biological activity and receptor binding characteristics similar to the unmodified peptide. The use of $N_2S_2$ and $N_3S$ for generating analogs of the apolipoprotein SP-4 for imaging atherosclerosis has also been reported (34).

Theoretically, *indirect* labeling of the peptide using bifunctional chelators affords a preferable product over that achieved with *direct* labeling. The chemistry of Tc-99m labeling is better defined and more predictable using the *indirect* or bifunctional chelate method.

**Indium-111 and Gallium-68**

Indium-111 is a Group III metal which possesses a chemistry and half-life which makes it ideal for radiolabeling intact immunoglobulin. This is due to the prolonged pharmacokinetics of these proteins and the ease of achieving rapid, stable labeling using bifunctionalized derivatives of the polyamino-polycarboxylate chelates, EDTA (35) and DTPA (36). DTPA has been used as a bifunctional chelating agent for radiolabeling alpha-melanocyte stimulating hormone (alpha-MSH) with Indium-111 for imaging melanoma (37), labeling formylated chemotactic peptides for infection imaging (38), labeling laminin fragments for targeting tumor-associated laminin-receptors (39), and labeling atrial natriuretic peptide (ANP) for imaging ANP receptors in the kidney (40). The positron-emitting isotope, Gallium-68 has been used for radiolabeling chemotactic peptides (41) and a somatostatin analog (42), using DTPA and desferrioxamine as bifunctional chelates, respectively.

**Fluorine-18**

In an effort to utilize positron emission tomography (PET) for peptide imaging, a number of reagents have been described. Proteins have been radiolabeled with $^{18}F$ using N-succinimidyl-4-[$^{18}F$]fluorobenzoate (43), methyl 3-[$^{18}F$]-5-nitrobenzimidate and 4-[$^{18}F$]fluoroacyl bromide (44) and more recently, N-succinimidyl-4-

(fluorobenzylamino) suberate (45) and [$^{18}$F]fluoropropionic acid and [$^{18}$F]fluoroacetic acid derivatives (46). Reports of $^{18}$F labeled peptides include $^{18}$F insulin labeled via a 4-(bromomethyl)benzoyl amine intermediate (47), $^{18}$F-fluoroproprionyl octreotide (48), and most recently, 4-[$^{18}$F]fluorobenzoate conjugated chemotactic peptide (49).

## BIOLOGICAL ROLE OF PEPTIDES

Naturally-occuring peptides function primarily in cell-signaling. The need for signaling or communication between cells comes from the need to regulate development and organize tissues, as well as to control growth and division and coordinate function. This communication can occur in three ways. One is remote signaling by secreted molecules, the second is contact signaling between plasma membranes of adjacent cells, and the third, is synaptic signaling, again between adjacent cells.

Types of remote signaling differ in the distance in which they operate. The first, endocrine signaling, occurs when hormones are secreted from a tissue and travel throughout the body allowing their messenger molecules to target cells in distant areas of the body. The second, paracrine signaling, occurs when cells secrete local chemical mediators which are so rapidly taken up and metabolized or immobilized that their actions affect cells only within a few millimeters of where they are secreted. The third, synaptic signaling, is confined to the nervous system. Cells secrete neurotransmitters--the chemical messengers which diffuse across a synaptic cleft and act only on the adjacent cells. Again, rapid immobilization or degradation of the neurotransmitter limits these compounds from acting in distant cells. In all cases, the chemical signal interacts with the receptor protein, which initiates the response.

In general, hormones are greatly diluted in the bloodstream and therefore must be able to act at very low concentrations (less than $10^{-8}$ M). Neurotransmitters are much less diluted and can achieve local concentrations approaching 5 x $10^{-4}$ M. Synaptic receptors have relatively low affinity, and as a result do not respond significantly to the very low concentrations of transmitter that may reach them via diffusion from remote sites. Peptide hormones act in a paracrine fashion as neurotransmitters. Most hormones and chemical mediators are water soluble.

## CLASSES OF PEPTIDES FOR IMAGING

For the sake of discusson we can broadly classify peptides in two ways based on the origin of the initial amino acid sequence of interest. The first group would include naturally occuring proteins and peptides acting in a cell signaling capacity (i.e., hormone, growth factors, neurotransmitters, etc.). These peptides act upon naturally-occurring target molecules such as receptors and would also include analogs of naturally-occurring peptides such as the somatostatin analog octreotide. The second group would be comprised of peptide analogs of immunoglobulins or MAbs. These would include low molecular weight fragments of MAbs, peptide analogs of MAb antigen-binding regions, as well as synthetically-derived peptides of epitope-binding region or hypervariable region peptides of MAbs.

Radiolabeled peptides targeted to the various receptors or cell membrane target proteins may allow for:

1. characterization of tumor biochemistry (receptor status)
2. tumor detection and staging (location and extent)
3. screening (at presentation for extent of disease and follow up)
4. receptor-guided delivery of cytotoxins (e.g., radionuclides, drugs, toxins)
5. therapeutic monitoring (of conventional or biological therapies)

In addition, therapeutic approaches involving the inhibition or disruption of autocrine growth factors could be studied in detail with radiolabeled peptide probes.

### Natural Biologically Active Peptides

In contrast to antibodies, low and intermediate molecular weight biologic peptides may be more suitable starting points for radiopharmaceutical development. Peptides are necessary elements in a variety of biologic processes, functioning as hormones, neurotransmitters, neuromodulators, growth and growth inhibition factors, and cytokines. In many cases, peptides display affinities for their receptors that are significantly greater than that of monovalent antibody fragments.

The molecular weights of biologically-active peptides are extremely diverse, ranging from 3-5 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 like 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.

**Single Chain Antigen Binding Proteins**

The large three-dimensional structure of immunoglobulins allows for a variety of functions, in addition to antigen recognition or binding. The variable region of the immunoglobulin's heavy and light chains make up the antigen recognition portion of the protein, whereas, the constant region (predominantly the Fc region) of the immunoglogulin confers its effector function. It should be noted that the molecular sequences recognized by antibodies are relatively small sequences (5-6 mer) of carbohydrates or amino acids. Hence, stripping down the antibody to its antigen binding site only would greatly reduce its molecular bulk as well as other biological functions not neccessarily related to antigen binding. Such approaches to improving MAb applications to imaging have been reported with some encouraging results.

