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Review Article

Tc-99m-labeled small biomolecules as potential radiopharmaceuticals for infection and inflammation imaging

D. Scott Edwards and Shuang Liu*

Medical Imaging Division, DuPont Pharmaceuticals Compan y, 331 Treble Cove Road, North Billerica, M A 01862, USA.
**Correspondence*

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Summary

Accurate and rapid detection of infectious and inflammatory foci is of great importance for elucidation of the cause of the disease, early prevention of the onset of complications and rapid implementation of a tailored therapeutic regimen. Numerous imaging procedures have been developed for the visualization of infectious and inflammatory lesions. Morphological and functional imaging modalities, including ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI), provide details of structural changes, variations in density and differences in proton content in tissues. Nuclear medicine procedures using Tc-99m-labeled small biomolecules can be used for *in vivo* characterization of cellular structure and function and for monitoring biological changes in the infectious tissues at the molecular level. This article will focus on those receptor-based, target-specific Tc-99m radiopharmaceuticals under investigation in preclinical or clinical studies for infection and inflammation imaging. These include Tc-99m-labeled chemotactic peptides, tuftsin receptor antagonists, LTB_4 receptor antagonists, antimicrobial peptides and a fragment of human platelet factor 4.

Introduction

Inflammation has been defined as “the reaction of the microcirculation characterized by movement of fluid and leukocytes from blood into the extracellular tissues” (1). The purpose of these reactions is to limit the spread of the injury and to repair or replace the injured tissues. Inflammation can be triggered by many stimuli, such as microorganisms or chemical, physical or immunological provocation. Infection is a specific inflammatory response to tissue damage caused by microorganisms (2). The inflammatory response is initiated and amplified by activation of mediators and systems such as cytokines, complement factors and vasoactive factors such as histamine and serotonin (3). As a response to inflammation, leukocytes migrate into the affected tissue. Vascular permeability is increased, allowing migration of large proteins and particles into the extracellular space. In addition, there is an overexpression of adhesion molecules on endothelial cells and of complementary adhesion molecules on leukocytes (3, 4). These reactions normally start within minutes from injury, resolve in hours or days and are typical of acute infections and inflammation.

If the inflammatory process persists, the character of the inflammatory lesion changes into that of chronic inflammation. In this situation, polymorphonuclear leukocytes (PMNLs) migrate out of the lesion, and vasodilation, vascular permeability and endothelial activation tend to normalize and many inflammatory symptoms may disappear. The infiltrate becomes predominantly mononuclear, consisting of lymphocytes and cells of the monocyte-macrophage series. Resolution of the process may take weeks or even years (4, 5).

Accurate and rapid detection of infectious and inflammatory foci is of great importance for elucidation of the cause of the disease, early prevention of the onset of complications and rapid implementation of a tailored therapeutic regimen. While in some cases infections are diagnosed on the basis of clinical history and physical examination of the patient, in others it is more difficult because the infections are asymptomatic or cause nonspecific symptoms. Numerous imaging procedures have

been developed for the visualization of infectious and inflammatory lesions. These include ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and nuclear medicine procedures. Ultrasonography, CT and MRI provide details of morphological changes, variations in density and differences in proton content in tissues (5). Nuclear medicine procedures can be used for *in vivo* characterization of cellular structure and function and for monitoring biological changes in the infectious tissues at the molecular level.

For a number of years, imaging inflammation and infection has been performed using Ga-67 citrate (6-8); however, the specificity is low and the exact mechanism of localization is still not well understood. In-111- and Tc-99m-labeled nonspecific polyclonal IgGs are also used for detection of infection foci (9-13). However, it usually takes 24 h to obtain diagnostically useful images due to their slow clearance from the blood pool. [Tc-99m]-HMPAO white blood cells (WBCs) (14-16) and [Tc-99m]-albumin colloid WBCs (16) have specificity for the infected foci since WBCs accumulate at the site of acute infection as part of the inflammatory response induced by bacteria. Imaging using both agents can be completed on the same day, but these procedures may impose significant risks to laboratory personnel and patients, particularly with the increasing prevalence of human immunodeficiency virus in the population (17, 18).

