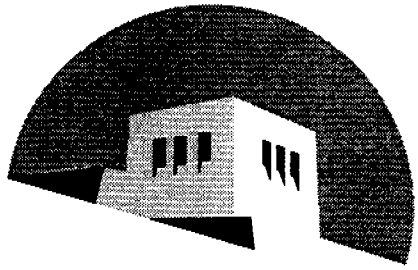

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The Development and Clinical Use of Radiolabeled Peptides for Therapy

By:

Geert J. Ensing, MD, PharmD, PhD
Department of Research and Development
Mallinckrodt Medical B.V.
Petten, The Netherlands

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Geert J. Ensing, MD, PharmD, PhD

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THE DEVELOPMENT AND CLINICAL USE OF RADIOLABELED PEPTIDES FOR THERAPY

STATEMENT OF OBJECTIVES

The purpose of this lesson is to provide a general review of the various aspects that must be considered during the development of a radiolabeled peptide intended for therapeutic use. An update of clinical trials involving therapeutic radiolabeled peptides is also provided. Clinical information is limited, however, due to the proprietary nature of these agents.

Upon completion of this continuing education unit, the reader should be able to:

1. Make a rational choice of the appropriate radionuclide to be used in a specific radiolabeled peptide for therapy.
2. Describe how radiolabeled peptides localize in target tumor cells.
3. Explain why internalization of radiolabeled peptides is advantageous.
4. Set specifications for the final labeled product.
5. Make reasonable decisions regarding the appropriate technique to be used to produce labeled peptides.
6. Describe some of the methods used to test the quality of radiolabeled peptides.
7. Discuss which pre-clinical toxicological studies are appropriate for radiolabeled peptides.
8. Understand the conditions under which labeled peptides can be used clinically.

COURSE OUTLINE

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II. RADIONUCLIDES FOR USE IN THERAPY

III. DEVELOPMENTAL ISSUES

- A. Stability of peptides
- B. Radionuclide binding ligands
- C. Analytical methods
- D. Pharmaceutical issues
- E. Aspects of toxicological testing

IV. CLINICAL ISSUES

- A. Measurement of efficacy
- B. Measurement of toxicity
- C. Dosimetry measurements
- D. Current status

V. CONCLUSION

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INTRODUCTION

Nuclear medicine differs from all other medical specialties because it uses the characteristics of radionuclides to detect and treat different diseases. The diagnostic gamma emitting properties of different radionuclides can be used to investigate the physiology of the human body without interfering in the physiologic process. This "tracer" principle is based on the very high detectability of gamma rays, requiring minute amounts of a radiolabeled physiologically active compound to obtain a measurable signal.

Therapy using beta-emitting radionuclides is still not firmly established in clinical practice despite a long and proven utility of radioactive iodine therapy for the treatment of thyroid disease. Other available treatments have gained only a limited clinical foothold. These include: ^{131}I-MIBG for pheochromocytoma and neuroblastoma; bone-seeking agents like ^{89}SrCl and ^{153}Sm-EDTMP for pain palliation in patients with metastatic prostatic cancer; ^{90}Y-colloids for radiosynovectomy, etc.

Radiolabeled peptides have been used diagnostically in nuclear medicine since the beginning of the 1990s, when ^{111}In-pentetreotide (Octreoscan, Mallinckrodt) was approved for the localization

of neuroendocrine tumors. Since then, a very small number of other labeled peptides have been approved in the US or Europe, all for diagnostic use. An example is ^{99m}Tc-apcitide (Acutect, Diatide) for the detection of acute venous thrombosis.

Radiolabeled peptides are the subject of intense research efforts.[1] A major advantage of (labeled) peptides over (labeled) monoclonal antibodies (Mabs) is, that the labeled peptides are small (molecular weight is in the range of 1-3 KDalton). This small size allows them to be cleared quickly from the body after intravenous administration and to rapidly penetrate into target tissues, including tumors. Rapid clearance from the blood combined with high specific binding to target tissues provides the high target/nontarget ratios normally seen with Octreoscan. In the therapeutic setting, these characteristics decrease the radiation dose to the non-receptor tissues. A major challenge for peptide imaging and therapy is to produce peptides that show a high (Kd>8-9) binding to the specific cellular targets in a manner similar to monoclonal antibodies. This is discussed in detail in the section on ***Developmental Issues***.

The use of the labeled peptides described above is based upon their ability to bind to naturally occurring receptors. In the case of ^{111}In-pentetreotide, the somatostatin receptor is the subtype-2 receptor (sst-2). In the case of ^{99m}Tc-apcitide, the target is the GPIIb/IIIa receptor.

The observed target-to-non-target ratio (i.e., tumor to normal tissue) when using ^{111}In-pentetreotide is very high (sometimes up to 6-10). Such high uptake ratios make it conceptually possible to use this compound not only for diagnosis of cancer, but also for therapy. At this time, neither this product, nor any other radiolabeled peptide is approved for therapeutic use. However, several companies and research groups currently are evaluating a number of peptides for potential therapeutic use in humans. It is the goal of this continuing education lesson to provide the reader with an overview of the process used in the development of radiolabeled peptides for cancer therapy, with particular emphasis on the pharmaceutical requirements.

The living body contains numerous receptor systems with myriad functions during homeostasis. In the future, it is hoped that the functions of many of these receptors will be elucidated. For the topic at hand, we will focus on cell membrane-bound receptors. In general, these receptors undergo a conformational change once the receptor is occupied extracellularly by an agonist, allowing induction of an intracellular action (often c-AMP formation). The expression of receptors on the cell membrane is regulated by feedback systems, so that the number reflects the local need for a certain action. Although this common path of intracellular action normally is present in tumors, the feedback systems that regulate the actual number of receptors on the cell membrane often is disordered. When cells undergo malignant change, genetic mutation can lead to suppression or over-expression of receptor regulation as well as a modification in the spectrum of receptors expressed. In the anaplastic end-phase, all characteristics of the cell of origin are lost, and the only thing the cell can still do is grow.

The specific features of receptor expression in tumors provide a wide array of possible points for interference with the cell function, allowing targeting specifically at systems that regulate tumor growth (e.g., neoangiogenesis).

By focusing on those receptor systems that are expressed in high numbers on certain tumors, one may expect to be able to treat (by local irradiation) the tumors, while exposing the rest of the body to relatively low amounts of radiation. This will be discussed further in the subsection on (tumor) dosimetry. Clinical trials are underway to evaluate this hypothesis.

The therapeutic index is greatest when the number of receptors on a human tumor is large in comparison to normal tissues. Therefore, the use of in-vitro studies to detect and quantify these receptors is especially important. However, ex vivo assessment of receptor expression using cell cultures from human cancers can be misleading, since cells in culture may express different receptors or different numbers of receptors than that detected in human tumor tissue. For this reason, autoradiography remains the "gold standard."

Although still in its infancy, work is underway to genetically modify the expression of certain receptors on target cells using viral vectors.[2] These approaches may become very important once a therapy with radiolabeled peptides becomes available. At present, many peptide receptor systems have generated interest as possible substrates for human diagnosis. These include:

- melanocyte stimulating hormone (MSH), used in the detection of melanoma;
- vasoactive intestinal peptide (VIP), used in the detection of adenocarcinomas;
- Substance P, used in the detection of glioblastomas;
- CholeCystoKinin (CCK)/gastrin, used in the detection of medullary thyroid carcinoma;
- neurotensin, for the detection of pancreatic cancer;
- somatostatin, for the detection of neuroendocrine tumors; and
- bombesin, used in the detection of breast and prostate cancer.