Recently, it has been suggested that imaging with radiolabeled Fv fragments which are about 50% smaller than Fab's, 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 (50), most single-chain binding proteins (sFv's) have been prepared by recombinant DNA techniques (51-55). 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 variable region of the light chain is linked to the amino terminus of variable region of the heavy chain by a 12-20 residue peptide. These proteins display similar antigen specificity as the parent antibody. Recent studies with these sFv's showed rapid tumor penetration (53, 56,57). For example, with the pancarcinoma antibody CC49, the time for maximal penetration of sFv is 30 min while intact IgG required 48-95 hr to attain similar concentrations. Autoradiographic studies comparing the penetration of intact IgG with $F(ab)_2$, Fab and sFv's revealed that sFv's distribute uniformly in tumors while intact antibodies concentrate in the region of, or immediately adjacent to, the blood vessel. The distributions of $F(ab)_2$ and Fab fragments showed intermediate penetration in a size related manner (56,57). When applied to *in vivo* imaging, sFv's clear rapidly from the circulation ($t_{1/2}$ 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 (53,58,59). Although in some cases absolute levels of accumulation in target tissues were lower than with larger fragments, target-to-background ratios were 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 sFv's. 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 (60). Similarly, by using nuclear magnetic resonance analysis in conjunction with molecular modeling, it has been possible to determine the effects of specific amino acid residues on antigen binding (61). Based on this type of information, peptides with improved binding properties have been designed and synthesized. With these techniques, it may be possible to modify the peptides to optimize biodistribution and pharmacokinetics. Unfortunately, these strategies are complex and require detailed knowledge of the three-dimensional structure of the antibodies and peptides. One important application of hypervariable region peptides to *in vivo* imaging has already been reported for thrombus imaging (62).

## CURRENT PEPTIDE RADIOPHARMACEUTICALS

Numerous biologically-active peptides have potential as imaging agents. Although imaging applications of these reagents are still relatively new, many important imaging agents are currently under development. The growing interest in peptides may be due in large part to the successful clinical application of the neuropeptide, somatostatin and its analogs(63). In the following sections, we will review the current status of radionuclide imaging with biologically-active peptides.

## TUMOR IMAGING

### Somatostatin Receptor Imaging with In-111 Pentetreotide

***Development.*** Although In-111 pentetreotide was only recently approved by the USFDA for clinical use (6/2/94), research which led to the compound's development began over twenty years ago with the isolation of a polypeptide from the hypothalamus of several animals (64). That same year, the peptide, named somatostatin because it inhibited the secretion of somatotropin from the pituitary gland, was structurally identified and synthesized (65,66). Years of research has shown the endogenous hormone somatostatin to process multiple functions in the endocrine homeostasis of the human body. Table 1 illustrates the various organs and body functions influenced by the naturally-occurring neuropeptide.

**Table 1. Effects of Somatostatin on Organ Systems**

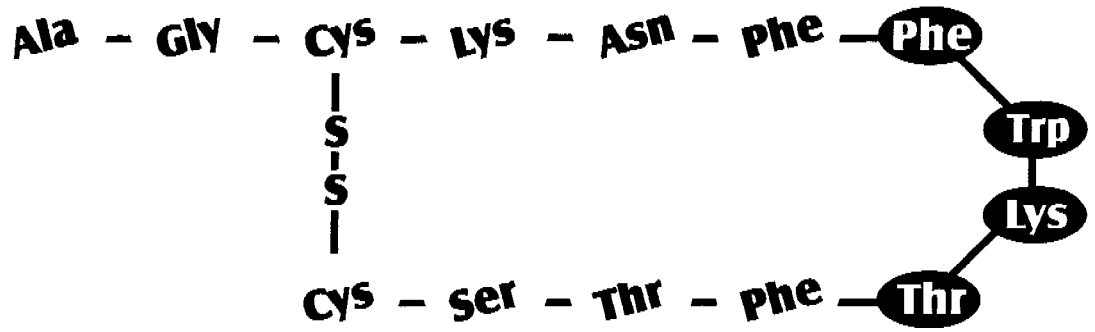
| Organ System | Effect |
|---|---|
| Anterior Pituitary Gland | Inhibition of secretion (growth hormone, thyrotropin) |
| Gastrointestinal Tract | Inhibition of gut hormone secretion, absorption, motility |
| Pancreas | Inhibition of secretion (insulin, glucagon, enzymes) |
| Liver | Decreased blood flow, inhibition of secretion of bile acid |
| Kidney | Fluid-electrolyte balance |

Adapted from O'Dorisio, Wood, O'Dorisio (Reference 81)

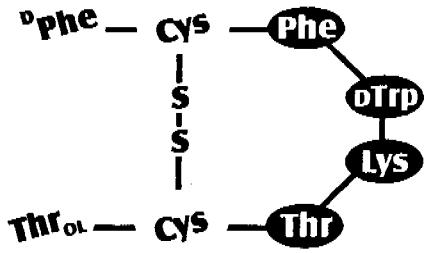
The promise of inhibiting growth hormone therapeutically led to the characterization and synthesis of the 14 amino acid structure seen in Figure 1A. Somatostatin had limited therapeutic value because of a short (2-3 minute) biological half-life. A long-acting analog of somatostatin was synthesized to use as a therapeutic agent to inhibit hypersecretion of hormones from a variety of neuroendocrine tumors (67). The octreotide analog, shown in Figure 1B, has only eight amino acids but maintains the sequence of Phe-Trp-Lys-Thr found essential for function. In addition, alteration of the terminal ends of the peptide led to longer circulation times and prolonged biologic activity.

