Development of Specific Radiopharmaceuticals for Infection Imaging by Targeting Infectious Micro-organisms

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Abstract: Infectious diseases remain a major health problem and cause of death worldwide. A variety of radiopharmaceuticals are used for the imaging of infections and inflammation in the practice of nuclear medicine. Long-term clinical use has shown that the majority of radiolabeled probes cannot distinguish between inflammation and infection. Gallium-67-citrate binds to bacteria, but also to proteins accumulating at both sterile inflammation and bacterial infection sites. Other agents are used to interact with receptors or domains on circulating and infiltrating leukocytes or to label them directly. However, these probes cannot distinguish between infection and inflammation because they are not specific to infectious micro-organisms. This review examines the recent developments and applications of radiolabeled specific agents, such as antiviral drugs, antifungal, antibiotics and antimicrobial peptides, to visualize infectious foci by targeting viruses, fungi or bacteria.

keywords: Radiolabeled antibiotics, antimicrobial peptides, antiviral drugs, technetium-99m-ubiquicidin, scintigraphy.

INTRODUCTION

Infectious diseases remain a major health problem and cause of death worldwide [1]. The sensitivity of nuclear medicine imaging makes it a suitable tool for the specific diagnosis of focal infections. It can be difficult to diagnose and localize a site of occult infection. If detected early, most infections can be cured with proper treatment, but a delayed diagnosis is associated with higher mortality. If localizing signs are present, anatomic imaging with ultrasonography or computed tomography is often used first. However, these modalities cannot usually differentiate between an infection, such an abscess, and a non-infectious process, such as a sterile fluid or inflammation. Indications for the use of nuclear medicine imaging of infections include fever of unknown origin, a suspected occult abscess, postoperative infection, suspected infection of a vascular implant, osteomyelitis, disk-space infection, and suspected infections in immunocompromised patients [2]. One advantage of nuclear medicine studies, is that the whole body can be imaged, which is important when there are no localizing signs.

A variety of radiopharmaceuticals are used to detect infection and inflammation. Long-term clinical use has shown that the majority of radiolabeled probes cannot distinguish between inflammation and infection. Gallium-67-citrate binds to bacteria and also to proteins accumulating at both sterile inflammation and bacterial infection sites [3,4]. Other agents are used to interact with receptors or domains on circulating and infiltrating leukocytes or to label them directly, such as ^{111}In-oxine, ^{99m}Tc- hexamethylpropylenamine-oxime, ^{18}F-fluordeoxyglucose, radiolabeled monoclonal anti-granulocyte antibodies (fragments), monoclonal antibodies against cytokines, tumor necrosis factor (TNF-α) and interleukin-8 or labelled chemotactic peptides and interleukins [5-16]. However, these probes cannot distinguish between infection and inflammation because they are not specific to target bacteria, fungi or viruses.

Infection specific radiopharmaceuticals can be used for diagnoses, decision-making in therapy and follow-up treatments [17]. In view of the large potential for applications in patients, the development of new and improved target-specific radiopharmaceuticals for infection imaging has been considered to be a very worthwhile aim for scientific research. This review examines the recent developments and applications of radiolabeled specific agents to visualize infectious foci by targeting viruses, fungi or bacteria.

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ANTIVIRAL DRUGS AS VIRUS-SPECIFIC IMAGING RADIOPHARMACEUTICALS

There are efficient drugs available that inhibit viral replication by binding to virion structural proteins or to the active sites of a viral enzyme. There are also antibodies that are active against viral proteins expressed on the surface of infected cells. Such drugs and antibodies have been proposed for use as probes for the detection of viral infections [18].

The recent development of recombinant oncolytic viruses encoding host reported molecules provides a proof of concept for virus-specific imaging. Images of herpes simplex virus (HSV)-infections are based on the phosphorylation of a radiolabeled thymidine analog by the viral thymidine kinase (TK), which traps radiothymidine derivatives within infected cells [19, 20]. Viral replication in cells has been mainly visualized using the following deoxythymidine analogs: [^{18}F]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-ethyluracil (FEAU) [19]; 9-(4-[^{18}F]-fluoro-3-[hydroxymethyl]butyl)-guanine (FHBG) [20]; or^{124}I-5-iodo-2′-fluoro-1-β-D-arabinofuranosyl-uracil (FIAU) [21]. Brader *et al.* [19] infected B16-F10 murine melanoma cells with replication-competent herpes simplex virus NV1023. The presence of tumor-targeting and reporter-expressing virus was assessed by [^{18}F]FEAU-positron emission tomography (PET) and confirmed by histochemical assays in an animal foot pad model of melanoma lymph node metastasis. The presence of virus-infected tumor cells was successfully imaged with [^{18}F]FEAU-PET and 8 out of 8 tumor-positive nodes were identified. There was no overlap between the radioactivity levels (ratios of lymph node to surrounding tissue) of tumor-positive and tumor-negative lymph nodes.

Based on bacteria possessing a TK whose substrate specificity is distinct from that of the major human TK, Diaz *et al.* [21] developed an imaging technique that can detect the presence of viable bacteria. Eight subjects with suspected musculoskeletal infections and one healthy control were studied by a combination of [^{124}I]FIAU-positron emission tomography and CT ([^{124}I]FIAU-PET/CT). All patients with proven musculoskeletal infections dem-

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onstrated positive [^{124}I]FIAU-PET/CT signals in the sites of concern 2 h after administration of the radiopharmaceutical. Importantly, no adverse reactions with FIAU were observed. Therefore [^{124}I]FIAU-PET/CT was sensitive for infection, however specificity has yet to be confirmed because in this study only 1 patient from a total of 9 did not have infection.