[F-18]-Fluorodeoxyglucose (FDG) PET has recently been proposed for imaging infection foci due to the fact that FDG-PET accumulates in various inflamed tissues. Recently, FDG-PET has been used for the detection of soft tissue and bone infection (19). The sensitivity for the patients with soft tissue and bone infections was very high (96% and 100%, respectively). The specificity is estimated to be in the range of 70 to above 90%. It was concluded that FDG-PET is a very sensitive tool for the detection and localization of infection. Infection imaging with FDG-PET relies on the fact that granulocytes and mononuclear cells use glucose as their energy source only during their metabolic burst, which takes place when activated by local triggers. In contrast to glucose, deoxyglucose cannot leave the cell once it is taken up. Therefore, it is not surprising that FDG accumulates in many types of inflamed tissue and many malignant tumors. Therefore, FDG-PET cannot be expected to be a highly specific marker for infection imaging (19).

Receptor-based radiopharmaceuticals

Diagnostic imaging largely relies on the localization of a radiotracer at the infected tissue and the contrast between the infected tissue and normal tissue. Many important biologic functions are controlled by receptors. Certain receptors are overexpressed in infected tissue compared to normal tissue. Changes in receptor concentration are also related to certain disease states. These provide the biological foundation for receptor imaging, a noninvasive way of assessing biochemical changes in disease states and monitoring the effect of treatment of infectious diseases.

Receptor imaging is usually reserved for localization of a radiolabeled compound that binds to receptors with high affinity and specificity (20-25). The high specificity of receptor binding results in selective uptake and distribution of the radiolabeled receptor ligand at the tissue, which contains a substantial concentration of the target receptor. It is this high receptor binding affinity and specificity that makes receptor imaging advantageous over traditional scintigraphic imaging using simple technetium complex radiopharmaceuticals and over other diagnostic modalities (20-25).

General considerations

Development of a specific agent for infection imaging remains a major challenge for investigators in nuclear medicine. The new target-specific infection imaging agent has to demonstrate biological efficacy, including high specificity and high uptake at the site of infection, and be able to rapidly delineate the infection foci and the extent of the lesion. It should have a favorable pharmacokinetic profile (fast and high focal uptake at the infection site, short blood residence time and rapid renal clearance). A kit formulation is often required due to the 6 h half-life of Tc-99m. Injection of the whole or part of the reconstituted kit should not cause any significant biological effect, such as neutropenia. The agent should have high radiochemical purity (RCP $\geq$ 90%) and high solution stability, with a shelf life of preferably $\geq$ 6 h.

Pharmacokinetic considerations

The main pharmacokinetic consideration in developing a target-specific radiopharmaceutical is that the radiolabeled receptor ligand is able to have high target uptake with a diagnostically useful target-to-background ratio (T/B) in a short period of time (20, 26). To achieve this goal, the new radiopharmaceutical should have a relatively short blood residence time. The fast blood clearance is necessary to minimize non-target radioactivity. The time for the radiolabeled receptor ligand to reach the target should also be short. Otherwise, it will take a long time to get diagnostically useful images. A major problem associated with radiolabeled antibodies is their slow kinetics to reach the targeted tissue and to clear from circulating blood. The receptor binding rate of the radiolabeled receptor ligand should be fast and the dissociation rate slow. In this way, the radioactivity accumulation at the target tissue can be maximized. The new radiopharmaceutical must have rapid renal clearance to avoid accumulation of radioactivity in the gastrointestinal tract, which may obscure visualization of abdominal infection or inflammation. If the radiolabeled receptor ligand does not have favorable pharmacokinetics, it will be difficult to develop it into a commercial product for routine clinical

applications even if it has high and specific receptor binding.

Choice of radionuclide

Nearly 80% of all radiopharmaceuticals used in nuclear medicine are Tc-99m-labeled compounds. This is largely due to the almost optimal nuclear properties of Tc-99m. The 6 h half-life is long enough to allow a radiochemist to carry out radiopharmaceutical synthesis and for nuclear medicine practitioners to collect useful images. At the same time, it is short enough to permit the administration of millicurie amounts of Tc-99m radioactivity without significant radiation dose to the patient. The monochromatic 140 KeV photons are readily collimated to give images of superior spatial resolution. Furthermore, Tc-99m is readily available from commercial Mo-99/Tc-99m generators at a low cost.