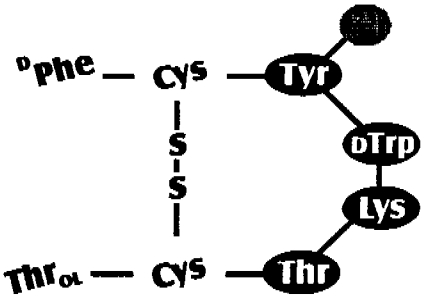
*In vitro* binding studies were conducted by radiolabeling somatostatin and octreotide with iodine-125 (68). The feasibility of nuclear medicine imaging of somatostatin receptor-containing neuroendocrine tumors was first shown using Iodine-123 $Tyr^3$-Octreotide (see Figure 1C), in which the third amino acid was changed from Phe to Tyr for subsequent iodination (63). Although I-123 provides excellent nuclear properties for external imaging, accelerator-production with its associated limited availability of high purity I-123 necessary for optimal radioiodination of $Tyr^3$-Octreotide, created difficulties in production. Clinical studies showed I-123 $Tyr^3$-Octreotide was cleared rapidly by the hepatobiliary system leading to excretion and accumulation in the intestines which caused difficulty interpreting abdominal and pelvic images (63). To overcome the production difficulties and problematic biodistribution patterns of I-123 $Tyr^3$-Octreotide, radioactive metal labeling of octreotide via chelation was introduced (70) Starting with octreotide, the DTPA moiety is attached to the d-phenylalanyl group at the N-terminus (Figure 1D). Four -COOH groups from DTPA are available for forming a metal-binding complex with the radioactive metal, indium-111. Attaching the large chelate group at the farthest distance from the receptor binding portion of the compound allowed the indium-111 radiolabeled species to retain the ability to bind somatostatin receptors. Indium-111 DTPA-D-Phe1-Octreotide (In-111 pentetreotide) has a longer physiologic half-life than the I-123 $Tyr^3$-Octreotide allowing more time for tumor localization. The longer physical half-life of In-111 allows delayed imaging. In-111 pentetreotide not bound to receptor sites is rapidly cleared through the kidneys reducing non-specific uptake and increasing the target to background ratios (T/S).

***Product Description.*** In-111 pentetreotide (OctreoScan®-Mallinckrodt) is a radiolabeled analog of somatostatin supplied as a single dose kit. Each kit includes a 10ml reaction vial containing a lyophilized mixture of 10$\mu$g pentetreotide, 2 mg gentisic acid, 4.9 mg anhydrous trisodium citrate, 0.37mg anhydrous citric acid, and 10 mg inositol. A second 10 ml vial contains In-111 chloride sterile solution in 1.1 ml of dilute (0.02N) hydrochloric acid (3mCi/ml In-111 chloride at calibration). The vial containing the In-111 also has 3.5$\mu$g/ml of ferric chloride. To complete the radiolabeling step, the contents of the In-111 vial is added to the reaction vial using the stainless

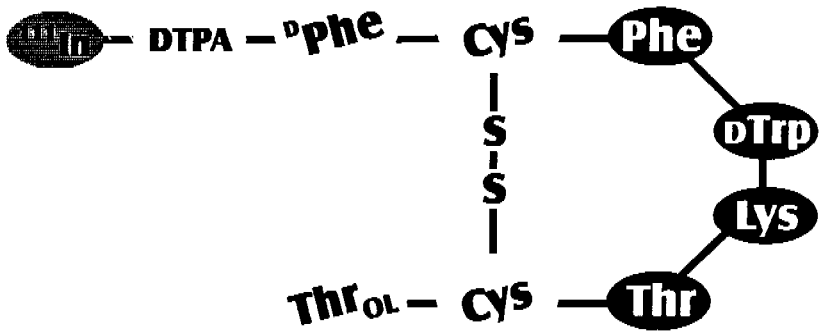



**A Somatostatin**




**B Octreotide** **C $^{123}$I Labeled Tyr$^{3}$-Octreotide**




**D Indium $^{111}$In Pentetreotide**

**Figure 1.** Somatostatin and its analogs. A. Native, human somatostatin. B. Synthetic analog of somatostatin with prolonged biologic activity used for control of symptoms associated with neuroendocrine tumors. C. Iodine-123 labeled somatostatin derivative first used for nuclear medicine imaging. D. Indium In-111 pentetreotide (OctreoScan®-Mallinckrodt Medical, Inc.).

**Table 2. Radiolabeling of OctreoScan® (Indium In-111 Pentetreotide).**

*Note: Read complete directions thoroughly before starting preparation.*

The Kit Contains:

One 10-ml reaction vial containing 10μg pentetreotide, 2mg gentisic acid, 4.9mg trisodium citrate, 0.37mg citric acid and 10mg inositol.

One 10-ml vial containing 1.1ml of 3.0 mCi/ml In-111 chloride in 0.02N HCl.

One 25G x 5/8" stainless steel needle.

One package insert and label.

**Procedure Precautions and Notes**

1. All transfers and penetrations of the vial stoppers by a needle must use aseptic technique.
2. Wear waterproof gloves during the entire procedure and while withdrawing the patient dosage from the OctreoScan® reaction Vial.
3. Transfer In-111 chloride sterile solution with an adequately-shielded, sterile syringe using the transfer needle in the kit.
4. Adequate shielding should be maintained at all times until the preparation is administered to the patient, disposed of in an approved manner, or allowed to decay to safe levels of radioactivity. A shielded, sterile syringe should be used for withdrawing and injecting the preparation.
5. Do not inject into TPN administration bags or associated intravenous lines.

**Procedure for the Preparation of Indium In-111 Pentetreotide**

1. Place the OctreoScan® reaction vial in a lead dispensing shield.
2. Swab the rubber stopper of the reaction vial with an appropriate antiseptic and allow the vial to dry.
3. Aseptically remove the contents of the In-111 chloride sterile solution vial using the needle provided and a shielded, sterile syringe.
4. Inject the In-111 chloride sterile solution into the OctreoScan® reaction vial.
5. Gently swirl the OctreoScan® reaction vial until the lyophilized pellet is completely dissolved.
6. Incubate the In-111 pentetreotide solution at or below 25°C (77°F) for a minimum of 30 minutes. Note: A 30-minute incubation time is required. Shorter incubation periods may result in inadequate labeling.
7. Using proper shielding, visually inspect the vial contents. The solution should be clear, colorless, and free of particulate matter. If not, the solution should not be used. It should be disposed of in a safe and approved manner.
8. Assay the In-111 pentetreotide solution using a suitably calibrated ionization chamber. Record the date, time, total activity, and patient identifier (e.g., patient name and number) on the radioassay information label and affix the label to the lead dispensing shield.
9. The labeling yield of the reconstituted solution should be checked before administration to the patient, according to the instructions given in Table 3. If the radiochemical purity is less than 90%, the product should not be used.
10. Store the reaction vial containing the In-111 pentetreotide solution at or below 25°C (77°F) until use. The In-111 pentetreotide must be used within six hours of preparation.
11. If desired, the preparation can be diluted to a maximum volume of 3mL with 0.9% Sodium Chloride Injection, U.S.P. immediately prior to injection. The sample should be drawn up into a shielded, sterile syringe and administered to the patient.