The HSV-1 TK gene should be able to be inserted into the genome of other viruses and conduct specific radionuclide imaging because the gene is comparatively small [22]. Additionally, the HSV-1 TK molecule has been used for SPECT or PET imaging of malignancies and in the assessment of gene therapy [23-25]. HSV has also been proposed to kill cancer cells, and its distribution and replication can be monitored with the [^{124}I]-FIAU radiopharmaceutical [26].

The beta herpesvirus cytomegalovirus (CMV) does not encode a TK, but employs its UL97 protein kinase to phosphorylate nucleosides. The drug acyclovir cannot be used to treat CMV infections because it can be recognized by UL97. Yet, ganciclovir is a compound with greater anti-CMV activity. [^{18}F]-FHPG (9-(1-[^{18}F]-fluoro-3-hydroxy-2-propoxi)methylguanine), an analog of ganciclovir, has been evaluated as a radiopharmaceutical to image CMV infections [27-28]. PET scanning using [^{18}F]-FHPG was shown to correctly identify CMV encephalitis in rats, as confirmed by autoradiography, but the utility of this approach was limited by the restricted entry of the radiopharmaceutical across the blood-brain barrier [29].

Bray *et al*. [18] identified a number of processes unique to viral replication that might serve as targets for radiolabeled pathogen-specific tracers. They reviewed nine different DNA and RNA virus families, identifying approved and experimental antiviral drugs that target virus-encoded molecules and might have potential as radiolabeled probes. Table **1** shows some of the potential targets and probes used to detect viral infections [18].

RADIOLABELED ANTIFUNGALS AND CHITIN-SPECIFIC AGENTS FOR FUNGAL INFECTION IMAGING

The ability to distinguish between fungal and bacterial infections is important for immunocompromised patients who are susceptible to fungal infections. Adequate antifungal treatment is often not administered at an early stage due to the low specificity of current diagnostic methods [30].

Fluconazole is a triazole drug that inhibits the fungal cytochrome P450 enzyme 14α-demethylase. Mammalian demethylase activity is much less sensitive to fluconazole than fungal demethylase. The inhibition of fungal demethylase prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols. Fluconazole has been labelled with ^{99m}Tc [30,31]. For example, in mice infected with *C. albicans* or *Aspergillus fumigatus* ^{99m}Tc-labelled fluconazole detects the active infections but not the tissues infected with bacteria or sites injected with endotoxin or heat-killed yeasts, indicating that this probe is specific to fungal infection. A good correlation between the accumulation of ^{99m}Tc-labelled fluconazole in *C. albicans*-infected thigh muscles in mice and the number of viable yeast present at the infection site was measured, indicating that ^{99m}Tc-fluconazole was suitable for monitoring the efficacy of antifungal therapy in *C. albicans* infections. However, this radiopharmaceutical was poor at detecting *A. fumigatus* infections.

Chitin is a long-chain polymer of a N-acetylglucosamine and is the main component of the cell walls of fungi. Chitinases are digestive enzymes that break down glycosidic bonds in chitin. ^{123}I-chitinase was developed in order to bind specifically to fungal cells [32]. The results revealed that this radioiodine-labelled enzyme accumulated in *C. albicans* and *A. fumigatus* infections in mice. These fungal infections can be visualized at 24 h after injection of the tracer and its accumulation correlates with the number of viable fungal cells, without visualizing bacterial infections or sterile inflammations [32]. Since radioiodinated peptides were rapidly dehalogenated *in vivo*, ^{123}I was rapidly taken up by the thyroid and stomach, resulting in disturbed scintigraphic images. Furthermore, chitinase, a 60 kDa protein, is not retained in kidneys.

The chitin-binding protein (CBP21; 21 kDa) produced by *Serratia marcescen*, binds chitin with high affinity and it has been labelled with ^{99m}Tc *via* the bifunctional chelating agent hydrazinonicotinamide (HYNIC) [33]. The maximum uptake of ^{99m}Tc-HYNIC-CBP21 was determined to occur between 5 and 7 h post injection of the radiopharmaceutical. Target to non-target (T/NT) ratios for *A. fumigatus* were significantly higher than T/NT ratios for *C. albicans*. *This difference* may be related to a different chitin percentage in their cell walls or a difference in the accessibility of the chitin. Similar results were found in *in vitro* binding studies. T/NT ratios for fungal infections were higher than T/NT ratios for

Table 1. Possible Probes for Virus-Specific Imaging that Interact Specifically with Virus-Encoded Molecules [18]

Target	Probe	Mechanism
Viral papain-like and chymotrypsin-like proteases in coronavirus	Chloropyridyl ester-derived and 2-methyl-5-amine-N-[1-(1-naphthyl)ethyl]benzamide	Inhibitors of viral papain-like and chymotrypsin-like proteases enzymes
Flaviviral proteases: NS3 protein from Dengue virus	Designed small molecules targeting the entrance of the RNA binding tunnel	Block viral replication
RNA-dependent RNA polymerase of bovine pestviruses	Pyrazolotriazolopyrimidinamine	Inhibits the RNA-dependent RNA polymerase enzyme
Hydrophobic fusion region of viral GP41 in retroviruses.	Polypeptide T-20 (HIV fusion inhibitor)	Prevents virus from undergoing the conformational change required for fusion with the cell membrane.
UL97 protein kinase of CMV (Herpesviruses)	Antiviral mirabavir	Inhibits the UL97 protein kinase enzyme
3A protein in picornaviruses	Benzimidazole analog enviroxime	Blocks viral RNA replication by inhibiting the action of the 3A protein.

bacterial infections and sterile inflammation between 5 and 7 h post injection. Radiopharmaceutical clearance occurred *via* the kidneys and urinary bladder.