Of the aforementioned receptor systems, only somatostatin, VIP, and bombesin are considered potential target receptors for treatment due to the limited number of other receptor types detected on human tumors. Since all the above-mentioned systems are naturally occurring receptors, the prospect of pharmacological effects, if an agonist is administered, is real. The natural ligands of these receptor systems are often degraded very rapidly (half-lives in blood of 1-3 min) by peptidases. The natural ligands often work via paracrine pathways, in which one cell produces the ligand, and the ligand is then bound on a neighbor cell through the specific receptor. Also it is known from all the aforementioned systems, that the receptors, once bound with agonists, are internalized, that is, that they are taken up by endocytosis into the cytoplasm and are broken down in endosomes.[3] In order to preclude pharmacological side effects, the use of receptor antagonists may be a good choice, since they generally are not internalized.

However, there is growing evidence suggesting that the internalization of radioactive (agonistic) ligands is advantageous, allowing retention of radioactivity in the cell. This is easy to understand by considering the kinetic pathways that occur upon receptor binding. Ligand and receptor bind according to their affinity (the "on" reaction). From that moment forward, an "off" reaction also takes place with a certain constant. If the ligand is internalized, the "off" reaction cannot take place, therefore the ligand (and its

radioactive label) will be associated with the cell longer. How much longer depends on the chemical characteristics of the radioactive label. For example, ^{111}In will, once in the endosome, transchelate to other proteins in the cell. This mechanism is most probably also true for ^{90}Y and lanthanides. Techetium- and rhenium-labeled compounds, on the other hand, are oxidized in the endosome to pertechnetate and perrhenate, respectively, and diffuse back out of the cell.

Since the radiation dose delivered is linearly dependent on the time a certain radionuclide is present in a structure, it is important for the outcome of a therapy to retain the radionuclide as long as possible. Of course, other parameters are also important (like physical half-life, radiation characteristics, radiation sensitivity of the tumor, etc.), but with a given radionuclide, it is advantageous to retain the radionuclide as long as possible in a certain target structure.

RADIONUCLIDES FOR USE IN THERAPY

There are currently more than 600 nuclides known that emit electrons (beta decay) or helium atoms (alpha decay). Because the energy deposition per unit of volume from particulates is high (in comparison with gamma rays), these nuclides are candidates for internal radiotherapy. In addition to the electrons emitted during beta decay, Auger and conversion electrons are emitted during the electron capture (EC) decay process. The main difference between the beta-decay electrons and the conversion and Auger electrons is their energy. "Beta" electrons have maximum energies of several hundreds of keV, sometimes even MeVs. Conversion electrons typically have energies in the range of a few keV; Auger electrons often have maximum energies below 1 keV. The nature of beta and alpha decay is also different with respect to the energy deposition along the path of decay. Beta particles typically deposit a large part of their energy at the end of their path, when their energy has decreased due to interaction with the matter between their point of origin (the decaying atom) and their current position. Since this path can be fairly long with respect to cell size (an electron with 2MeV energy has a path length in water of about 5 mm), the majority of energy will not be delivered in the cell where the beta decay took place. Alpha particles have a short path length (often not more than micrometers when the energy is a few MeV), so they will deposit their energy often in the same cell, if the decay took place in the cell or at the cell membrane.

Because of their low energy, Auger electrons also have path lengths in the order of 0.1-1.0 micrometer. Accordingly, they also are good candidates for therapy. The number of Auger electrons per decay can be greater than 10, which may also enhance radiation delivery. If Auger electrons are emitted from a site within the target cell, the cell nucleus can be reached.

This difference in energy deposition also establishes that alpha particles (and Auger electrons) probably are best suited for small (several cells at most) tumors/metastases ("micrometastases"), while beta particles are better suited to deliver a radiation dose to larger tumors, or to tumors that are not homogeneous with respect to their receptor presence. This appears to be the case in breast cancer (JC Reubi, personal communication). Due to the phenomenon known as "crossfire," a cell undergoing irradiation also is irradiated by electrons emitted from surrounding cells and, as a result, receives a high radiation dose.

In order to select an optimal radionuclide (see Table 1), apart from the aforementioned discussion regarding differences in energy and species of decay, it is important to consider the following features:

- Presence or absence of gamma rays during decay. The presence of gamma rays permits scintigraphic imaging of the patient, which provides evidence whether there is radioactive uptake in the targeted tumors. Gamma rays also permit dosimetric calculations. A disadvantage of the presence of gamma rays is that the people in close proximity to the patient also may be exposed to radiation. Accordingly, many countries have established guidelines regarding the maximum permissible amount of gamma radiation emitted by a patient at the time they are discharged from the hospital. These guidelines may require that the patient be hospitalized in a special therapy room, resulting in additional expenses related to treatment. At present, the number of therapy rooms available for gamma radiation treatment is low and the majority of these rooms are used for thyroid patients.[4]

It is important to note that "pure" beta-emitters also emit gamma rays. Also known as "brehmsstrahlung," this phenomenon is caused by the interaction of the electrons with atoms having a large atomic mass number. It has been demonstrated that scintigraphic pictures are possible with brehmsstrahlung radiation.[5]

- Half-life. The physical half-life should generally be more or less similar to the retention time within the target tissue. If the physical half-life is much longer, both the patient and the environment are burdened with an excessively high (not tumor related) radiation dose. On the other hand, if the half-life were shorter, more frequent dosing would be required in order to achieve an optimal tumor dose. Another aspect has to do with the logistics of shipments from the production site to the customer. Here, a longer half-life (in the order of several days) is advantageous. When nuclides of short half-life are used (for example ^{188}Re or ^{213}Bi) production of the radiopharmaceutical final product will have to be performed at the site of treatment (in the hospital or in a near-by radiopharmacy). With the longer half lives (>3day), production at a centralized production facility is the best option.
- The cost and availability of radionuclide therapy agents. The goal of developing labeled peptides for tumor therapy is to be able to provide a treatment with high efficacy and a minimum number and severity of side effects for patients with a range of tumors. For this goal to be feasible, drug approval is needed, so that patients all around the world can use these compounds. The drug approval process is a very costly one, up to several million dollars. Expenses are related to the need to garner enough clinical data. Accordingly, the potential patient population that could benefit from such treatment must be large (e.g., a minimum of several thousands of patients per year) in order for the manufacturer of the labeled compound to make a profit.

The actual amount of radioactivity per patient treatment is often large (currently, patients with thyroid cancer are sometimes treated with 200 mCi ^{131}I-NaI).

Table 1: Factors That Must Be Considered When Selecting a Radionuclide To Be Incorporated Into a Therapeutic Radiopharmaceutical

- Physical half life of the radionuclide
- Type of radiation(s) emitted by the radionuclide
- Energy of radiation(s) emitted by the radionuclide
- Chemistry and chemical reactivity of the element/isotope/radiochemical containing the radionuclide
- Cost of the radionuclide
- Availability of the radionuclide

The above factors relate to the following considerations:

- What is the range that the radiation must travel in the tumor?
- How large is the tumor?
- What is the distance between the site where the radiotracer binds to the tumor (in or on the cell) and the structure that must be irradiated (the nucleus)?
- What type of tumor is being treated and how metabolically active is the tumor?
- Does the physical half-life of the radionuclide closely match the biological half-life and rate of tumor localization of the carrier molecule so that the tumor cells are irradiated for an optimal amount of time?
- How destructive is the radiation emitted? (low or high LET?)
- Does the radionuclide emit a gamma photon that can be used to screen patients and/or monitor therapy outcome? (or, Can the therapeutic radionuclide be "paired" with a diagnostic, photon-emitting radionuclide?)
- Can the radionuclide bind readily to the carrier molecule and remain stable in vivo?
- Can a sufficient quantity of the radionuclide be combined with an appropriate amount of carrier molecule?
- Is the cost of the radionuclide such that it could be used and marketed clinically at a reasonable price?
- Is there a reliable source of the radionuclide in quantities sufficient for clinical demands?