**Table 3. Recommended Method for Determination of Labeling Yield of Indium In-111 Pentetreotide**

**Required Materials**

1. Waters (Division of Millipore) Sep-Pak® C18 Cartridge, Part No. 51910.
2. Methanol, 15mL (Caution: toxic and flammable. Exercise due caution)
3. Distilled water, 20mL
4. Disposable syringes:
   2-10mL, no needle required
   2-5mL, no needled required
   1-1mL, with needle
5. Three disposable culture tubes or vials, minimum 10mL capacity
6. Ion Chamber

**Preparation of the Sep-Pak Cartridge**

1. Rinse the Sep-Pak cartridge with 10mL methanol as follows:
   Fill a 10mL syringe with 10mL methanol, attach the syringe to the longer end of the Sep-Pak cartridge, and push the methanol through the cartridge. Discard the eluate in a safe and approved manner.
2. Similarly, rinse the cartridge with 10mL water. Ensure that the cartridge is kept wet and that there is no air bubble present. If an air bubble is present, rinse the cartridge with additional 5mL of water. Discard the elute.

**Sample Analysis**

1. Using a 1mL syringe with needle, withdraw 0.05 - 0.1 mL In-111 pentetreotide from the OctreoScan® reaction vial. Apply the preparation to the Sep-Pak cartridge through the longer end of the cartridge. Make sure that the sample is migrating on the column of the cartridge. Note: After this step, the cartridge and all solutions eluted from it will be radioactive.
2. With a disposable 5mL syringe, slowly (in dropwise manner) push 5mL water through the longer end of the cartridge, collecting the eluate in a counting vial or tube. Label this eluate as "Fraction 1."
3. Similarly, elute the cartridge with 5mL methanol. Be sure that this solution is pushed slowly through the longer end of the cartridge so that the elution occurs in a dropwise manner. Collect this fraction in a second culture tube or vial for counting. Label it as "Fraction 2." Push two 5mL portions of air through the longer end of the cartridge and collect the eluate with Fraction 2.
4. Place the Sep-Pak cartridge in a third culture tube or vial for assay.

**Assay**

1. Assay the activity of Fraction 1 in a suitably-calibrated ionization chamber. This fraction contains the hydrophilic impurities (e.g., unbound In-111).
2. Assay the activity of Fraction 2. This fraction contains the In-111 pentetreotide.
3. Assay the activity of the Sep-Pak cartridge. This component contains the remaining non-elutable impurities.
4. Dispose of all of the materials used in the preparation, the sample analysis, and the assay in a safe and approved manner.

**Calculations**

1. Percent In-111 pentetreotide = (Fraction 2 activity/Total activity) X 100%
   Where Total activity = Fraction 1 + Fraction 2 + activity remaining in Sep-Pak
   Note: If this value is less than 90%, do not use the preparation. Discard it in a safe and approved manner.
2. Percent hydrophilic impurities = (Fraction 1 activity/Total activity) X 100%
3. Percent non-elutable impurities = (Activity remaining in Sep-Pak cartridge /Total activity) X 100%

steel needle provided in the kit (See Table 2). After an incubation period of 30 minutes at room temperature, the finished In-111 pentetreotide can be diluted to a maximum volume of 3 ml with 0.9% Sodium Chloride for Injection USP prior to quality assessment (see Table 3) and patient infusion which must be done within six hours of preparation.

In addition to the radiolabeled somatostatin receptor binding peptide, the finished product contains citrate as a buffer and inositol and gentisic acid as stabilizers.

***Clinical Pharmacology and Pharmacokinetics.*** Pentetreotide is a long-acting, cyclic octapeptide analog of the human hormone, somatostatin, that has been coupled with a DTPA moiety. The DTPA chelate enables radiolabeling with In-111 while the octapeptide analog confers somatostatin receptor recognition and binding.

In-111 pentetreotide binds to somatostatin receptors on cell surfaces throughout the body, concentrating in tumors that contain a high density of somatostatin receptors such as those listed in Table 4. Within an hour of injection, the radiopharmaceutical primarily distributes from plasma to extravascular body tissues. Visualization of somatostatin receptor-rich tissues including normal pituitary, thyroid, liver, spleen and urinary bladder is seen (71). Lymphocytes are also thought to possess somatostatin receptors which led to clinical investigation of the usefulness of the radiopharmaceutical to detect malignant lymphomas (71). The 10$\mu$g of pentetreotide found in the radiopharmaceutical is 5-20 times less than that of therapeutic dosages of octreotide and is not expected to exact clinically-significant somatostatin-like effects (72).

**Table 4. Neuroendocrine tumors known to have somatostatin receptors.**

| |
|---|
| Gastrinoma |
| Insulinoma |
| Carcinoid |
| Small Cell Lung Cancer |
| Paraganglioma |
| Glucagonoma |
| Neuroblastoma |
| Pheochromocytoma |
| Pituitary Tumors |
| Medullary Thyroid Carcinoma |
| Lymphomas |
| Meningiomas |
| Astrocytomas |
| Merkel Cell Tumors |

After intravenous injection, In-111 pentetreotide radioactivity leaves the plasma rapidly with only one-third of the administered dosage remaining in the circulation at ten minutes post-infusion. Blood levels continue to drop with less than 1% of the radioactive dose found in the blood pool twenty hours post-infusion (72). Radioactivity levels in the liver and spleen remain low (2-3%) over a 48-hour period. Despite this, the spleen is the organ that receives the highest radiation absorbed dose (2.46 rads/mCi). Imaging of intestinal tumors is aided by the use of laxatives to diminish abdominal background radioactivity from the small amount of hepatobiliary clearance of the drug.

In-111 pentetreotide clearance is primarily by renal excretion with 25% of the injected dosage recovered in urine at three hours, 50% at six hours, 85% at 24 hours and over 90% by two days (72). The biological half-life of In-111 pentetreotide is approximately six hours. This rapid biodistribution and systemic clearance coupled with urinary excretion lead to high T/B ratios for imaging. Hepatobiliary excretion accounts for less than 2% of the injected dosage over three days. *In vivo* stability studies indicate that plasma and urine radioactivity is predominantly in the parent form (72).