Choice of biomolecules

Monoclonal antibodies and their fragments have been studied for their potential applications in both diagnostic and therapeutic nuclear medicine. Although considerable progress has been made in this area (9-16), clinical studies with radiolabeled antibodies have often demonstrated limited accumulation in the target and slow blood clearance due to their high molecular weight, resulting in only modest T/B ratios. Antibodies have high receptor binding affinity and high specificity. However, if the radiotracer cannot be delivered to the target efficiently and clear from the non-target organs fast enough, they are not ideal targeting molecules.

Compared to antibodies, small biomolecules often show equivalent or higher binding affinity for the targeted receptor. They can tolerate harsher chemical conditions for modification or during radiolabeling. Small biomolecules are easy to synthesize and modify, less likely to be immunogenic and can have rapid blood clearance. The faster blood clearance results in adequate T/B ratios earlier so that it is practical to use Tc-99m, the preferred radionuclide for diagnostic nuclear medicine.

Tc-99m-labeled chemotactic peptides

The chemotactic peptide fMLF is a bacterial product that initiates leukocyte chemotaxis by binding to high-affinity receptors on inflammatory cells. Many synthetic analogs of fMLF analogs bind to neutrophils and macrophages with equal or greater affinity when compared to the native peptide. Fischman and coworkers (27-32) have investigated a series of radiolabeled chemotactic peptides as infection/inflammation imaging agents. These peptides were modified with a 6-hydrazinonicotinamide (HYNIC) (27-32) or a diaminedithol chelator (33) for Tc-99m-labeling at the C-terminus (Fig. 1). The Tc-99m-labeling of HYNIC-modified chemotactic peptides can be achieved using coligands such as glucoheptonate, mannitol and glucamine. Very high specific activity (> 20,000 mCi/μM) could be achieved using glucoheptonate as the coligand. Recently, van der Laken and coworkers (34) also reported the use of the Tc-99m-labeled fMLFK-HYNIC for imaging acute infection and sterile inflammation. Animal studies have shown evidence of binding to leukocytes and localization at sites of infection. However, the binary ligand technetium complexes [Tc-99m(fMLFK-HYNIC)$(L)_2$] (L = glucoheptonate and tricine) often show poor stability and the presence of multiple species in solution. Thus, it would be difficult to develop them into a product for routine clinical use even though animal studies showed that these agents were able to localize at the site of infection (27-32, 34).

Using a combination of fMLFK-HYNIC, tricine and TPPTS, the complex [Tc-99m(fMLFK-HYNIC)(tricine)-(TPPTS)] (Fig. 1, RP463) can be synthesized in high yield and high radiochemical purity (RCP > 90%), as well as high specific activity (>10,000 mCi/μM fMLFK-HYNIC) without the postlabeling HPLC purification (35). RP463 is stable for at least 6 h in solution. Apparently, the addition of TPPTS coligand dramatically reduces the number of isomeric forms and significantly increases the solution stability of Tc-99m-labeled chemotactic peptide.

In a rabbit infection model, RP463 rapidly cleared from the blood mainly through the renal system (35). As a result of rapid blood clearance and increased uptake, the T/B ratios continuously increased from 1.5 ± 0.2 %ID/g at 15 min to 7.5 ± 0.4 %ID/g at 4 h postinjection. The infected area could be visualized as early as 2 h postinjection. A transient decrease in WBC count of 35% was observed during the first 30 min after injection of the HPLC-purified RP463 in the infected rabbit. Similar neutropenic response was also reported for the Tc-99m-labeled thienylalanyl-leucyl-phenylalanyl chemotactic peptides (36), Tc-99m-RP050 and Tc-99m-RP056 (37).

The neutropenic effect is probably due to the fact that RP463 is a highly potent agonist for chemotactic peptide receptors on leukocytes. In an *in vitro* binding assay, Tc-99m-RP463 was able to effectively compete with fMLF for receptors on PMNs with an IC_{50} value of 2 nM (35). Like the unlabeled peptide, fMLFK-HYNIC (IC_{50} = 3 ± 2 nM; EC_{50} = 0.8 ± 0.4 nM) (34), Tc-99m-RP463 also induced superoxide release of PMN with an EC_{50} value of 0.2 ± 0.2 nM (35). It is clear that the Tc-99m-labeling did not significantly alter the binding affinity even though the molecular weight after radiolabeling was more than doubled. Future research should focus on developing highly potent antagonists for chemotactic peptide receptor or other receptors on PMNLs, neutrophils and monocytes.