Clinical experience to date indicates that treatments must be repeated several times. Therefore, the actual amount of radioactivity needed per year is staggering: several thousands of Curies per radionuclide. Within the aforementioned >600 nuclides that emit beta or alpha particles, only a few viable candidates remain that can be produced economically and in large enough quantities: ^{90}Y, ^{131}I, and ^{177}Lu.

Other potential candidates, which may, under certain circumstances, also be produced in large amounts are ^{188}Re and ^{213}Bi. All other radionuclides can be produced in amounts that permit a limited clinical efficacy study, but they are not candidates for a large-scale radiotherapeutic product.

- Diagnostic/therapeutic pairs.

Experience gained with the treatment of thyroid cancer suggests that a diagnostic scan with either ^{123}I or a diagnostic dose of ^{131}I be performed before therapy is initiated with ^{131}I.

The same principle is applicable to radiolabeled peptide treatment of other tumor types. Therefore, combinations of diagnostic and therapeutic radionuclides that share certain chemical properties are needed to identify those patients who may benefit most (based upon a calculated radiation dosimetry to the tumor and critical organs) from the therapy.

Several such combinations are possible:

Diagnostic	Therapeutic
^{99m}Tc	^{186}Re, ^{188}Re
^{123}I	^{131}I
^{111}In	^{90}Y (^{177}Lu, ^{213}Bi)

An important consideration to be addressed when making choices between these possible combinations is the half-life of the radionuclides in the combinations. Since the mass of a carrier-free radionuclide is inversely proportional to its half-life, large differences in half-life can mean that the chemistry that binds the radionuclide to the ligand (peptide) may vary over time. The best example is the difference between ^{99m}Tc and ^{186}Re. The literature is replete with data demonstrating that the chemistry that allows ^{99m}Tc to be easily incorporated into several compounds is unfavorable for ^{186}Re.[6] This is due to both the difference in half-life of the two radionuclides (6 hours versus 90 hours, respectively) and the fact that ^{186}Re can not easily be obtained in carrier-free state. The first factor also plays a role in the $^{123}I/^{131}I$ combination, but is less important in the $^{111}In/^{90}Y$ combination.

Lastly, there are differences in the chemical behavior of elements that share the same column in the periodic system. For example, Re oxidizes much easier than Tc. Therefore, the synthesis of Re products requires a more potent reducing environment than needed for Tc products. Similarly, the chemical behavior of ^{90}Y is different from that of ^{111}In. For example, diethylene triamine penta acetic acid (DTPA) is an excellent ligand for ^{111}In but not for ^{90}Y. When DTPA is labeled to ^{90}Y, some 'free' (non-ligand-bound) radionuclide is always present a few hours after administration, causing enhanced bone-marrow uptake/radiation dose. The solution is to seek ligands that are capable of binding both the diagnostic and therapeutic isotope with high efficiency, like DOTA (1,4,7,10-tetra-azacyclododecane-N',N'',N'''-tetraacetic acid) instead of DTPA for the $^{111}In/^{90}Y$ combination.

DEVELOPMENTAL ISSUES

This segment of the lesson focuses on several aspects of the development of radiolabeled peptides.

Stability of peptides

As mentioned previously, the human body has several very effective systems that degrade peptides. The main reason for this metabolic capacity lies in the often very potent pharmacological action of naturally occurring peptides, so that, in order to keep this action in check, a rapid clearance of the peptides in (especially) blood and tissues is needed.

One of the differences between "natural" peptides and labeled peptides is the point of entry in the human body. Natural peptides are produced in certain organs, often in a longer form, that is degraded to yield the active, smaller peptide. Their action is focused on other (or the same) organs that have a relationship with the production site through blood flow. The labeled analogs are administered in a peripheral vein, so their journey toward the target organ or organs is mostly via the arterial system.

For labeled peptides to be of use, a certain blood-half-life is needed in order to reach the target (tumor). Whether this optimal half-life is the same for all peptides remains to be proven. The experience with somatostatin receptor binding peptides is that a blood half-life of 1-2 hours is sufficient to be taken up in the tumor. Work by the Rotterdam group[7] indicates that approximately 30 minutes is required to permit binding and subsequent internalization of the somatostatin receptor-bound peptide ligands.

The half-life in blood of natural, non-stabilized peptides is on the order of minutes, so that it is clear that some form of stabilization is necessary. Possible ways of stabilization include the following:

- Cyclization. Stabilization is possible with an S-S bond (as performed by Sandoz to stabilize Octreotide); by normal peptide bonds (as accomplished by DuPont and Diatide in their somatostatin-receptor agents); by an aliphatic chain; by an amine bond,[8] as well as others.
- The use of non-natural amino acids. For example, D-forms of natural amino acids, as well as synthetic amino acid look-alikes are currently under investigation.
- The use of non-peptide bonds.[9]
- The exchange of certain amino acids that are known to be reactive in certain environments. One example is the substitution of methionine for isoleucine, so that the resulting peptide will not be oxidized as easily.

Of course, a combination of these methods are often required to synthesize a peptide that is stable, while still maintaining a high binding affinity to the receptor of choice. Ideally, the goal of computer mapping programs is to map the three-dimensional structure of certain peptides with the corresponding receptor structure. However, a major limitation to this approach is that the three-dimensional structure of many receptors is not fully known.

Heat resistance is another important attribute for peptides that are used in radiolabeling. Radionuclides must be able to withstand a heating step (often up to 100°C), if the labeling process requires it. This is especially important for iodinations (^{131}I and ^{123}I), as well as ^{90}Y and lanthanide labeling methods. This is less important for Tc and Re chemistry. Possible improvements can be found in ligand systems that allow the incorporation of radioactive nuclides at room temperature or at a marginally enhanced temperature.

Lastly, the peptides must not be overly susceptible to radiolysis. This

aspect can be improved by changing the formulation of the final product (addition of antioxidants) and/or dilution of the solution.

Radionuclide binding ligands

Over the years, several articles have been written about the characteristics of the "ideal" ligand to bind a radioactive atom to a peptide or a protein.[10,11]

The most important aspects of an ideal ligand are listed as follows:

- There must be strong, irreversible binding of the radionuclide;
- The ligand must be chemically versatile, so that it can be (de)protected during synthesis of the peptide-ligand system at will, permitting labeling at room temperature;
- The ligand should allow binding of both the diagnostic and the therapeutic isotope in a diagnostic/therapeutic pair;
- The ligand should allow high specific activity.

For the ^{111}In (diagnostic) and ^{90}Y/^{177}Lu (therapeutic) pair, DOTA, which has an N4 "cage" structure, is the most preferred at present. Ongoing research continues in an attempt to fine-tune the charge/lipophilicity of this kind of ligand by adding one or more acetate groups and/or making the binding cage larger/smaller by adding/deleting a carbon atom.

It is questionable whether the established procedure for the ^{123}I/^{131}I labeling for monoclonal antibodies (reacting with tyrosine amino acids) can be used for the much smaller peptides also. In this case, novel iodination methods that would diminish the frequently occurring deiodination reactions are advisable.[12]

For the ^{99m}Tc/^{188}Re pair, one might use the MAG3 (mercapto acetyl triglycine) technology,[6] the HYNIC (hydrazino-nicotinamide)[13] or the newly developed carbonyl approach.[14]

The DOTA compound has many of the ideal characteristics mentioned previously. Even though labeling at room temperature does not seem feasible, it is the best ligand available at present. Other approaches are still in the early stages of development and are therefore not yet suitable for therapeutic compounds.