***Approved Indications and Potential Uses.*** In-111 pentetreotide is indicated for the scintigraphic localization of primary and metastatic neuroendocrine tumors bearing somatostatin receptors (72). Table 4 lists those neuroendocrine tumors that have been localized in clinical investigations. In addition, breast cancer, non-Hodgkin's lymphomas, Hodgkin's disease, sarcoidosis, tuberculosis, and gliomas have been visualized with In-111 pentetreotide scintigraphy (71,74)

High dose octreotide therapy may lead to tumor regression in acromegaly, islet cell tumors, and metastatic carcinoids (75). In-111 pentetreotide scintigraphy has been used to identify those patients with uptake at somatostatin receptors, and therefore, more likely to respond to octreotide therapy for tumor regression and control of hormonal hypersecretion syndromes such as secretory diarrhea. Patients with negative studies would be spared the expense and bothersome daily injections of octreotide.

***Clinical Efficacy.*** In-111 pentetreotide is the first drug approved by the FDA based entirely on European trial data (76). Clinical studies totaling 365 patients indicated greater than 86% success in detecting neuroendocrine tumors. A subgroup of 39 patients evaluated with tissue confirmation provided a sensitivity and specificity rates of 85.7% and 50%, respectively, for In-111 pentetreotide imaging compared with 68% sensitivity and 12% specificity for CT/MRI.

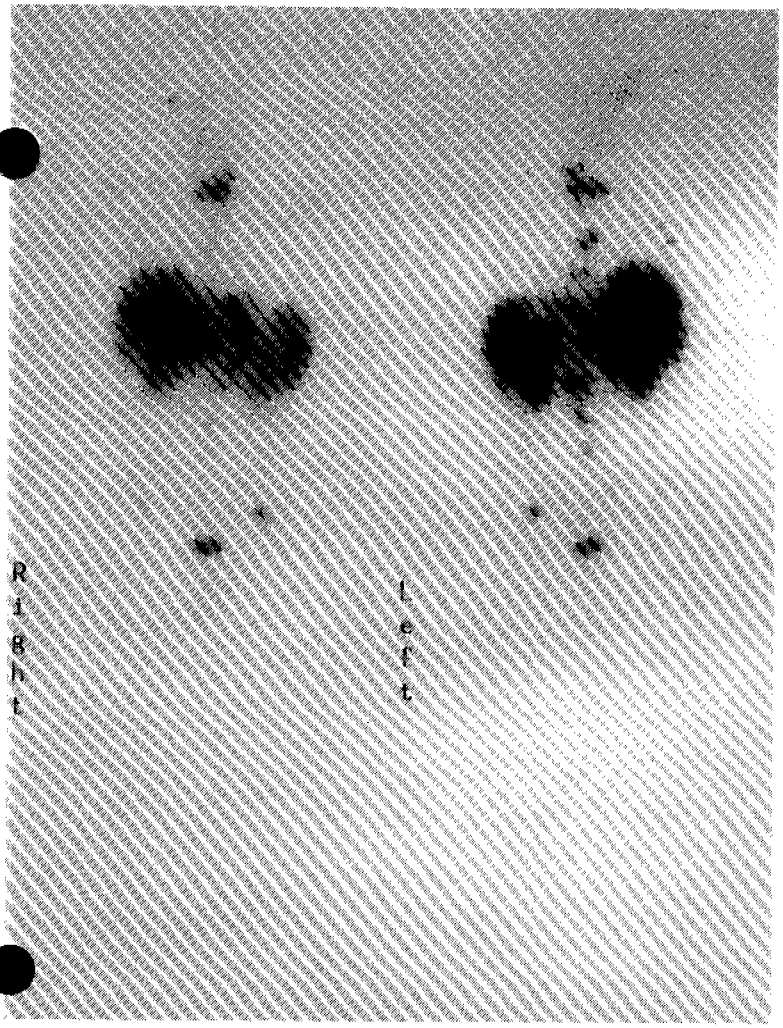



**Figure 2.** Whole body anterior (on left) and posterior (on right) images of a 43 y.o. male with metastatic VIPoma with unknown primary cancer taken 24 hours after infusion with 6.6mCi of In-111 pentetreotide. Patient was receiving 150$\mu$gm of octreotide three times daily for symptomatic control. This study was conducted to determine if all lesions were somatostatin-receptor positive. Along with normal uptake in the kidneys, liver, spleen, and thyroid, there are numerous lesions seen in the thoracic, lumbar, and cervical spinal skeleton, as well as ribs, pelvis, and right side of the head. In addition, there are numerous lesions seen in the liver and the subhepatic region.

### Vasoactive Intestinal Peptide (VIP)

The neuropeptide vasoactive intestinal peptide (VIP) has recently been shown to be a promising agent for tumor imaging (77). VIP is a 28 amino acid peptide (3326 daltons) of the glucagon-secretin family, first characterized from porcine duodenum (78). VIP has a range of biological activities depending on the target tissue. First characterized as a vasodilatory agent with potent effects on peripheral and pulmonary blood pressures (75), this peptide also stimulates secretion of various hormones (79,80). VIP also acts as an immunomodulator (81) and promotes growth and proliferation of normal and malignant cells (82).

VIP receptors are found on cell surface membranes throughout the body, peripheral blood cells (83), the gastrointestinal tract (84) lungs (85) and kidneys (86). Recently, VIP receptors were identified on various human tumors in amounts exceeding that of peripheral blood cells (87). This finding led to an initial study of I-123 labeled VIP in 79 patients with various tumors. I-123 VIP showed good sensitivity for primary and recurrent colorectal, pancreatic and gastric adenocarcinoma as well as detecting liver and lymph node metastases. This study also compared I-125 octreotide in 38 patients. VIP was superior to I-125 octreotide in detecting adenocarcinomas and was equivalent in detecting intestinal endocrine tumors.

Subsequent to these findings, Reubi evaluated the presence of VIP receptors on a variety of human cancers (88). Over three hundred tumors were studied for VIP receptor expression by *in vitro* receptor autoradiography. For comparison somatostatin receptors were also studied in the same tissue samples using I-125-[Tyr$^3$]-octreotide. The results of this study showed a high incidence of VIP receptors present on numerous tumor types as described in Table 5. However, VIP receptors were found on only half of undifferentiated pheochromocytomas, small-cell lung cancers, neuroblastomas and gastroentero-pancreatic tumors.