RADIOLABELED OLIGOSACCHARIDES BINDING PROTEINS OF BACTERIAL MEMBRANES

Breast milk oligosaccharides act as soluble receptors for different pathogens, protecting a newborn child from infection. Shukla et. al. [34] labelled hydroxypropyl-β-cyclodextrin with technetium-99m (^{99m}Tc-HPβCD) *via* a direct method. Docking studies demonstrated the interaction between HPβCD and bacterial maltose binding protein and biodistribution studies in rats of ^{99m}Tc-HPβCD revealed that the radiolabeled oligosaccharide was eliminated mainly *via* renal excretion [34].

RADIOLABELED ANTIBIOTICS

Radiolabeled antibiotics have the potential to differentiate between sterile inflammation and infection due to their interaction with pathogens.

Ceftizoxime is a cephalosporin belonging to the β-lactam class of antibiotics. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in peptidoglycan synthesis is facilitated by transpeptidases, known as penicillin binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. β-lactam antibiotics mimic this site and competitively inhibit PBP crosslinking of peptidoglycan. Ceftizoxime has been successfully labelled with ^{99m}Tc with a labelling efficiency of 95%. The ^{99m}Tc-labelled ceftizoxime complex was stable, but a high uptake was observed in both inflamed and infected abscesses in rats [35,36]. Furthermore, high accumulation of ^{99m}Tc-labelled ceftizoxime in the liver and intestine made this compound less suitable for abdominal imaging.

Another cephalosporin, cefoperazone, was effectively and directly labelled with ^{99m}Tc [37]. Rats with *Staphylococcus aureus*-infected thigh muscles were injected with ^{99m}Tc-cefoperazone and within 1 h after injection the infected lesions were visualized.

Ciprofloxacin is the generic international name for the synthetic antibiotic belonging to the group of fluoroquinolones. Its mode of action depends upon blocking bacterial DNA replication by self-binding to an enzyme called DNA gyrase, thereby causing double-stranded breaks in the bacterial chromosome. Ciprofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Infecton®, the first ^{99m}Tc-labelled ciprofloxacin, has been substantially studied in clinical trials. The largest trial of about 900 patients was conducted in the framework of an International Atomic Energy Agency-Coordinated Research Project. In this multicenter clinical trial involving eight countries, the overall sensitivity for ^{99m}Tc-ciprofloxacin was 88% while the specificity was 82% [38]. These encouraging results promoted studies with other fluoroquinolones like norfloxacin, sparfloxacin, enrofloxacin, levofloxacin, pefloxacin, lomefloxacin, ofloxacin, moxifloxacin and rifampicin. However, none of these compounds could be used to visualize bacterial infections [39-44]. Infecton® is supposed to bind *via* an interaction of the radiometal with the nitrogen or the C=O groups of the carboxyl region of two fluoroquinolone molecules. For this interaction to occur, the reduction of ^{99m}Tc and ciprofloxacin is carried out with formamidine sulphinic acid (FSA); however, FSA may decompose during heating and produce secondary complexes with reduced ^{99m}Tc. Some works have tried to improve the production methods of ^{99m}Tc-ciprofloxacin [45,46]. Nevertheless, parallel studies showed no specific binding of ^{99m}Tc-ciprofloxacin to bacteria and additionally demonstrated controversial results regarding its uptake in aseptic inflammation [47-49]. Given that the complex structure of the Infecton® is still unknown, controversial results could be attributed mainly to the formation of various radiolabeled chemical species with different biodistribution. This problem can be overcome with the development of well-characterized technetium-99m complexes in freeze-dried kit formulations and validated analytical methods (HPLC, TLC) that give robust information about the radiochemical purity and stability of these radiopharmaceuticals. In most cases, including the patient studies with Infecton®, the presence of ^{99m}Tc-colloid, a potential by-product trapped in inflammation sites, was not investigated. The presence of ^{99m}Tc-colloid could lead to a higher number of true positives and a lower number of false positives (in cases where the infection was not confirmed by other methods). Thus, the development of a freeze-dried kit, as well as analytical methods capable of distinguishing potential radiochemical impurities are required to avoid these discrepancies. Additionally, solid testing in laboratory animals is warranted in order to obtain insight on the specificity of radiolabeled compounds. There are two different definitions of specificity: clinical specificity is a measure of the number of false-positive results whereas radiopharmaceutical specificity is related to whether an agent localizes in a lesion through a well-defined molecular process for which it is designed. If radiopharmaceutical accumulation (i.e., target-to-background ratio) continues over 24 h, the agent must be specific by the criteria of the radiopharmaceutical [17]. Recently published data report a Phase II clinical study carried out in the USA sponsored by Draximage (Draximage Inc., Quebec, Canada), the manufacturer of Infecton® kit formulation. In spite of the enhancements in standardizing the ^{99m}Tc-labelled ciprofloxacin preparation, this radiopharmaceutical showed poor specificity and accuracy in patients with osteomyelitis images taken at 2 (early) and 24 h (late) [50]. The tracer was observed to disappear from infection and inflammation sites at equal rates, raising the question of whether the uptake is non-specific and simply a blood pool effect. As Infecton® shows the detection of bacterial infections with poor specificity and accuracy, this research group seriously considered it unlikely that this radiolabeled antibiotic would be a viable method for imaging infections. Moreover, the recent press release from Draximage clearly stated that the formulation development of Infecton® targeting orthopaedic indications has, to date, not been successful. Furthermore, Draximage will allocate the resources devoted to this product to other projects [*http://www.draxishealth.com/pdf/Draxis_Q3_PR_US_GAAP.pdf*].