Analytical methods

Analytical methods used to assess the quality of the labeled peptide can be divided into non-radioactive methods (e.g., characterization of the peptide, etc.) and radioactive methods (e.g., labeling yield and radiochemical purity of the peptide). The following section will identify some of the different methods presently available.

Non-radioactive analytical methods for radiolabeled peptides

The following methods are used to characterize and assess the purity and content of the peptide preparation.

- The characterization of the peptide should be done by determining the amino acid sequence by controlled degradation with carbo-peptidase. In addition, it is advisable to produce ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra and infrared (IR) spectra to be used as "fingerprints" to assess batch-to-batch variability.
- Determination of solubility in water at 25°C at neutral, low, and high pH, and (sometimes) in dimethyl-sulfoxide (DMSO), can give valuable information for the routine use of the peptide.
- The purity (typical specifications are 97%-99.9%) is defined as the amount of pure peptide in the peptidic material (i.e., the desired peptide and peptidic impurities from synthesis and purification). Impurities from synthesis include "deleted" sequences due to uncompleted coupling reactions,

fragmented sequences, etc. An example of an impurity from the purification process is ester formation due to chromatography in the presence of primary alcohols under acidic conditions.

Methods to assess peptide purity. The method of choice to determine the ratio of desired peptide to peptidic impurities is reverse phase (C18 or C4) high pressure liquid chromatography (HPLC). A rule of thumb (especially from a regulatory point of view) is that all impurities being present above 1% should be identified and be tested in an acute toxicity test.

Using acidic conditions, (e.g., water-acetonitril gradients with 0.1% tri-fluoro-acetic acid (TFA), all peptides are fully protonated and thus can be detected most easily. The most common detection method is UV spectrometry. The most common wavelengths used are 280 nm (measuring tryptophane-like amino acid residues) and 215 nm (measuring amino acids). The sensitivity of the 215 nm signal is much greater than the 280 nm signal, but many other compounds also show absorbance at 215 nm. A very sophisticated method consists of a liquid chromatography-mass spectrometry (LC-MS) system, where all relevant peaks eluting from the chromatograph are evaluated for their mass by the connected MS system.

Thin layer chromatography (TLC) may still be used to get a quick idea of overall purity (using ninhydrin spray as detector), but quantification is generally not possible.

Another method that is quickly gaining popularity is capillary electrophoresis and electrofocusing. These methods provide an independent second method from the HPLC methods that can be used to detect impurities not seen in the HPLC profiles (e.g., when an impurity cannot be eluted from the HPLC column). Electrofocusing can also be used to measure the pKi values of the peptides.

Another important aspect to be aware of is the possibility of racemization due to hydrolysis or due to the production method. For example, cyclic peptides with ring-closure via a chiral amino acid can show significant racemization of that amino acid

- The peptide content is the amount of peptidic material in the total preparation (typical specifications are 80%-95%). The remainder consists mainly of (defined) counter-ions and water. This latter impurity can be relatively high; 5% to 15% of water is not uncommon.

Methods to assess peptide content. The method of choice is a total amino acid determination after hydrolysis in 6N HCl for 24 hours at elevated temperature (110°C). This method needs to be corrected for labile amino acids (i.e., Cys, Trp, Tyr) and "difficult to hydrolyze" peptide bonds (e.g., Val-Val).

Separate nitrogen and sulphur determinations can provide valuable data about the accuracy of the total amino acid determination by hydrolysis. A disadvantage of this method is the high amount of peptide needed (several hundred milligrams).

Once the total peptide content is known, it can be considered along with the results of the method used to assess peptide purity to make an estimate of the desired-peptide content.

For routine analysis, using a standard of the desired peptide, one can perform a HPLC assay, comparing the absorbance of the standard with that of a sample of the "to-be-determined" peptide.

Another interesting method is to make use of a saturation assay. This procedure involves the labeling of a known amount of the desired peptide with an excess of a (known) mixture of non-radioactive isotope and a radioactive

isotope of the same element. The radioactive content of the peptide peak after HPLC separation becomes a measure of the amount of peptide present in the sample to be determined.

The two latter methods discussed above use only minimal amounts of peptide (micrograms) for the determination.

Determination of water content. In general, the final step in any preparation is a lyophylization of a filtered (0.22 micrometer) aquaeous solution containing the peptide, so the water content of the final, lyophilized material needs to be assessed, and can be seen as a good method to look at reproducibility of the lyophilization process.

Methods used to determine the water content include the Karl-Fischer titration (0.5 mg -2 mg of water are needed for a reliable result); integration of water signals in ^{1}H-NMR spectra (about 1 mg of peptide, i.e., 50 micrograms - 100 micrograms of water is needed) is performed and compared with the amount of the peptide's aromatic hydrogen atoms as internal standard. If no aromatic amino acids (Phe, Tyr, Trp, Pro, etc.) are available, an internal standard has to be added. Relative accuracy of this method is 5%-10%.

Another method that may be of use is NIR (Near-InfraRed) spectrometry. This method has a significant advantage in that it is a non-destructive method, however, its reproducibility is rather low (3%-5%). This method has to be calibrated by another technique such as a Karl-Fischer titration.

Typical specifications for water content are 5% -15%.

Determination of the non-peptide constituents. This procedure should always be performed in each batch. The counter-ions (acetate or chloride) can be analyzed using gas chromatography (for acetate) or conventional analytical methods such as Volhard's or Mohr's titration (for chloride). The specifications for these counter-ions typically are fairly high (3% -8%).

Since metal impurities are often a problem in radiolabeling, a total metal content using, for example, atomic absorption spectrometry (AAS) or induced coupled plasma (ICP) is needed. Typical specifications are <10 ppm (heavy) metals.

Non-peptide organic impurities (solvents) can be detected by gas chromatography; typical levels are <0.1%.

Finally, the appearance of the lyophylisate and (absence of) odor should always be determined on each batch.

Radioactive analysis

The analysis of the final, labeled product should consist of two different assays: a determination of the radiochemical purity and a determination of the content of labeled peptide.

The radiochemical purity is a measure of the amount of desired labeled peptide versus other radiolabeled peptides. These byproducts can still bind the radionuclide since they consist of peptide (fragments) that still bear the nuclide-binding ligand. The specification for radiochemical purity should be >90%, or higher if possible (based upon several analyses). See Figure 1 for an example of results from HPLC testing of radiochemical purity.

If specific radioactive impurities with a content > 1% of total radioactivity are found, an effort should be made to chemically characterize this impurity. Since the absolute amounts of labeled compounds are so low (often not more than nano- or even pico grams), it is often advisable to try to produce the impurity using non-radioactive isotopes (^{89}Y, when ^{90}Y is the radioactive isotope,

^{185}Re for ^{186}Re or ^{188}Re, ^{176}Lu for ^{177}Lu, etc.) and then perform "cold" chemical analyses.

It is therefore advisable to perform a biodistribution study with HPLC-purified impurities to assess whether these impurities have a biodistribution that is different from that of the desired peptide (particularly with regard to the rate and route of clearance). If this is the case, then a specification for the allowed amount of this impurity should be set at a low level.

Of course, assurance is needed during validation of the method that the recovery of the radiolabeled peptide approaches 100%, so that no important amounts are retained on the HPLC column.

The labeled peptide content is a measure of the total amount of "free," non-peptide-bound radionuclide and ideally all radioactivity should be accounted for. A TLC method is often the easiest to perform. The specification for this assay should typically be very high. Depending on the radiotoxicity of the nuclide used, it may have to be as high as >99.5% (i.e., < 0.5% of the radionuclide is not bound to the peptide). See Figure 2.