**Table 5. TUMORS FOUND TO EXPRESS VIP RECEPTORS BY AUTORADIOGRAPHIC MEANS.**

| Tumor Type |
|---|
| Carcinomas |
| breast |
| endometrium |
| prostate (and prostate cancer metastasis) |
| bladder |
| Adenocarcinomas |
| ovarian |
| colonic |
| pancreatic |
| Other |
| gastrointestinal squamous cell carcinoma |
| non small cell lung cancers |
| lymphomas |
| astrocytomas |
| glioblastomas |
| meningiomas |
| All differentiated neuroendocrine tumors. |

When compared with the results obtained with I-125 [Tyr3] octreotide, significantly more tumors were

found to express VIP receptors than somatostatin receptors. These data suggest a strong likelihood that VIP receptor targeting may be useful for tumor imaging *in vivo*.

## INFECTION IMAGING

Despite the success of several agents for imaging infection, in most cases localization requires 24 hours before lesions can be visualized. This is a serious clinical deficiency. The problems of the prolonged interval between *in vitro* cell labeling, injection, and lesion detection could be reduced by imaging with an agent capable of binding *in vivo* to both circulating white blood cells (WBCs) as well as WBCs already present at the site of inflammation. Candidate molecules with these characteristics include: Interleukin-8 (89), platelet factor-4 (90) and the peptide, N-formyl-methionyl-leucyl-phenylalanine (Formyl-MLF) (91-93). Of these potential agents, Formyl-MLF is particularly promising. The following is a review of the development of chemotactic peptides as new agents for imaging infection.

### Chemotactic peptides

Leukocytes follow a chemoattractant signal to migrate to focal sites of inflammation. Formyl-MLF is a bacterial product that initiates leukocyte chemotaxis by binding to high-affinity receptors on the white blood cell membranes. These receptors are present on both polymorphonuclear leukocytes and mononuclear phagocytes. As granulocytes respond to a chemoattractant gradient, the affinity of the receptors decreases as additional receptors are expressed, until the site of inflammation is reached (94-96). It has been previously demonstrated that many synthetic analogs of Formyl-MLF bind to neutrophils and macrophages with equal or greater affinity compared to the native peptide (92,97,98). Due to the very small molecular size of Formyl-MLF (M.W. 437), its molecular structure can be readily manipulated to design an optimal imaging agent.

The first use of a radiolabeled chemotactic peptide for abscess localization was reported in 1982 (99,100). Unfortunately, the radiolabeling methods available at the time of these studies yielded agents of relatively low specific activity, requiring pharmacological amounts of peptide for imaging. These doses of peptide were shown to produce profound transient reductions in peripheral leukocyte levels in rabbits and dogs (100-104).

Chemotactic peptides of the class formyl-MLF are somewhat ideal for developing into radiodiagnostics. It is known that C-terminal modification can be done without significantly altering the binding properties of the peptide. This has led investigators to derivatize the sequence by adding C-terminal groups that contain a free amino group (e.g., Lys or alkyldiamine) (105). A small series of such peptides have been prepared and derivatized with DTPA for labeling with In-111. These peptides maintained their ability to bind to the Formyl-MLF receptor (7-50 nM affinity) and maintain biological activity (3-150 nM for $O_2^-$ production). Upon labeling, the agents were shown to localize in intramuscular injections in a rat model. Accumulation of the radiolabel was rapid as was blood clearance. Such properties suggested that Tc-99m would be better suited for this application than In-111.

Recently, the preparation of hydrazino nicotinamide (HYNIC) derivatives of a series of chemotactic peptides has been reported, based on Formyl-MLFK (106). HYNIC had been previously shown to be a facile and stable bifunctional chelating agent for Tc-99m (107). Peptides with HYNIC covalently linked through a C-terminal amide bond maintained biological activity ($EC_{50}$ 's:12-500 nM for $O_2^-$ production) and receptor binding ($EC_{50's}$: 0.12 - 40 nM binding affinity). Labeling was achieved by incubation of the peptide in acetate buffer with equal volume of Tc-99m glucoheptonate. Using reverse phase HPLC, labeled peptide can be separated from unlabeled peptides and resulting specific activities > 10,000 mCi/$\mu$Mole could be obtained. The Tc-labeled peptides retained receptor binding with $EC_{50}$ < 10 nM. Biodistribution of the individual peptides were similar with low levels of accumulation in most normal tissues. In rats, all of the peptides concentrated at the site of infections (T/B = 2.5-3.0:1) by one hour. In rabbits outstanding images of infection were obtained with T/B ratios > 20:1 at 16 hr. after injection.

In an effort to more clearly define the mechanisms of localization and the relative ability of Tc-labeled chemotactic peptides to image infection, the biodistribution and infection imaging properties of Tc-99m-labeled-Formyl-Met-Leu-Phe-Lys-HYNIC (Tc-99m HP) were compared with In-111-labeled leukocytes (In-111 WBC's) in rabbits with intramuscular *E. coli* infections (108). After co-injection, the distributions of Tc-99m HP and In-111 WBCs were similar and the sites of infection were well visualized with both radiopharmaceuticals. The T/B ratios of the labeled peptides were approximately three times greater on average than that of In-111 WBC. Absolute accumulation at the site of infection was responsible for the significantly higher T/B calculated from direct tissue sampling (33:1 vs. 8:1 for Tc peptide and In-WBC, respectively). Activity in normal tissue (skeletal muscle) was similar for both agents.

Blood fractionation studies demonstrated that, only ~ 25% of the circulating Tc-99m radioactivity was

associated with WBC's, whereas, greater than 85% of the circulating In-111 radioactivity was cell associated. For both radionuclides binding to red blood cells was minimal (<2%). In contrast, fractionation of the pus demonstrated that for both Tc-99m and In-111, approximately 90 % of the radioactivity was associated with WBC's. Column chromatography of the plasma demonstrated that >95% of the plasma associated Tc-99m radioactivity eluted at an apparent molecular weight of ~150,000 (IgG fraction) with no radioactivity detected at the elution position of the free peptide.