Langer *et al*. [51] used an alternative approach to label ciprofloxacin, synthesizing this molecule with ^{18}F for PET/CT imaging. [^{18}F]-ciprofloxacin is structurally identical to the unlabelled drug. PET/CT fusion imaging is advantageous to differentiate bone from soft tissue infections. However, [^{18}F]-ciprofloxacin showed rapid radiopharmaceutical wash-in and wash-out of the infected areas and non-specific uptake in bacterial infected lesions, suggesting that ciprofloxacin was not a suitable agent for bacteria-specific imaging [51,52].

Current studies are focused on developing other well-defined ^{99m}Tc-labelled complexes that carry fluoroquinolone antibiotics [53-55] (Fig. (**1a**) and Fig. (**1b**)). The synthesis and characterization of rhenium and technetium-99m tricarbonyl complexes with norfloxacin derivative was reported by Kyprianidou *et al*. as the first example of well-characterized complexes in the development of infection imaging agents using antibiotics. Critical evaluation (e.g. interaction with host cells and adequate control experiments), with this investigational compound is required to prove its value for specific infection imaging [54] Fig. (**1b**).

Another well-characterized fluoroquinolone-complex is the norfloxacin dithiocarbamate (NFXDTC) [55]. It was synthesized and radiolabeled with a $[^{99m}TcN]^{2+}$ intermediate to form the ^{99m}TcN-NFXDTC complex in high yield. The radiochemical purity of ^{99m}TcN-NFXDTC was over 90%, as measured by TLC and HPLC, without any notable decomposition at room temperature over a period of 6 h. The partition coefficient and electrophoresis results indicated that ^{99m}TcN-NFXDTC was lipophilic and neutral.

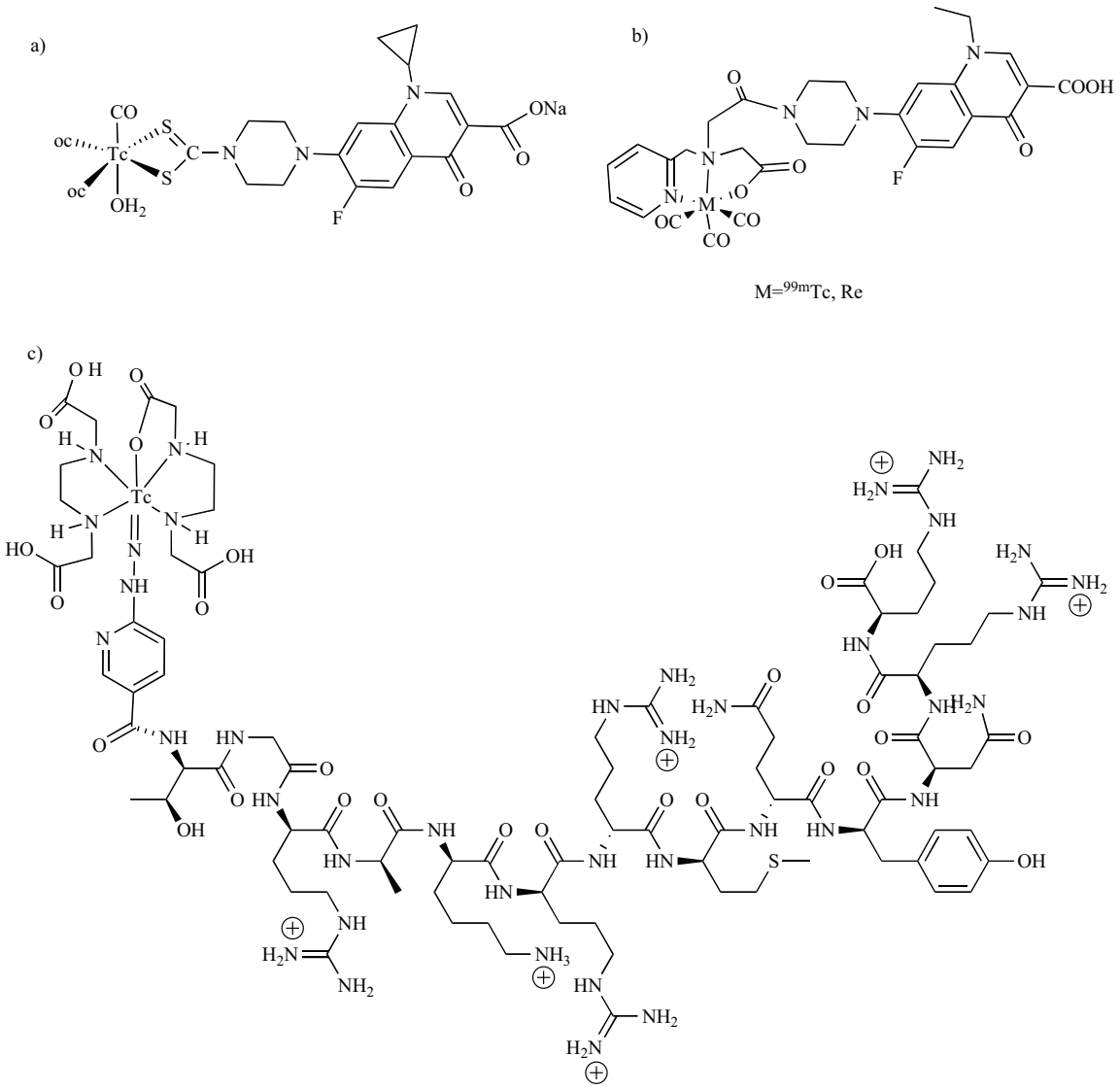

Fig. (1). a) Structure of the $^{99m}Tc(CO)_3$-dithiocarbamate ciprofloxacin [53] **b**) Structure of the $^{99m}Tc(CO)_3$-norfloxacin derivative [54] **c**) Structure of the ^{99m}Tc-UBI 29-41 cationic antimicrobial peptide. The initial mechanism of binding between the antimicrobial peptide and the bacteria is based on the interaction of the cationic domains of the peptide with the negatively charged surface of the micro-organism, but the mechanism responsible for the intracellular accumulation is the binding to a cytoplasmatic specific site on a bacterium target protein by virtue of the stereospecificity of the amino acid sequence [70].