Pharmaceutical issues

Peptide production. The current method of choice is solid-phase synthesis. This method relies on the use of commercially available peptide synthesizers capable of producing various amounts of peptides ranging from milligrams to grams. In order for these peptides to be suitable for administration in humans, care must be taken to ensure that the production takes place according to good manufacturing practice (GMP) methods.

The essence of GMP is to have a total quality system for all aspects of the synthesis. For example, this would require that:

- The synthesizer be in a controlled/validated condition;
- The starting materials be described and their quality assured;
- The production and the purification steps must be described in detail as part of standard operating procedures (SOPs);
- The analysis of the quality of the final product should be described as part of an SOP;
- The site of production must be confined to an area in which the environment is controlled, including both the microbiological quality and number of particles. It also is essential that a separation of space and/or time be possible when the production of more than one peptide is to take place in this area.

The purification of the crude peptide mixture is done by preparative HPLC methods. Since, during synthesis, the reactive groups of many amino acids will be protected by protecting groups like t-BOC or f-MOC, a typical step before or after crude purification is to use TFA to de-protect the different amino acids.

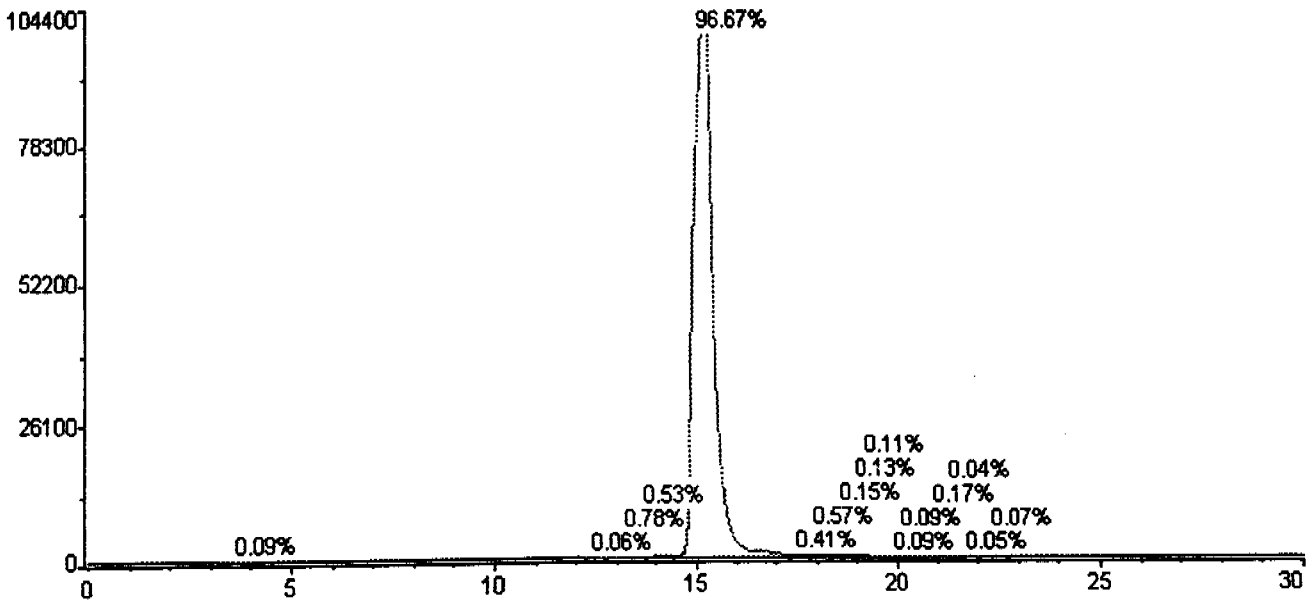

Figure 1. Results from the analysis of a ^{90}Y-labeled peptide by HPLC ('radiochemical purity'). The eluent used is a gradient of 0.05 mol Na-acetate pH5.5 and methanol. At r.t. of 15-16 min, the main, labeled peptide peak can be seen. A number of small, labeled impurities can be seen eluting around the labeled peptide. The activity eluting around 4 min is "free," non-peptide-bound ^{90}Y.

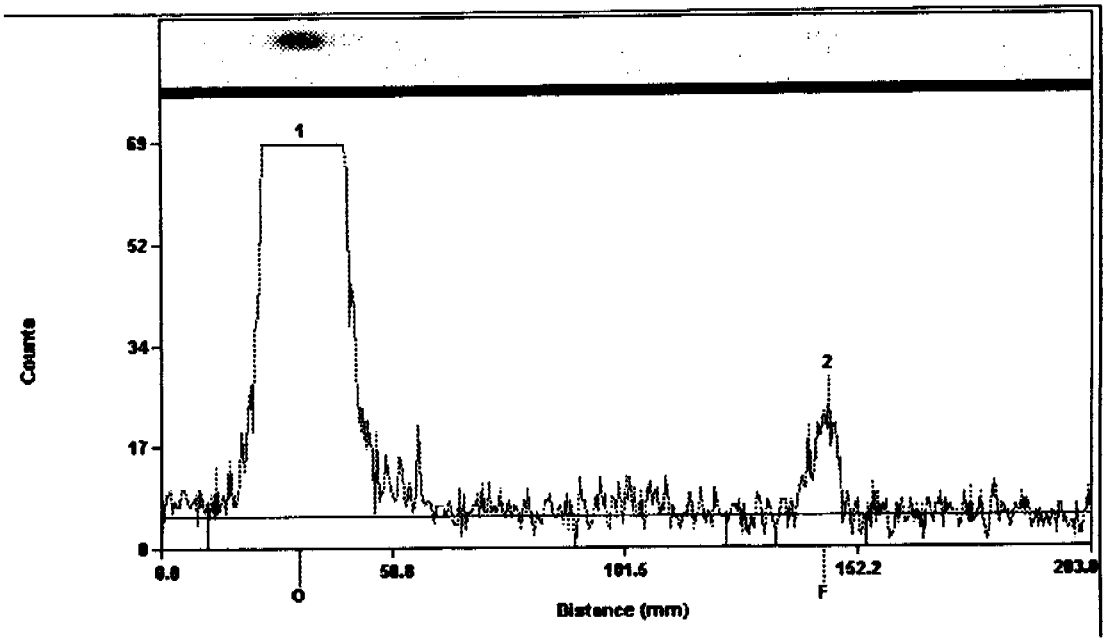

Figure 2. Results from the analysis of a ^{90}Y-labeled peptide by TLC ("labeled peptide content"). The eluent consists of saline; the sample has been diluted with 4 mmol DTPA solution pH4 in order to elute any free ^{90}Y. In the upper panel, a visualization of the TLC trace is given. At Rf 0, the ^{90}Y-labeled peptide is seen At Rf 1.0 ("F") the free ^{90}Y is seen.

This TFA must be exchanged with acetate or chloride ions using ion-exchange columns if the material is to be used in humans.

As discussed above, the water content of the final, lyophilyzed product is relatively high. Furthermore, it is often impossible to reduce the water content, due to strong binding of water molecules (via hydrogen bonds) to the peptidic backbone. It may therefore be advisable to have the lyophylisate equilibrate in an atmosphere of defined humidity (10%-20%) in order to reduce the variability of the water content.

Development of the final formulation. Outlined below are several important aspects.