Further studies (109) comparing Tc-99m peptide and In-111 DTPA-IgG in rabbits with *E. coli* infection indicated that Tc-99m-labeled chemotactic peptides localize to a significantly greater extent than In-111 IgG and the correlation between the degree of localization of these two tracers is not statistically significant, suggesting that non-specific mechanisms do not play a major role in accumulation of Tc-99m peptide at sites of infection. At all imaging times, the T/B for the peptide was significantly higher than for IgG and much lower levels of peptide accumulated in non-target tissues.

As chemotactic peptides are known to produce transient neutropenia in rabbits and dogs, the dose-response of Formyl-NleLFYK-DTPA in monkeys has been evaluated (110). The peptide induced a clear dose-dependent reduction in peripheral leukocyte levels in the animals. The reduction in leukocyte number occurred almost immediately after injection and rapidly returned to baseline. No significant effects on differential white blood count, blood pressure, pulse rate, or respiration rate were detected. Despite these results, the fact that HYNIC-conjugated chemotactic peptides can be labeled at high specific activities (>10,000 mCi/$\mu$Mole) allows imaging experiments to be perfomed at doses of cold peptide that are more than 1,000-fold lower than the dose that elicits a significant but transient reduction in white blood cell count. This finding supports the safety of these compounds for use in humans when labeled at high specific activities.

The data presented to date indicate that Tc-99m labeled chemotactic peptides possess several characteristics which make them attractive for infection imaging. Recent data has also shown that Tc-99m labeled chemotactic peptides are capable of high quality visualization of infection in both dogs (111) and primates (112). Further efforts in this area should lead to clinically-usable infection imaging agents.

## THROMBUS IMAGING

Vascular thrombosis occurs in about 2.5 million people annually in the United States alone. Thrombosis formation is frequently associated with patients who undergo hip replacement or suffer from hip fracture. Vascular thrombosis can lead to pulmonary embolism, a potentially lethal condition. New ways of detecting vascular thrombosis or deep venous thrombosis (DVT) are needed. Two methods now widely used are contrast venography and B-mode (compression) ultrasonography. These techniques have the limitation of not being able to determine if the thrombus is hematologically active and therefore likely to continue to grow or embolize.

The current radionuclide test for DVT is radionuclide venography. Several tracers such as Xe-133, Kr-81m, Tc-99m labeled colloids, macroaggregated albumin or erythrocytes have been used for this application. Advantages to radionuclide venography are that it makes use of readily-available radiopharmaceuticals and results can be obtained quickly. The test, however, is not specific for thrombi. The images only show cold spots distal to sites of obstruction and not specific binding to thrombi. Also the technique cannot distinguish between acute and chronic thrombi.

Newer agents have been developed based on the chemical nature, mode of formation and fate of thrombi. The current understanding of thrombus formation begins with platelet deposition behind a venous valve followed by platelet aggregation and release of thrombin (113). Fibrin deposits form on the platelets and retract. The thrombus may resolve or may continue to recruit more platelets and more fibrin or the thrombus may dislodge where it will find its way to other vessels (i.e., pulmonary vessels). Agents have therefore been directed against fibrin or platelet targets.

Fibrin directed targeting includes radiolabeled fibrinogens (the precursor to fibrin) (114). Fibrinogen is converted to fibrin monomer by action of thrombin. Advantages to radiolabeled fibrinogen include significant accumulation on fresh thrombi and lack of antigenicity of this protein. However, fibrinogen is insensitive to pre-existing thrombi, anticoagulants are known to interfere with binding and blood clearance is slow.

Monoclonal antibodies (MAbs) have also been used in thrombus imaging as well (115). MAbs have the advantage of being produced to recognize specific components of the thrombus complex. Once raised against a target epitope, the MAbs can be enzymatically cleaved to form smaller fragments with different biological clearance rates. Several MAbs against fibrin have been developed (116) which bind the central chain of fibrin (anti- $\alpha$ or anti- $\beta$ MAbs), which bind the carboxy terminal domains of

fibrin/fibrinogen (Anti- D antibodies) and which recognize fibrin dimers.

**RGD and RYD peptides**

Glycoprotein-IIb/IIIa(GP-IIb/IIIa) are transmembrane proteins on the surface of platelets. They comprise a heterodimer complex which changes conformation in response to platelet activation. The complex does not bind fibrinogen until after activation, a neccessary phenomena for cell-cell attachment during platelet aggregation. The GP IIb/IIIa complex belongs to the family of cell adhesion molecules called integrins. The subfamily of compounds that recognize the GP IIb/IIIa complex generally contain the tripeptide sequence Arg-Gly-Asp (RGD). Fibrinogen contains this peptide sequence in two locations of the alpha chain.

The IgM, PAC1, binds stimulated platelets with an affinity of 5 nM but does not bind unstimulated platelets (117) (similar to fibrinogen). The epitope binding region of this IgM has been mapped and the 21 amino acid sequence contains the tripeptide sequence, Arg-Tyr-Asp (RYD). The synthetic 21 amino acid peptide was shown to inhibit fibrinogen-dependent platelet aggregation as well as PAC1 binding to platelets. Substitution of G (glycine) with Tyr in the RYD sequence of the peptide resulted in a peptide with increased anti-platelet potency over the original sequence (118).

Knight and co-workers have evaluated a series of RGD and RYD containing peptides as thrombus imaging agents (119). In this study 16 to 31 residue analogs of the hypervariable sequence of the IgM, PAC1, which binds stimulated platelets with an affinity of 5 nM (but not unstimulated platelets) were prepared. All of the peptides contained a tripeptide binding group (RYD or RGD) and a metallothionein-derived sequence (KCTCCA) for Tc-99m 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 hours after injection. In control experiments, a Tc-99m labeled nonspecific peptide did not image the thrombi. These investigators concluded that the use of RGD peptides is technically feasible but that further improvements are neccessary for reliable thrombus imaging.

## OTHER PEPTIDES

**Atrial Natriuretic Peptide (ANP)**

ANP is a 28 amino acid peptide which is produced in the cardiac atrium. Receptors for ANP are found in the kidneys, adrenals, and lungs (120). In the kidney these receptors are most numerous in the glomeruli and play an important role in fluid, electrolyte, and blood pressure homeostasis (121). Various types of binding sites have been reported for ANP (122,123) and these different ANP receptors have now been cloned (i.e., A, B and C).