Tc-99m-labeled tuftsin receptor antagonists

Tuftsin is a natural macrophage activator tetrapeptide (TKPR) which is part of the Fc-portion of the heavy chain of leukophilic immunoglobulin G (IgG: residues 289-292).

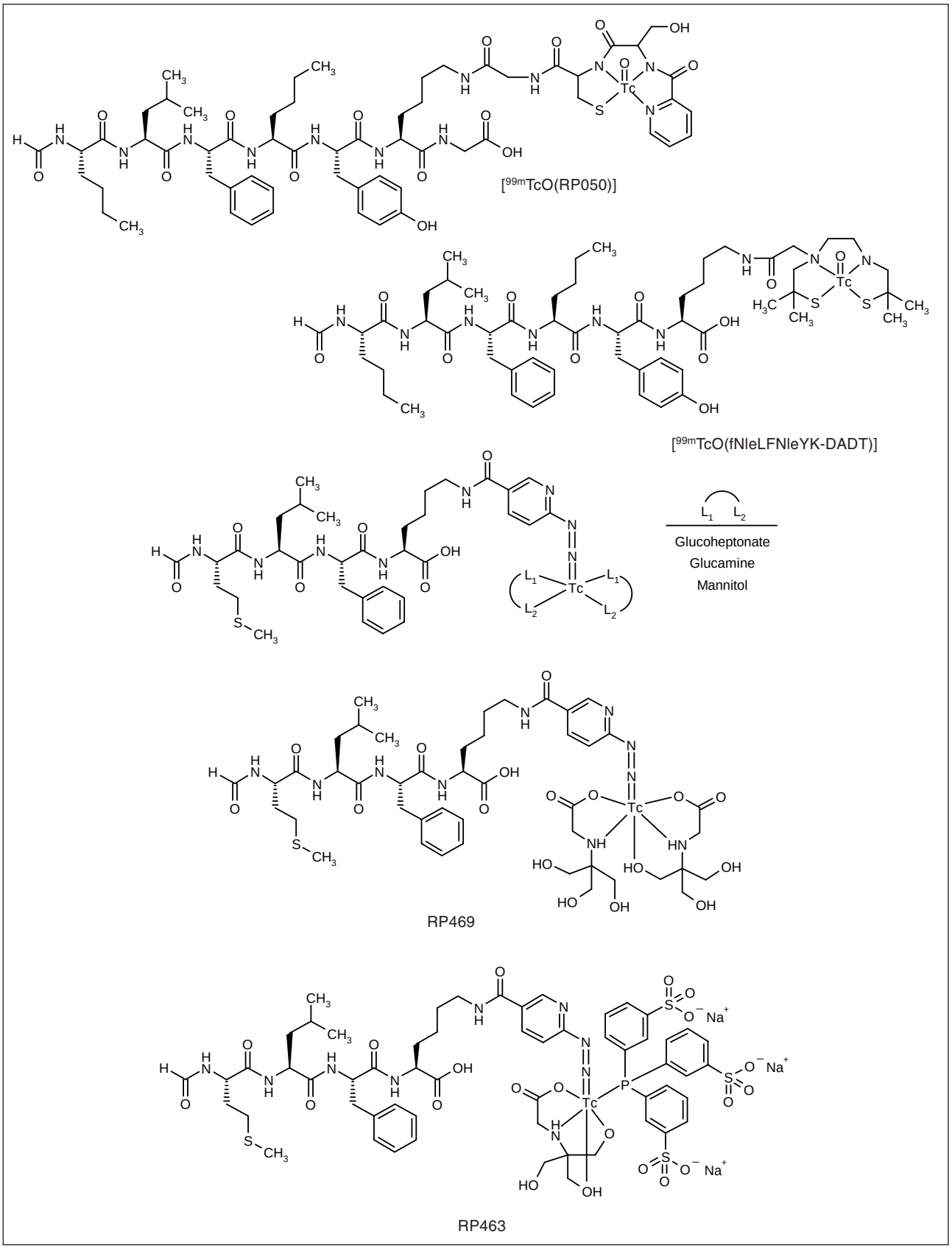

Fig. 1. Tc-99m-labeled chemotactic peptides.