The bacterial binding assay studies showed that ^{99m}TcN-NFXDTC had a good binding affinity. The biodistribution results showed that the abscess uptake of ^{99m}TcN-NFXDTC (3.43 ± 0.53 % ID/ g) was nearly two times better than that of ^{99m}Tc-ciprofloxacin (1.76 ± 0.20 % ID/g) at 4 h postinjection. Moreover, the abscess/ blood ratio (4.08) of ^{99m}TcN-NFXDTC was much higher than that of ^{99m}Tc-ciprofloxacin (0.62). The biodistribution results of ^{99m}TcN-NFXDTC in bacterially infected mice and in mice with turpentine-induced abscesses indicated that ^{99m}TcN-NFXDTC was suited to be a bacteria-specific infection imaging agent. Single photon emission computed tomography (SPECT) image studies showed there was a visible accumulation in infection sites [55]. Nevertheless, the lung and liver uptakes of ^{99m}TcN-NFXDTC are appreciable, thus making it unsuitable to localize the abscess in liver and lung.

Kanamycin isolated from *Streptomyces kanamyceticus* is an aminoglycoside antibiotic used to treat a wide variety of infections. Kanamycin works by affecting the 30S ribosomal subunit, causing a frame shift mutation or preventing RNA translation. Bacteria are destroyed because they cannot correctly produce any of their proteins. Kanamycin has been directly labelled with ^{99m}Tc [56]. The labelling efficiency was >97% and the complex was stable up to 6 h. About 20% of injected radioactivity accumulated in liver and 50% of the radioactivity accumulated in the urinary bladder. *S. aureus*-infected thigh muscles were visualized from 30 min to 24 h after tracer administration, indicating that ^{99m}Tc-kanamycin detected bacterial infections. However, the usefulness ^{99m}Tc-kanamycin has not been studied in animals with sterile inflammatory lesions.

Sulphanilamide is a broad-spectrum antibiotic used routinely for medical treatment of various infections. Sulphonamide is bacteriostatic, inhibiting the synthesis of bacterial folic acid synthase dihydrofolic. The N-sulphanilamide ferrocene carboxamide (N-SFC) was chemically synthesized and labelled with technetium-99m [57]. *In vitro* investigations were conducted and high stability in serum was observed up to 24 h of testing. The uptake of the tracer with living and heat/killed bacteria was compared under physiological conditions and was determined to be about 69% and 61.9% for *Escherichia coli* and *Staphylococcus aureus* strains, respectively. Biodistribution studies demonstrated that ^{99m}Tc-N-SFC rapidly accumulated at significant levels at infection sites [57].

A detail review including the structure of various antibiotics used for radiolabeling and imaging of infections has been recently published [17].

RADIOLABELED ANTIMICROBIAL PEPTIDES

Because some antimicrobial peptides selectively bind to the bacterial cell membrane, they have been proposed as potential agents to distinguish bacterial infection from sterile inflammation. On the basis of the biological features of domains present in natural antimicrobial peptides, various potential domains have been identified such as ubiquicidin (UBI), human lactoferrin (hLF) and human β-defensin-3 (HBD-3) [17].

Antimicrobial peptides are generally composed of less than 60 amino acid residues with a positive net charge, amphipathic and, in most cases, membrane active [58]. Cationic antimicrobial peptides can be broadly categorised on the basis of their secondary structure into the following four classes: a) amphipathic α-helices, such as cecropin, magainins, melittin and ubiquicidin; b) peptides with two or more disulphide bridges, such as defensins, cathelicidins, protegrins; c) peptides with one intermolecular disulphide bond have a loop/hairpin like structure; d) linear peptides lacking cystein with a high content of certain residues, such as tryptophan-rich indolicidin or proline-arginine-rich PR39 [58]. Antimicrobial peptides exhibit rapid killing often within minutes *in vitro* and a broad activity spectrum against various targets, including Gram-positive and Gram-negative bacteria, fungi, parasites, enveloped viruses and tumor cells [59]. Their mode of action against invading pathogens, rather than against mammalian cells, is based upon the architectural and biochemical composition of the cellular membrane [60]. Cationic antimicrobial peptides have a net positive charge due to an excess of basic residues, such as lysine and arginine, and are predominantly produced by phagocytes, epithelial and endothelial cells. The induction occurs upon contact with micro-organisms or microbial products like lipopolysaccharides or pro-inflammatory cytokines. The basis of antimicrobial activity is the interaction of the peptide´s cationic (positively charged) domains with the surface (negatively charged) of micro-organisms. The membranes of the latter expose negatively charged lipoteichoic acid and phospholipids, while in normal mammalian cells negatively charged lipids face the cytoplasm. These properties explain the poor binding of cationic peptides to mammalian cells at physiological conditions [58]. The interaction of these peptides with the bacterial cytoplasmic membrane results in destabilization and pore formation in the membrane, allowing leakage of cellular constituents such as potassium ions; thus, destroying the proton gradient across the membrane, resulting in bacterial death. Also, intercellular activity leading to disturbed metabolic processes, binding to DNA, inhibiting DNA synthesis and reducing mitochondrial metabolism have been reported [60]. These properties cause varying degrees of antimicrobial toxicity *via* perforation, membrane destabilization, metabolic inhibitors and triggering of bacteriolysis, making intercellular activity an important component of innate immunity against pathogenic infections [61,62]. Antimicrobial peptides display activity against bacteria, viruses and fungi *in vitro* [63-65], as well as experimental infections in laboratory animals [66-68].