The ability to image ANP receptors has recently been demonstrated in monkeys (124) and rabbits (125). In the monkey, I-123 labeled ANP was rapidly bound to ANP receptors in the kidneys and lungs. The observed uptake was demonstrated to be receptor mediated by competition studies using simultaneous injection of unlabeled ANP. In a more recent report (126), after intravenous administration to monkeys, I-123 ANP was shown to localize primarily in the kidney and lungs, and blood clearance was rapid. This study demonstrated that localization was receptor mediated based on displacement studies using cold ANP and C-ANP. Biodistribution in the monkey correlated well with the presence of ANP receptors determined by *ex vivo* receptor binding assays.

The diagnostic implication of ANP receptor imaging are not firmly established as yet but likely will allow assessment of renal glomerular diseases. Since ANP receptor density and affinity are influenced by various physiological and pathological conditions, clinical and diagnostic applications seem possible.

## SUMMARY

Over the past several years there has been a large body of data accumulated on the use of somatostatin analogs for tumor imaging (73). FDA approval of In-111 pentetreotide may signal a turning point in radiopharmaceutical design and development. In addition to In-111 pentetreotide, other peptides have found their way into preclinical and clinical studies, such as ANP (126) and VIP (77), insulin (47), captopril (127), platelet factor 4 (128), interleukin-1 (129), aM2, a synthetic peptide mimicking the complementarity determining region of an anti-tumor monoclonal antibody (130). Also a significant amount of preclinical data is available on the the use of chemotactic peptides for infection imaging (131). Clearly, the scope for future applications of peptide-based radiopharmaceuticals is vast. As natural bioactive peptides continue to be implicated in disease processes, greater interest in using them as imaging probes will no doubt follow.

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## QUESTIONS

1. Radiolabeled peptides are composed of which of the following?

   a. fatty acids
   b. sugars
   c. lipids
   d. amino acids

2. Which of the following radionuclides has been used for radiolabeling peptides for diagnostic imaging?

   a. iodine-123
   b. technetium-99m
   c. indium-111
   d. all the above

3. Which of the following structures has been shown to provide the greatest penetration into tumors?

a. single-chain antigen binding proteins (sFv's)
b. Fab antibody fragments
c. $F(ab)_2$ antibody fragments
d. whole, intact antibodies

4. Which of the following fractions from the SEP-PAK quality assessment of indium In-111 pentetreotide contains the hydrophilic impurities from the product?

a. SEP-PAK cartridge
b. fraction number 1 eluted in water
c. fraction number 2 eluted in methanol
d. all the above

5. Which of the following amino acid residues must be present in a peptide structure before direct radioiodination labeling can occur?

a. tyrosine
b. aspartic acid
c. serine
d. valine

6. Somatostatin is involved in which of the following types of cell signaling?

a. paracrine
b. endocrine
c. synaptic
d. all the above

7. Which of the following components of the In-111 pentetreotide kit acts as a stabilizer?

a. citric acid
b. ferric chloride
c. gentisic acid
d. hydrochloric acid

8. Which of the following blood cells are thought to possess somatostatin receptors?

a. erythrocytes
b. platelets
c. stem cells
d. lymphocytes

9. Radiolabeled atrial natriuretic peptide receptor imaging should allow diagnosis of which of the following?

a. renal glomerular disease
b. myocardial infarction
c. small cell lung carcinoma
d. Hodgkin's and non-Hodgkin's lymphoma

10. Which of the following chemical classes does HYNIC (hydrazino nicotinamide) come under?

a. biologically active polypeptide
b. monoclonal antibody
c. bifunctional chelate
d. amino acid

11. Technetium-99m labeled chemotactic peptides have been shown to possess high binding affinity to which of the following types of blood cells?

a. erythrocytes
b. lymphocytes
c. platelets
d. none of the above

12. Both RGD and RYD peptides for thrombus imaging contain how many amino acids in their sequence?

a. 14
b. 8
c. 5
d. 3

13. Which of the following tumor types are known to have somatostatin receptors on the tumor cells?

a. small cell lung cancer
b. carcinoid
c. pheochromocytoma
d. all the above

14. Which of the following is the primary route of clearance of radioactive In-111 pentetreotide?

a. hepatobiliary system
b. reticuloendothelial system
c. renal excretion
d. none of the above

15. Which of the following tumor types are known to have vasoactive intestinal peptide (VIP) receptors on the tumor cells?

a. non-small cell lung cancer
b. breast carcinoma
c. colon adenocarcinoma
d. all the above

16. Technetium-99m labeled chemotactic peptides have been indicated for nuclear medicine imaging of which of the following disorders?

a. infection
b. myocardial infarction
c. colorectal cancer
d. stroke

17. Which of the following amino acids is included in the structure of RGD peptide?

a. tyrosine
b. glycine
c. glutamic acid
d. serine

18. Radiolabeled peptides may allow for which of the following clinical indications?

a. tumor detection and staging
b. therapeutic monitoring
c. characterization of tumor biochemistry
d. all the above

19. Which of the following is the expiration time for prepared indium In-111 pentetreotide?

a. 30 minutes
b. 120 minutes
c. 6 hours
d. 18 hours

20. Which of the following has been studied for its potential as a thrombus imaging agent in nuclear medicine?

a. radiolabeled anti-fibrin antibodies
b. radiolabeled fibrinogen
c. radiolabeled colloids
d. all the above

21. The quantity of pentetreotide in the In-111 pentetreotide kit is _____ times less than that of therapeutic dosages of octreotide used to control the symptoms of neuroendocrine tumors.

a. 1-4
b. 5-20
c. 20-50
d. 50-80

22. Which of the following adjunct medications is suggested for use in patients undergoing nuclear medicine imaging with In-111 pentetreotide in order to improve the images?

a. dexamethasone
b. morphine
c. furosemide
d. laxatives

23. Which of the following is considered the critical organ (receives the highest radiation absorbed dose) for In-111 pentetreotide?

a. urinary bladder
b. kidneys
c. spleen
d. liver

24. Which of the following radioiodination methods provides greater stability when radiolabeling octreotide analog with iodine radionuclides?

a. Chloramine-T
b. N-succinimydyl-5-iodo-3-pyridine carboxylate
c. iodine monochloride
d. Iodogen

25. According to the literature, which of the following peptide compounds has been radiolabeled with the positron emitting radionuclide, fluorine-18?

a. chemotactic peptide
b. octreotide
c. insulin
d. all the above