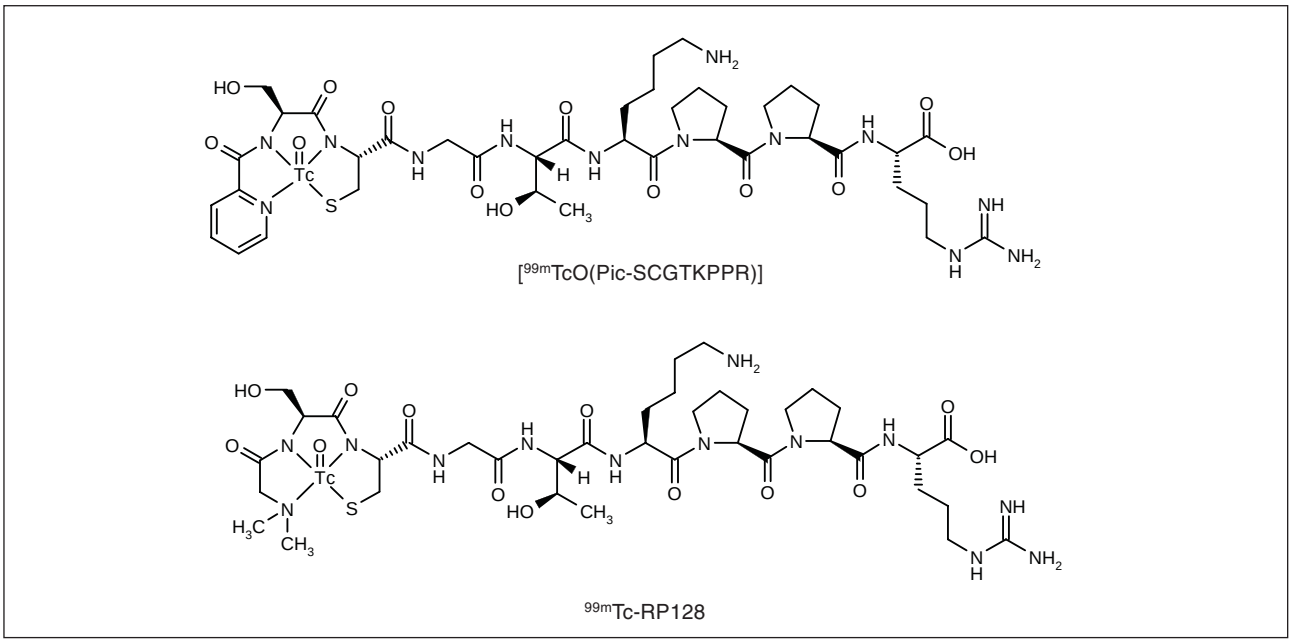

Fig. 2. Tc-99m-labeled tuftsin receptor antagonists.

The TKPR tetrapeptide is released physiologically after enzymatic cleavage and is known to bind specifically to macrophages, monocytes and PNM leukocytes, and possesses a broad spectrum of activities related primarily to the immune system fuction. It promotes phagocytosis and chemotaxis of neutrophils and monocyte/macophages through and following binding to specific receptors present in virtually all phagocytic cells. The features of tuftsin, coupled with its low toxicity, make the TKPR tetrapeptide a promising biomolecule useful for imaging infectious and inflammatory diseases.

Recently, a Tc-99m-labeled tuftsin receptor antagonist, Pic-SC(Acm)G-TKPPR (Fig. 2), was used for imaging inflammation (38). Pic is a picolinic acid residue, and the PicSC tripeptide sequence forms an N_3S chelating unit for Tc-99m-bonding. Radiolabeling was achieved by ligand exchange with Tc-99m-glucoheptonate. Animal studies showed that the Tc-99m-labeled tuftsin antagonist was able to give excellent images with T/B ratios of 3.6, 5.0 and 16.2 at 0.5, 3 and 17 h postinjection, respectively (38). Another Tc-99m-labeled tuftsin receptor antagonist is [Tc-99m-O(RP128)] (Fig. 2, RP128 = dimethylGSCGTKPPR). In an inflammatory bowel disease (IBD) model, [Tc-99m-O(RP128)] showed much better imaging quality than In-111-oxine WBCs (39). Target (inflamed terminal colon) to background (proximal noninflamed colon) ratios of 2.14, 2.51, 2.90 and 1.90 were obtained at 0.5, 1, 3 and 18 h postinjection, respectively. Both agents were rapidly excreted via the renal system. A pilot phase II clinical study demonstrated that [Tc-99m-O(RP128)] could be used to visualize inflammatory lesions in patients with Crohn's disease within 4 h (40).