The technetium-99m labelling of the antimicrobial peptide ubiquicidin was initially proposed by a research group at the Leiden University in Holland [69]. Several studies have shown that the synthetic antimicrobial peptide fragment ^{99m}Tc-ubiquicidin 29-41 (^{99m}Tc-UBI 29-41), prepared by direct and indirect methods, is highly stable both *in vitro* and *in vivo* and is also a very sensitive and specific agent for the scintigraphic detection of bacterial and fungal infections in animals and humans [69-76].

UBI 29-41 is a cationic human antimicrobial peptide fragment (MW 1.69 kDa) with the amino acid sequence Thr-Gly-Arg-Ala-Lys-Arg-Arg-Met-Gln-Tyr-Asn-Arg-Arg (TGRAKRRMQYNRR), therefore with 6 positively charged residues (5 Arg + 1 Lys). A specific mechanism exists for the bacterial intracellular accumulation as it does not concentrate in tumor cells [70]. Considering that the inhibition of bacteria protein functions requires stereospecificity, it has been proposed that the first interaction of ^{99m}Tc-UBI 29-41 with the bacterium membrane lipids is based on the interaction of the cationic domains of the peptide with the negatively charged surface of the micro-organism in order to transverse, but that the mechanism responsible for the intracellular accumulation is the binding to a cytoplasmatic specific site on a bacterium target protein by virtue of the stereospecificity of the amino acid sequence Fig. (**1c**) [70].

After effective and stable technetium-99m labelling of UBI 29-41 through either the amine groups of arginine and lysine (direct method) or N_3S, N_2S_2 or HYNIC chelators, technetium-99m labelled UBI 29-41 accumulates at sites of infection with a rapid background clearance (renal excretion), minimal accumulation in nontarget tissues and detection of rapid infection in animals and humans with no adverse reactions [72-82]. ^{99m}Tc-UBI 29-41 allows rapid visualization of gram-positive and gram-negative bacterial infections with little or no accumulation in sterile inflammatory processes, indicating that this peptide directly tags the microorganisms at the site of infection [72-82].

Data of recent clinical trials with ^{99m}Tc-UBI 29-41 regarding its sensitivity, specificity and accuracy in detecting various types of infections have been reported Table **2**. The preparation of ^{99m}Tc-UBI 29-41 as a kit formulation is an easy, rapid, and reproducible process, and the radiopharmaceutical is very well tolerated by patients [73, 83]. After ^{99m}Tc-UBI 29-41 has been injected into patients, the biodistribution and dosimetry are favorable over other radiopharmaceuticals used for imaging of infections [72]. Patients with fevers of unknown origin, osteomyelitis, diabetic foot, prosthesis infection, septic arthritis or bacteremia were successfully imaged with ^{99m}Tc-UBI 29-41 scintigraphy (Fig. (**2**) and Fig. (**3**)) [83]. Data from 198 patients demonstrate that ^{99m}Tc-labeled UBI 29-41 is a promising agent for the specific detection of infections in humans because of its high sensitivity (96.3%), specificity (94.1%) and accuracy (95.3%) with high positive predictive (95.1%) and negative predictive values (95.5%) [83].

In particular, the absolute and relative frequencies of ^{99m}Tc-UBI 29-41 to detect infection foci in patients with fevers in this study or FUO were determined [84]. Images (207) from 196 patients with FUO acquired with ^{99m}Tc-UBI 29-41 and SPECT were analyzed by two nuclear medicine physicians and classified as being either positive or negative for infection foci. The diagnostic value was corroborated with bacterial cultures of biopsies, blood and urine, plus laboratory studies, morphological images (radiographs, nuclear magnetic resonance, and computed tomography) and the clinical history of each patient. The specificity of ^{99m}Tc-UBI 29-41 for localizing infection foci and for discarding sterile inflammation was 95.35%, the sensitivity was 97.52%, the positive predictive value was 96.72%, the negative predictive value was 96.47%, the accuracy was 96.62% and the observed agreement between the bacterial culture and the molecular image was 96.62% [84].

The non-invasive diagnosis of vertebral osteomyelitis is difficult. Recently, Dillmann-Arroyo *et al* [85] evaluated the utility of ^{99m}Tc-UBI 29-41 in the diagnosis of 27 patients with suspected vertebral osteomyelitis. The final diagnosis was obtained with a histopathologic study, a microbiologic culture, or with the clinical findings after a follow-up of at least six months. The sensitivity, specificity, positive and negative predictive values and the positive and negative probability ratio were determined using a 95% confidence interval (CI). Twenty patients had positive scans. The sensitivity and the specificity for detecting pyogenic vertebral osteomyelitis were 100% and 87.5%, respectively. [85].