- Radiolysis. Radiolysis is the destruction of chemical structures by the radiation emitted by the labeled peptide. Since the patient dose is relatively large, the radiation dose in the raw product (after labeling of the peptide) is often quite high; levels of several hundreds of Sieverts are not uncommon. In aqueous solutions, this radiation dose can cause radical formation (OH· and H·), which will react, mostly in an oxidative way, with other chemical compounds. The use of anti-oxidants (like ascorbic acid, gentisic acid, etc.), absence of oxygen during dispensing, and enlargement of final volume (by dilution with saline or glucose 5%) are all good methods to decrease the effect of these radicals.
- Isotonicity. Since the labeled peptides are almost always administered intravenously, isotonicity is an important aspect. If the dilution with saline or glucose 5%, as mentioned above, is performed, isotonicity is achieved more or less immediately, but in all other cases, care has to be taken to assure an isotonic (or slightly hypertonic) end-solution. Of course, one can also achieve isotonicity by co-administration in a running isotonic infusion solution.
- "Ready-to-inject" vs. locally produced radiotracers. There is a growing pressure from regulatory authorities to achieve a 100% assurance regarding the quality of the product. This demands that quality control (QC) standards be met prior to administration of the drug to humans. It is essential to establish a quality assurance (QA) system for the total production and QC process, and obtain a signed release from a responsible pharmacist. This process, whether accomplished on paper or with an electronic system, can be quite time consuming, posing a real and significant burden on small radiopharmacies. Therefore, it has been suggested that these QC procedures be incorporated into the already-existing QA systems used by manufacturers of radiopharmaceuticals.

However, this approach is possible only when the half-life of the radionuclide used is long enough to permit shipment from the production site to the customer. Since shipment can take as long as 2-3 days (an absolute requirement if the product in question is to be used in a truly global fashion), physical half-lives should be at least 50 hours. Of course, the use of short-lived nuclides, such as ^{188}Re or ^{213}Bi, may be considered optimal in the treatment of certain patients. In this case, each center responsible for producing the final product must have a QA system in place to oversee production of these agents.

Specific activity of the peptide. In this context, the specific activity of the peptide refers to the ratio of radioactive peptide molecules to total peptide molecules. There is growing evidence to suggest that a carrier-free, labeled peptide product is sub-optimal.[15] Due to local production of receptor-binding

natural peptides, local (non-tumor) presence of receptors, and/or aspecific binding, the biodistribution of the labeled peptide is changed in such a way that receptor-positive tumors are not (or not as readily) visualized.

Moreover, carrier-free labeled peptides often cannot be prepared because of labeling yield problems. As such, the only possibility lies in a separation (by HPLC) of labeled and non-labeled peptides.

Based upon experience obtained with somatostatin receptor agents, there seems to exist a broad optimum in specific activity. Peptide amounts ranging from 10 micrograms to 500 micrograms per administration are known to allow a good visualization of somatostatin-positive tumors. There are some small differences in biodistribution between high-specific activity (10 microgram) and low-specific activity (500 microgram), for example in spleen uptake.

Another noteworthy aspect regarding specific activity is that the pharmacological activity of a certain peptide often will limit the amount of peptide that can be administered. If this limitation means that too little radioactivity can be given by bolus intravenous injection, then it is important to look for other ways of administration, like a slow infusion, or several administrations within a short period of time (1-2 hours) to increase the total amount of radioactivity to a therapeutic level. In this case, it seems advisable to determine any differences in biodistribution quantitatively by, for example, positron emission tomography (PET) imaging, using a PET isotope that has characteristics similar to the therapy isotope.

Production of the final product. It is clear that the reproducibility of any production is better if the number of uncertainties is as low as possible. Therefore, it is advisable to devise a production protocol using as many as possible fully analyzed intermediate products.

In general, production should be performed according to GMP rules in a fully validated production environment. After production and QC, the product must be assembled in a packaging system that will comply with all applicable transportation guidelines. For beta- (or alpha-) emitting nuclides, a plastic container is typically used to absorb the beta particles and a limited outer layer of high Z material (steel, lead) is recommended to absorb any brehmstrahlung that may be formed.

Stability. The stability of the final product should be tested at various temperatures with appropriate methods, up to at least one physical half-life. If needed, the influence of (UV) light should also be tested. Tests should at least include appearance, radiochemical purity, labeling yield, container-closure integrity, and sterility; the temperatures tested should include 2°-8°C and room temperature according to ICH guidelines. Since the end products will be used quickly (normally within a small number of days, sometimes within hours), higher temperatures and longer periods of testing should only be done for special reasons.

Sterility. The sterility of the final product is an absolute must, even though the intended stability time is usually short. This goal can be reached by either autoclaving or filtration of the solution intended to be administered to the patient. Of these two possibilities, autoclaving is preferred because it can be done in the final container and the environmental requirements, once the process is validated, are less stringent

than with filtration. Furthermore, parametric release, if sufficiently validated, can be an option.

The greatest problem is that the thermic energy administered may break the labeled peptide into fragments that may be labeled or non-labeled. The labeled fragments (the radiolabel, bound to its ligand, plus one or more amino acids, or the "free" radiolabel) will cause an unwanted radiation dose, because they will not bind (or bind to a lesser extent) to the receptors for which they are intended. Most probably, these labeled fragments will be excreted quickly by the kidneys. The eventually formed free radiolabel, if administered, will have a biodistribution that is typical for the chemical nature of the specific isotope. For example, yttrium and the lanthanides will appear in the bone marrow, rhenium isotopes in thyroid, stomach and salivary glands, and iodine will also appear in the thyroid and stomach. The advantages of autoclaving are so significant that a test is needed to determine whether autoclavability is possible. The analytical systems used to assess stability over time may be used to determine whether the labeled peptide can be autoclaved. For both sterilization methods, it is important to know the microbiological burden [in colony forming units (CFUs) per volume] before sterilization; <2 CFU per 100 mL may be a good specification when autoclaving is possible. When using filtration as the sterilization method, the limit should be set lower (< 1 CFU per 100 mL). When filtration is the method of choice, environmental demands are quite stringent. The use of a HEPA-filtered, closed cabinet of class A is required. In addition, the area in which the cabinet is placed must conform (minimally) to class B requirements. The integrity of the filter must be verified after the filtration process is completed.

Since nearly all radionuclides used have a short half-life with regard to the time it takes to perform a sterility test, they will be administered to patients before microbiological data on sterility are available. This underscores both the need for standardization and the importance of validating the sterilization process.

Pyrogens. Although the chances of bacterial growth and the presence of pyrogens are generally small in a typical labeled peptide formulation, it remains essential that testing for pyrogens be performed during the developmental phase. However, if these tests [lumulus amebocyte lysate (LAL) test, validated for positivity] are consistently negative and the number of CFUs measured also are low (<1 per 100 mL), this test is not required for all batches, but instead may be performed at random.

Aspects of toxicological testing

Each component of the peptide complex (i.e., the ligand, linker and radionuclide) or each combination of components can have significant toxicological properties. Furthermore, the metabolites of the components and the decay products (radioactive or stable) can be of toxicological significance. If the labeled peptide is found to be stable in both in-vitro and in-vivo tests, it may be argued that investigating the kinetics of the labeled peptide through the tracer principle, also will investigate the kinetics and metabolism of the non-labeled component in the final product. Therefore, the biodistribution profile and the in-vitro and in-vivo stability of the labeled peptide should help identify the tests needed. For example, if the labeled peptide shows an excretion pattern

through the urine only and there does not seem to be any important metabolism (i.e., if after a period of several hours/days, the recovered labeled peptide in blood and urine is the same as the one administered), the number of toxicological tests that should be performed before a first study in humans is started can be relatively small. Such testing may include:

- Acute toxicity of the non-labeled peptide (1-1000 times the expected clinical dose per kg weight in 3-5 levels plus control) in at least two mammalian species (both sexes). The aspects to be examined are clinical observations and body weights. After sacrifice (day 15), gross pathology and histopathology of selected organs is needed.
- Pharmacodynamic studies are required in one species to assess the influence of the peptide administered on relevant biochemical parameters.
- Biodistribution studies in at least one species to assess the excretion pattern and to perform a first evaluation of the dosimetry; the values obtained in this study should be extrapolated to humans when possible. Of course, in the Phase 1 human studies, dosimetry calculations based upon human biodistribution should also be performed.