Tc-99m-labeled LTB_4 receptor antagonists

Leukotriene B_4 (LTB_4) is a proinflammatory mediator which is derived from the action of 5-lipoxygenase on arachidonic acid and is synthesized primarily by PMNLs, monocytes and macrophages. LTB_4 is known to stimulate degranulation, aggregation, chemotaxis and chemokinesis of PMNLs, as well as promote superoxide generation. LTB_4 also promotes the adhesion of neutrophils to the vascular endothelium. LTB_4 receptors are highly expressed on a number of inflammatory cells such as neutrophils, monocytes, macrophages and lymphocytes. Numerous LTB_4 receptor antagonists have been evaluated for their potential in the treatment of various infectious and inflammatory diseases.

Recently, we reported a series of the Tc-99m-labeled LTB_4 receptor antagonists for imaging infection and inflammation (41-44). RP517 (Fig. 3) was evaluated in 2 animal models (guinea pig and rabbit) of focal infection. In the guinea pig peritonitis model (40), RP517 showed uptake of 0.6 ± 0.05 %ID/g at the site of infection 1 h postinjection. The radioactivity was retained with uptake of 0.5 ± 0.08 %ID/g at 4 h postinjection. RP517 is excreted mainly via the hepatobiliary route. In the rabbit infection model (41), RP517 showed similar uptake at the infection site with the T/B ratio (infected muscle/normal muscle) of 16:1 at 1 h postinjection. A primate dosimetry study indicates that the dose-limiting organ is the gall bladder. RP517 is under clinical investigation as a new imaging agent for acute infection and inflammation.

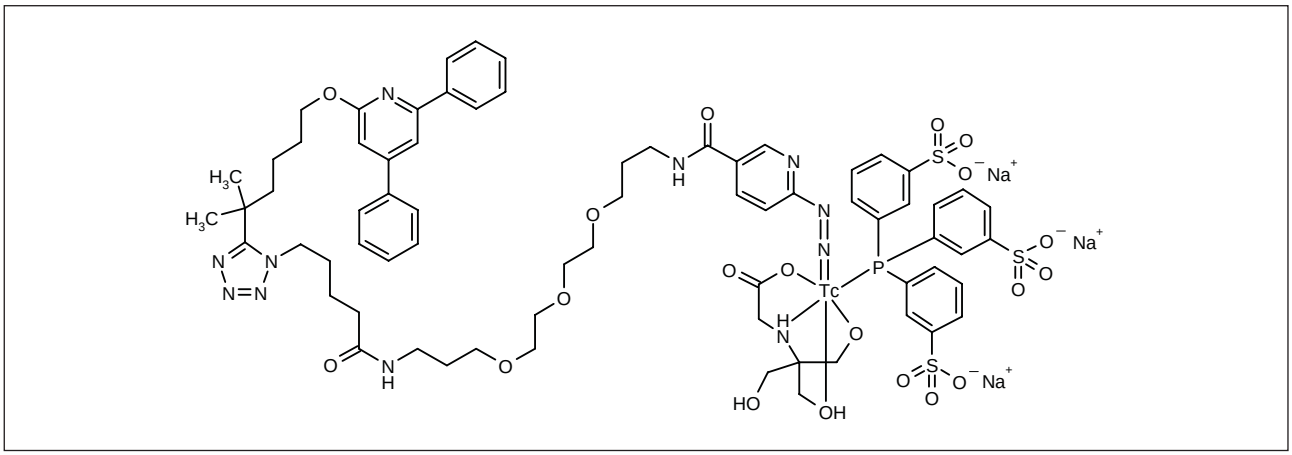

Fig. 3. A Tc-99m-labeled LTB_4 receptor antagonist.

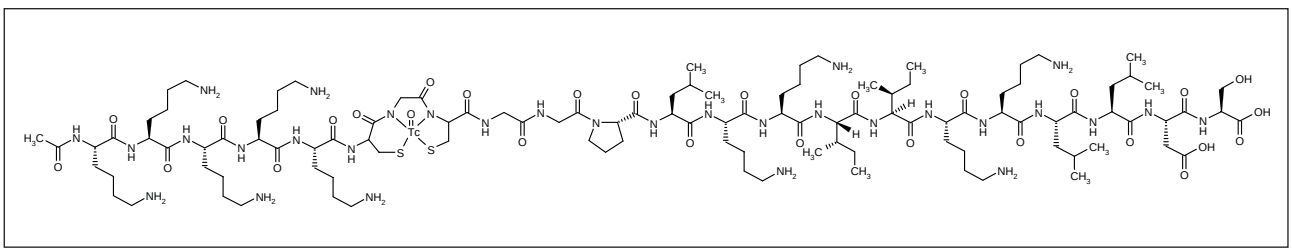

Fig. 4. A Tc-99m-labeled tridecapeptide fragment of platelet factor-4.