Vallejo *et al*. [86] evaluated the clinical utility of ^{99m}Tc-UBI 29-41 for the detection of mediastinitis after cardiac surgery. Thirteen

Table 2. Clinical Trials of the ^{99m}Tc-UBI 29-41 Antimicrobial Peptide to Detect Infections in Patients by Nuclear Imaging

Disease [Ref]	Number of Patients	Sensitivity (%)	Specificity (%)	Accuracy (%)
Osteomyelitis, diabetic foot, prosthesis infection, septic arthritis, or bacteraemia [83]	198	96.3	94.1	95.3
Fever of unknown origin [84]	196	97.5	95.3	96.6
Vertebral osteomyelitis [85]	27	100.0	87.5	96.3
Mediastinitis after cardiac surgery [86]	13	83.0	100.0	92.0
Osteomyelitis [87]	20	100.0	100.0	100.0
AVERAGE		95.4	95.4	96.0

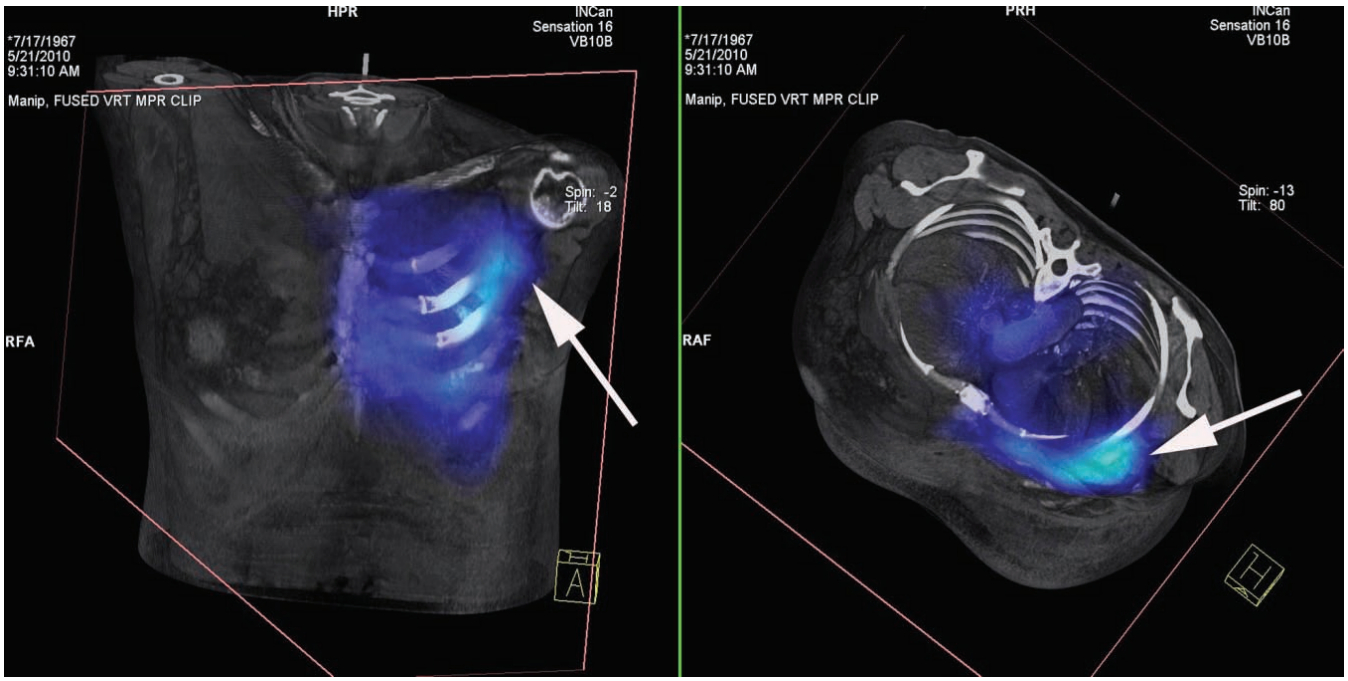

Fig. (2). SPECT/CT images of radiopharmaceutical uptake (indicated with the arrow) in the left pectoral muscle from a patient with myositis and cellulitis 2 h after administration of ^{99m}Tc-UBI 29-41. The infection process was confirmed by biopsy.

patients were included in this study. Mediastinitis was confirmed by bacterial culture. The sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy for detecting patients with mediastinitis were 83%, 100%, 100%, 87 % and 92%, respectively [86].

A study to determine the accuracy of the ^{99m}Tc-UBI 29-41 scan in the detection of osteomyelitis and to compare it with ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP) scan and magnetic resonance imaging (MRI) was recently reported [87]. Twenty patients with suspected osteomyelitis were included in this study. MRIs, ^{99m}Tc-UBI 29-41 and ^{99m}Tc-MDP scans were performed. In total, osteomyelitis was detected in the ^{99m}Tc-UBI 29-41 scans of 17 patients, indicating 100% accuracy, as compared with an accuracy of 90% for osteomyelitis detected in three-phase bone scans (^{99m}Tc-MDP). MRI showed 75% accuracy. The authors concluded that for fast imaging with high accuracy, ^{99m}Tc-UBI 29-41 was a suitable choice for the detection of osteomyelitis [87].

Nazari *et al.* [88] evaluated the potential of ^{99m}Tc-UBI 29-41 to assess response to antibiotic therapy in orthopedic infection. A total of 12 patients with suspected orthopaedic infection (bone, soft tissue, or prosthesis) and positive ^{99m}Tc-UBI 29-41 scans for infection were included in the study. One day after the ^{99m}Tc-UBI 29-41 scan, a bone scan (^{99m}Tc-MDP) was also performed. Eleven treated cases were analyzed in this study and divided in two groups: (a) 9 treated responders and (b) 2 treated non-responders. The erythrocyte sedimentation rate and C-reactive protein were measured in all patients, and wound cultures were also assessed. Quantitative analyses of erythrocyte sedimentation rates, C-reactive protein, and bone scans before and after the 10-14-day interval showed no significant change in either group. However, a quantitative ^{99m}Tc-UBI 29-41 scan at 30, 60, and 120 min indicated a significant reduction in radiopharmaceutical uptake after the 10-14-day interval as compared with the ^{99m}Tc-UBI 29-41 scan before this interval in the responder group. Furthermore, there was no significant change in the non-responder group. Thus, the authors concluded that the ^{99m}Tc-UBI 29-41 scan can determine response to antibiotic therapy in orthopedic infection in humans [88].