In later stages of development, other studies should be executed. These include:

- Acute toxicity of the labeled peptide (1-20 times the expected clinical dose per kg weight in 2-3 levels plus control) in one mammalian species, if possible in primates. The aspects to be monitored are clinical observations and body weights. After sacrifice (4-6 weeks), gross pathology and histopathology of selected organs are performed.
- Acute toxicity study of the non-labeled final formulation (1-50 times the expected clinical dose/kg in 2-3 levels plus control) in one species of mammal.
- Subacute toxicity of the non-labeled peptide (dose level dependent on the results of the acute toxicological study, but at least in 3-4 dose levels plus control), administered every other day for 8 to 12 doses. Aspects to monitor are clinical observations, body weights, gross pathology, and histopathology of selected organs.
- Immunotoxicity of the non-labeled peptide in one mammalian species (2 dose levels, dependent on the results of the subacute toxicity tests), administered every other day for a total of 8-12 doses. Aspects to be assessed are natural killer cell (NK) assay, plaque forming cell (PLC) assay, and mixed lymphocyte reaction (MLR) test.
- Local tolerance of the non-labeled peptide in one species; the local administration should be done with the concentration of the expected human dose and a vehicle control every other day for a total of 8-10 days. The local aspect of the skin should be examined both grossly and microscopically.

It may be expected that the radioactive component of the labeled peptide will induce genetic toxicity. Although there is a small risk of carcinogenesis associated with the use of these peptides, it is important to weigh the potential risk of carcinogenesis with the potential therapeutic benefit. Since these patients already have cancer, these factors must be carefully weighed and an informed decision made regarding treatment.

As stated previously, this list of tests is the minimum. If the labeled peptide is not stable, if its biodistribution is not simple, etc., other tests may be required

to elucidate the metabolism, not only of the labeled peptide, but also of the non-labeled peptide component.

CLINICAL ISSUES

Once the necessary toxicological studies are performed, it is time to considered the clinical application(s) of labeled peptides.

The purpose of these clinical applications is to treat patients with receptor-positive tumors within acceptable toxicity levels. The following parameters must be considered: measurements of efficacy, measurements of toxicity, and dosimetry measurements.

Measurement of efficacy

The result of treatment with any drug should, if possible, be tested in a double-blind fashion, and compared with the currently best treatment. However, it is difficult to perform double-blind studies using radioactive drugs since it is possible to detect whether a patient is receiving a radioactive drug simply by measuring the level of radiation in that patient.

Therefore, it may be better to compare the result obtained with a radioactive drug with that of the comparison drug using objective measurements such as the size of tumor (verified by computed tomography, magnetic resonance imaging, or scintigraphy) or tumor activity (biochemical markers in serum). Furthermore, quality of life measurements continue to gain importance in decisions with respect to new treatments.

Measurements of toxicity

Although it is important to consider all possible causes of toxicity, radioactive compounds in general, and radiolabeled peptides in particular, can cause hematological toxicity due to (temporary) binding in the bone marrow. Experience now suggests that the platelet number is an especially important parameter. The number of platelets tends to decrease after each treatment cycle (nadir, 3-8 weeks post injection), with a rebound to (nearly) the level before the treatment. In general, patients with platelet numbers of <80,000 per microliter should be evaluated carefully prior to treatment because of the risk of internal bleeding if further significant reduction in platelet counts occurs. At this moment, not enough data are available to judge, whether the use of platelet infusions or platelet growth factors can be of assistance with this problem.

Dependent on the biodistribution pattern of the labeled peptide, other organ systems may be targets for radiation toxicity. For example, experience gained with compounds like ^{90}Y-DOTA-tyr(3)-octreotide (reviewed later) indicates that the kidneys also can be a target organ for toxicity.

The mechanism of toxicity lies in the handling of the peptides by the kidney. Because of their size, the peptides will be filtered in the glomerulus and into the primary urine. These peptides will then be partly reabsorbed in the proximal tubule. Here a small percent of the total administered radioactivity (1%-4%) is retained in the cells of the proximal tubules. This radioactivity can cause radiation damage to the nearby glomerulus, causing a decrease in glomerular function.

Work by several groups[16] have shown that continuous administration of high amounts (grams) of amino acids, lysine in particular, will decrease the amount of radioactivity that is retained in

the kidneys, possibly by saturation of the re-absorption mechanism. This may minimize toxicity to the kidneys and thereby improve the therapeutic index.

Dosimetry measurements

The dosing of the labeled peptides should be determined by the radiation dose that can be delivered to the tumor. The dosimetry can be done only by measuring, through gamma rays, the biodistribution of the peptide in a diagnostic test. This diagnostic/ therapeutic pair approach was described earlier.

An interesting approach has recently been described by Pauwels and associates,[17] who used PET scanning with ^{86}Y-DOTA-tyr(3)-octreotide to predict the dosimetry of the ^{90}Y-analogue. Although this is probably the most scientifically sound approach (since the use of PET allows the calculation of absolute quantifications), it is unfortunately not very practical, because the number of patients with access to PET is low and ^{86}Y is not easy to produce. Therefore, if quantification with a "normal" gamma-emitter, like ^{111}In, can provide reliable numbers, it is the method of choice.

Current status

At present, no radiolabeled peptide is approved, although several clinical trials are currently underway. Organized by Novartis, trials have been initiated in Albuquerque, New Mexico (Prof. L. Kvols), Rotterdam (Prof. E. Krenning) and Brussels (Prof. S. Pauwels) with ^{90}Y-DOTA-tyr(3)-octreotide (Octreother). Patients enrolled in these trials must have somatostatin-positive tumors as demonstrated by positive imaging with ^{111}In-pentetreotide. The status of these trials is as follows: 22 patients have entered a trial in New Mexico, which is a Phase I, uncontrolled, open-label, vertical (per-cycle) and horizontal (number of cycles) dose escalating study. The initial dose of ^{90}Y-Octreother to be administered is 25 mCi/m^2/cycle. Vertical dose escalation will proceed in 25 mCi/m2/cycle intervals and will be permitted for the next group of patients pending completion of the prior subject's Cycle 1 therapy provided there is no excessive toxicity. Horizontal dose escalation allows a subject to receive as many as four successive cycles of therapy. Up to this point, two partial responses (PR) and stable disease (SD) in six patients have been recorded; no dose limiting toxicity was seen.

Using the same compound, a similiar study is underway at the Kantonsspital in Basle.[18,19] This study also is being conducted on patients with somatostatin-positive tumors. The dose escalation in this case is "intra-patient" (i.e., patients get more ^{90}Y each time they undergo a new administration). This is in contrast to the Novartis trial, in which dose escalation is "inter-patient"(e.g., between groups of patients). In the Basel study, dosimetry is performed by co-injecting ^{111}In-DOTA-tyr(3)-octreotide. In a large number of patients, symptomatic relief of symptoms such as pain (10 out of 30 patients); tumor size reductions (7 out of 30 patients) and stabilization of disease (20 out of 30 patients) were noted after multiple administrations up to a total dose of 300 to 400 mCi ^{90}Y. Side effects seen were hematological (temporary decreases in number of platelets and lymphocytes) and nephrological (decreases in GFR in 2 patients).

The third ongoing clinical trial is using ^{90}Y-DOTA-lanreotide.[20] It is important to note that ^{90}Y-DOTA-lanreotide has a somewhat different biodistribution than ^{90}Y-DOTA-tyr(3)-

octreotide, in that its blood clearance is slower, leading to a generally higher total body dose and less rapid urine excretion (42% after 24 hours vs > 80% with ^{90}Y-DOTA-tyr(3)-octreotide).