Tc-99m-labeled peptide P483H

Peptide P483 is a fragment of human platelet factor 4. It contains a Cys-Gly-Cys tripeptide chelating unit for Tc-99m-labeling. The pentalysine sequence on the N-terminus was used to promote renal clearance. It was reported that the combination of Tc-99m-P483 (Fig. 4) with heparin (Tc-99m-P483H) enhanced the binding to WBCs and resulted in improved uptake in sites of infection in a rabbit infection model (45). *In vitro* distribution in human blood suggests that Tc-99m-P483H associates with specific WBCs, particularly monocytes. In a rabbit infection model, Tc-99m-P483H showed slightly higher infection uptake (0.062 ± 0.022 %ID/g) than In-111-oxine-WBCs (0.051 ± 0.008 %ID/g) and a 6-fold higher T/B ratio, probably due to rapid blood clearance. Tc-99m-P483H has been studied in patients to test its usefulness as a new infection and inflammation imaging agent (46). The sensitivity and specificity are 82% and 77%, respectively. However, thyroid uptake was observed in some patients, indicating instability of the technetium chelate.

Tc-99m-labeled antimicrobial peptides

Neutrophil defensins (human neutrophil peptide [HNP]) are stored in the granules of neutrophils. In addition to their direct antimicrobial activity, the peptides have chemoattractive activity for various monocytes and lymphocytes (47). Although the various antimicrobial peptides are chemically different, most of them are cationic. It has been hypothesized that their cationic charge facilitates binding of these peptides to various microorganisms. HNP-1 was labeled with Tc-99m using a direct radiolabeling method by reducing the S-S disulfide linkage of the molecule (48). With this agent, experimental thigh infections in mice were visualized within 5 min postinjection. The abssess uptake and abscess-to-background ratios were relatively low. Most of Tc-99m-labeled antimicrobial peptides are excreted via both renal and hepatobiliary routes (49). The pharmacokinetic characteristics are not ideal for infection imaging. It was reported that Tc-99m-HNP-1 binds to bacteria rather than to leukocytes and might be able to distinguish between bacterial infection and sterile inflammation.

Conclusions

For the last several years, a large number of radiolabeled receptor ligands have been synthesized and studied for potential use as target-specific radiopharmaceuticals. Tc-99m-labeled small biomolecules have become an important class of imaging agents for the detection of various diseases, such as thrombosis, infection or inflammation and tumor. Examples described in this review

represent a new generation of diagnostic radiopharmaceuticals based on receptor binding of radiolabeled biomolecules. Results from preclinical and clinical studies have demonstrated high specificity, high uptake in the target organ and high target-to-background rations due to high receptor binding affinity and rapid blood clearance.

A major challenge in developing a new receptor-based radiopharmaceutical is the "fine tuning" of the pharmacokinetics. For a receptor-based radiopharmaceutical to be successful, it must have a favorable pharmacokinetic profile (fast and high target uptake, a relatively short blood residence time and rapid renal clearance). There are several ways to modify the pharmacokinetics of a radiopharmaceutical. These include chemical modification of the targeting molecule or technetium chelate and the use of a linker as the pharmacokinetic modifier. The chemical modification of the targeting molecule and the technetium chelate can be achieved by introducing a poly anionic or cationic peptide sequence such as polylysine, polyglycine or polyaspartic acid. The ultimate goal is to reduce the background activity and to increase the target-to-background ratio.

Development of a target-specific radiopharmaceutical requires a good understanding of fundamentals in biology, chemistry and nuclear medicine. As advances in science have led to the characterization of cellular structure and function, the evolving face of molecular nuclear medicine has mandated that we all to some extent become molecular biologist in order to understand the fundamental biochemistry of receptor-based imaging. On the other hand, studies on various diseases using receptor imaging techniques will lead to more detailed characterization of cellular structure and function and to further advances in molecular biology.

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