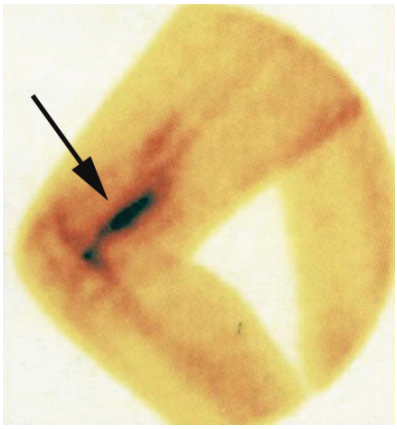

Fig. (3). SPECT image from a patient with an infectious process of the distal left femur (indicated with the arrow) 2 h after administration of ^{99m}Tc-UBI 29-41. The infection process was confirmed by biopsy.

Radiolabeled synthetic peptides derived from the N-terminus of human lactoferrin (hLF) have been less favorable for infection imaging as they displayed microbicidal activity at low concentrations. Additionally, radioactivity uptake was observed in liver, gall bladder, or intestines, making these tracers less suitable for imaging abdominal infections [89,90]. Recent data showed that the first eleven N-terminal amino acids of hLF (hLF 1-11, GRRRRS VQWCA, Mw 1,375 Da, a linear peptide comprising the first cationic domain on hLF), was more effective against a wide variety of bacteria and fungi than the full hLF protein [91]

Human β-defensin-3 (HBD-3) is an antimicrobial peptide with bactericidal effects on many gram-positive and gram-negative bacteria and some yeast species. Liberatore *et al*. [92] developed a method for radiolabeling HBD-3 with ^{99m}Tc and performed preliminary *in vivo* studies of ^{99m}Tc-HBD-3. *Staphylococcus aureus*-induced infections were used to evaluate the capability of ^{99m}Tc-HBD-3 to distinguish between an infection and aseptic inflammation in rats. Twenty to 40 mg of recombinant HBD-3 were labelled with ^{99m}Tc-hexa-coordinated with 3 molecules of CO and H_2O following subsequent separation from free pertechnetate *via* a column. ^{99m}Tc-HBD-3 was added to cultures of a bacterial suspension of *Staphylococcus aureus* and *Escherichia coli* to evaluate *in vitro* antibacterial activity. Induced infection and sterile inflammation were developed in opposite thighs of 9 adult rats. The radioactivity in tissue samples from the infected sites was significantly higher than that in samples of either induced inflammation or normal control muscle (ratio 3:1) at 3 and 5 h after injection, whereas similar radioactivity counts were observed for tissue samples from aseptic inflammation sites and normal control muscle. Thus, ^{99m}Tc-HBD-3 retained antibacterial activity and distinguished the infection from aseptic inflammation in adult rats [92].

CONCLUSIONS

The development of infection imaging agents will help clinicians monitor the success of antimicrobial therapy of infections from multi-drug resistant pathogens. The specific and fast accumulation of well-characterized complexes of ^{99m}Tc-labelled antiviral, antifungal, antibiotics and antimicrobial peptides makes them the infection-seeking agent of choice. They have now been successfully applied in clinical settings and further evaluation with different types of infections in humans will sort out the future for these promising compounds. So far, from this review it is evident that the radiopharmaceutical for infection imaging with encouraging results in clinical studies is the ^{99m}Tc-UBI 29-41 antimicrobial peptide.

Discriminating between infections and sterile inflammation by imaging with radiopharmaceuticals and monitoring antimicrobial therapy effect based on the number of bacteria is really a unique tool considering that neither CT nor MRI is able to detect micro-organisms. In clinical settings, it is important to correlate functional scintigraphic studies with anatomical imaging. SPECT as well as PET provides images for direct correlation to anatomical modalities, such as CT. These fusion methods include side-by-side, software and hardware fusion [93,94]. Fusion imaging is advantageous to differentiate bone from soft tissue infections; therefore it is being undoubtedly an important method for increasing the specificity for detecting infections using nuclear medicine procedures.

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ABBREVIATIONS

CBP21	=	Chitin-binding protein 21
CMV	=	Cytomegalovirus
CT	=	Computer tomography
FEAU	=	[^{18}F]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-ethyluracil
FHBG	=	9-(4-[^{18}F]-fluoro-3-[hydroxymethyl]butyl)-guanine
FHPG	=	9-(1-[^{18}F]-fluoro-3-hydroxy-2-propoxi)methylguanine
FIAU	=	^{124}I-5-iodo-2′-fluoro-1-β-D-arabinofuranosyl-uracil
FUO	=	Fever of unknown origin
HBD-3	=	Human β-defensin-3
hLF	=	Human lactoferrin
HPLC	=	High performance liquid chromatography
HSV	=	Herpes simplex virus
HYNIC	=	Hydrazinonicotinamide
ITLC	=	Instant thin layer chromatography
MDP	=	Methylendiphosphonate
MRI	=	Magnetic resonance imaging
NFXDTC	=	norfloxacin dithiocarbamate
PET	=	Positron emission tomography
SPECT	=	Single-photon emission computed tomography
TK	=	Thymidine kinase
UBI	=	Ubiquicidin antimicrobial peptide

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