Furthermore, ^{90}Y-DOTA-lanreotide may have different binding to the somatostatin receptor subsets (binding to subtypes 2, 3 and 5, versus binding to subtype 2 only with ^{90}Y-DOTA-tyr(3)-octreotide). It may, therefore, be possible to treat intestinal adenocarcinomas with this compound.

Dosimetry is being performed using ^{111}In-DOTA-lanreotide. Using this compound, tumor doses of up to 60 mGy/MBq (220 rad/mCi) could be obtained,[20] with normal organ doses ranging from 2mGy/MBq (7 rad/mCi) for kidneys to 0.3 mGy/MBq(1 rad/mCi) for bone marrow.

Clinical efficacy was reported to be encouraging. After four administrations of 1 GBq (27 mCi) over a six-month period, liver metastases had decreased 25% in size, while the uptake of ^{111}In-DOTA-lanreotide in the primary gastrinoma was remarkably reduced.

Lastly, several groups around the globe are using repeated administrations (up to 20 times) of high doses (up to about 200 mCi per administration) of ^{111}In-pentetreotide (Octreoscan)[21] to treat patients with somatostatin receptor positive tumors. An important finding is that the number of responses seems to be positively correlated with the number of receptors present on the tumor.

Here too, clinical responses could be seen in a high percentage of patients, with tumor reductions of up to 50% in a smaller number of patients. Also of interest is that up to now, no kidney toxicity is being reported in these patients, despite the huge amounts of ^{111}In administered. It is postulated that the therapeutic effect seen is caused by the Auger electrons of ^{111}In, which cause DNA damage after internalization into the receptor positive tumor cell. The lack of glomerular kidney damage also is explained by the low range of these Auger electrons. The glomerulus cannot be reached by the Auger electrons that are emitted in the proximal tubule, where reabsorption of the labeled peptide is taking place.

CONCLUSION

Radiolabeled peptides are already available for diagnosis and whether they will be used for treatment of patients will depend on the results of the ongoing (and future) clinical trials.

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QUESTIONS

1. Which of the following statements is valid?
A. In general, monoclonal antibodies have a short (5-60 minutes) biological half-life in the blood.
B. In general, labeled peptides have a short (5-24 hours) biological half-life in blood.
C. In general, labeled peptides have a short (30-180 min) biological half-life in blood.
D. In general, labeled peptides have a long (1-10 days) biological half-life in blood.

2. Radiolabeled peptides are cleared by the kidneys because _____.
A. They have high affinity to albumin
B. They have a size that will allow glomerular filtration
C. They inhibit the anti-diuretic hormone
D. They have low affinity to albumin

3. "Natural"peptides are degraded:
A. Quickly (minutes) by peptidases
B. Quickly (minutes) by the spleen
C. Slowly (hours) by peptidases
D. Slowly (hours) by the spleen

4. Once intracellular, the ^{99m}Tc of ^{99m}Tc-labeled peptides will be:
A. Transchelated to other peptides
B. Oxidized to pertechnetate
C. Reduced to Tc(I) species
D. Decay faster than when present extracellular

5. Labeled CCK can be used to diagnose/treat:
A. Medullary thyroid cancer
B. Breast cancer
C. Colon cancer
D. Melanoma

6. The mean energy of Auger electrons is in the range of:
A. GeV.
B. MeV.
C. keV.
D. eV.

7. The range in water of betas with a maximum energy of 2 MeV is:
A. 5 micrometer.
B. 50 micrometer
C. 500 micrometer
D. 5000 micrometer

8. To treat "micrometastases," which of the below mentioned radionuclides is best?
A. ^{90}Y (beta decay, max. energy 2 MeV).
B. ^{131}I (beta decay, max. energy 800 keV).
C. ^{177}Lu (beta decay, max. energy 500 keV).
D. ^{213}Bi (alpha decay, max energy 6 MeV).

9. Can radionuclides that decay by pure beta emissions be detected outside of the body by scintigraphy?
A. Yes, because they emit brehmsstrahlung.
B. Yes, because the range in tissue of the betas is enough for detection.
C. No, because they will not emit brehmsstrahlung.
D. No, because the range in tissue of the betas will not be enough for detection.

10. Which of these therapy radionuclides can be produced in high volume?
A. ^{67}Cu.
B. ^{177}Lu.
C. ^{161}Tb.
D. ^{111}Ag.

11. ^{125}I has a half-life of 60 days, ^{123}I a half-life of 13 hours. Which statement is correct?
A. The mass of 1 mCi ^{123}I is larger than that of 1 mCi ^{125}I.
B. The mass of 1 mCi ^{123}I is the same as that of 1 mCi ^{125}I.
C. The mass of 1 mCi ^{123}I is less than that of 1 mCi ^{125}I.
D. Both isotopes have no mass, because they are radioactive.

12. Which of the following methods to stabilize peptides CANNOT be used to produce metabolically stable peptides:
A. Distillation.
B. Cyclization.
C. Use of non-peptide bonds.
D. Use of non-natural peptides.

13. Which of the following characteristics for an "ideal" radionuclide-binding ligand is NOT
valid?
A. Strong binding of the radionuclide.
B. Allow labeling at room temperature.
C. Allow high specific-activity labeling.
D. Inexpensive.

14. The specification for peptide content in a non-labeled peptide preparation is typically:
A. >99%.
B. >95%.
C. 80%-95%.
D. <80%.

15. Which methods CANNOT be used to assess water content?
A. Volhard's titration.
B. Karl-Fischer's titration.
C. NIR.
D. NMR .

16. In peptides used for radiolabeling, strict specifications for heavy metals are necessary because:
A. They can be toxic to the patient.
B. They can act as a catalyst for radiolysis.
C. They can act as a catalyst for peptidolysis.
D. They can negatively influence radiolabeling.

17. Typical specifications for the 'labeled peptide content' are:
A. <99.5%.
B. >99.5%.
C. >95%.
D. >90%.

18. Which method used to decrease radiolysis is NOT valid:
A. Dilution of final product.
B. Addition of anti-oxidant.
C. Addition of anti-reductant.
D. Dispensing under nitrogen.

19. A packaging system for a pure beta emitter should consist of:
A. Lead outer system with plastic primary container.
B. Lead outer system with lead primary container.
C. Plastic outer system with lead primary container.
D. Plastic outer system and plastic primary container.

20. Carrier-free radiolabeled peptides are expected to show:
A. High target-to-non-target ratios.
B. Low target-to-non-target ratios.
C. High liver uptake.
D. Low liver uptake.

21. Which of the following toxicological studies are NOT needed for initiation of Phase I clinical studies?
A. Acute toxicity of the non-labeled peptide.
B. Biodistribution studies to assess dosimetry.
C. Pharmacodynamic studies to assess the influence of the peptide on relevant physiological systems.
D. Subacute toxicity of the non-labeled peptide.

22. Which toxicity can be expected to be important when clinically using radiolabeled peptides?
A. Fever.
B. Nausea.
C. Hyperbilirubinaemia.
D. Thrombocytopenia.

23: Which method can be used best to assess dosimetry?
A. Measuring beta emissions.
B. Measuring gamma emissions.
C. PET.
D. SPECT.

24. The probable mechanism of kidney toxicity after administration of radiolabeled peptides is:
A. Absorption of the radionuclide in the glomerulus.
B. Reabsorption of the radionuclide in the proximal tubulus.
C. Reabsorption of the radionuclide in the distal tubulus.
D. Absorption of the radionuclide in Henle's loop.

25: Which method can be used to decrease the kidney toxicity of radiolabeled peptides?
A. Administration of amino acids.
B. Administration of furosemide.
C. Administration of ascorbic acid.
D. Administration of glucose.