Risk factors and new markers of pulmonary fungal infection

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Chapter 8

RADIOTRACERS FOR FUNGAL INFECTION IMAGING

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Abstract

Invasive fungal infections are recognized as an important cause of morbidity and mortality in the immunocompromised host. Rapid initiation of adequate antifungal treatment is often hampered by the limitations of current diagnostic methods. This review encompasses the promises and limitations of newer tracers (believed to target the infectious agents), i.e. radiolabeled antimicrobial peptides, antifungals and chitin-specific agents, for fungal infection imaging by scintigraphy. In mice ^{99m}Tc-labeled peptides derived from human ubiquicidin (UBI29-41) and lactoferrin (hLF1-11) distinguished local *Candida albicans* and *Aspergillus fumigatus* infections from sterile inflammatory processes, but not from bacterial infections. Clinical trials showed that ^{99m}Tc-UBI29-41 can distinguish infectious from inflammatory lesions with 80% specificity and 100% sensitivity. ^{99m}Tc-hLF1-11 was able to monitor the antifungal effects of fluconazole on *C. albicans* infections. Moreover, ^{99m}Tc-fluconazole proved to be an excellent tracer for *C. albicans* infections as it did not accumulate in bacterial infections and inflammatory processes. However this tracer poorly detected *A. fumigatus* infections. Furthermore, ^{123}I-chitinase and ^{99m}Tc-HYNIC-CBP21 accumulated in both *C. albicans* and *A. fumigatus* infections in mice at later time points. In conclusion, despite the recent advances in radiolabeled imaging techniques for invasive fungal infections, the search for better tracers for fungal infection imaging should be continued.

Abbreviations: hLF1-11: human lactoferrin peptide corresponding to residues 1-11, UBI29-41: ubiquicidin peptide corresponding to residues 29-41, LPS: lipopolysaccharide, CBP21: chitin-binding protein (21 kDa), HYNIC: hydrazino nicotinamide

Introduction

Positive blood cultures have remained the gold standard for the diagnosis of invasive candidiasis, a condition still associated with high morbidity and mortality [1]. Whether attempted treatment is successful depends on the choice of the antifungal agent and duration of therapy as well as on the elimination of the primary focus [2]. However, patients may have no evident clinical signs or symptoms pointing to the origin of the candidemia or new foci that developed through hematogenous spread. In general, intravascular catheters are most frequently involved as a primary or maintaining focus of candidemia and should then promptly be removed [3, 4]. In surgical patients, infection with *Candida* spp. and subsequent candidemia can be related to the type and site of operation, e.g. intra-abdominal abscesses, mediastinitis, vascular grafts or joint prosthesis. In patients with hematological disorders requiring treatment with bone-marrow transplantation or intensive chemotherapy fungal infections may originate from the gastro-intestinal tract, where the growth of yeasts like *C. albicans* is restricted by the microbial flora, the actions of host's immune defenses, and other local environmental conditions. Disturbance of this balance in severely immunocompromised patients, e.g. loss of the gastro-intestinal mucosal integrity and neutrophil depletion [5], can result in uncontrolled growth of *C. albicans* and lead to invasion of deeper mucosal tissue or dissemination to other organs [6]. Due to an increase in the numbers of these patients it can be anticipated that the absolute number of patients with invasive candidiasis is increasing.

It is often a major challenge for clinicians to determine the primary focus of the infection, the extent of dissemination or whether the surgical site is involved. Important clinical consequences may include extended antifungal treatment, abscess drainage or even removal of the graft or prosthesis [2, 7, 8]. Currently available techniques such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) are anatomically oriented. These are highly sensitive and sophisticated, yet lack specificity for infection, especially in early phases, when anatomic structures have not been altered. Furthermore, in presence of distortion of the normal anatomy, i.e. postsurgical changes, scarring, or the presence of implants and/or vascular grafts, the diagnostic role of these techniques is limited. Visualization of fungal infections with fluorine 18- (^{18}F)-fluoro-2-deoxy-D-glucose (^{18}F-FDG) by positron emission tomography (PET) and PET/CT procedures in patients with malignancy has been reported [9]. However, this tracer suffers from serious drawbacks, including its high radiation burden for patients and medical personnel, the requirement of a dedicated imaging equipment (PET scanner), and its short half life (110 min) puts strains on the infrastructure and logistics. No imaging technique that specifically and accurately assesses the presence of *Candida* spp. (or other yeasts/fungi) at different anatomical locations in the human body is currently available to assist clinical decision making. Scintigraphic detection of fungal infections would have the advantage of a whole body image. Thus, nuclear medicine could contribute to fungal infection imaging provided that good tracers are available [10]. The

ideal tracer for fungal infection imaging should fulfill the following criteria: *i*) rapid uptake at sites of fungal infection with little or no accumulation at sites of sterile inflammation and bacterial infections; *ii*) good stability of the labeled complex under physiological conditions; *iii*) preservation of binding activity upon labeling; *iv*) rapid clearance from the circulation with little or no accumulation in unaffected tissues, and *v*) little or no adverse effects, such as toxicity and immunological reactions [11]. Unfortunately, none of the currently available radiopharmaceuticals, e.g., gallium-67-citrate (^{67}Ga), indium-111 (^{111}In), and technetium-99m (^{99m}Tc)-labeled polyclonal human immunoglobulins or monoclonal antibodies, and ^{111}In- or ^{99m}Tc-labeled autologous leukocytes discriminates between infection and sterile inflammation [12-14]. Nevertheless, the visualization of radiolabeled leukocytes has taken a central role in infection imaging during the last decades in clinical practice, resulting in high accuracy for infection diagnosis in selected clinical settings. For obvious reasons, novel tracers for fungal infection imaging are urgently needed.

This review encompasses the current knowledge and future promises as well as the limitations of radiolabeled antimicrobial peptides, antifungals and chitin-binding agents for scintigraphic imaging of invasive fungal infections.

Antimicrobial peptides

Antimicrobial peptides are important components of innate immune system of all living organisms. They are evolutionarily ancient molecules that fend off a wide range of infections. They often contain hydrophobic and cationic amino acids, which are organized in an amphipatic structure. Most antimicrobial peptides interact through their cationic (positively charged) domains with the (negatively charged) surface of microorganisms [15, 16]. Differently from mammalian cells, where the negatively charged lipids are segregated into the inner leaflet facing the cytoplasm, microorganisms expose the negatively charged headgroups, e.g., lipopolysaccharide (LPS), lipoteichoic acids, mannoproteins, to the outer world. Main features of antimicrobial peptides are described in Box 1 and an online catalogue on cationic peptides can be consulted at: *http://www.bbcm.univ.trieste.it/~tossi/antimic.html*.

Difficulties in purifying natural antimicrobial peptides from various sources have prompted the recombinant production of antimicrobial peptides by genetically engeneered bacteria [35] or by peptide synthesis [36, 37]. Sufficient amounts of antimicrobial peptides can be produced under good laboratory practice conditions. The latter is essential for future approval to employ the (radiolabeled) peptides in clinical practice. Peptide synthesis offers the possibility to make chemical variants, such as D-enantiomers, peptides having amino acid substitutions at various positions, peptides in which chelators can be introduced on desired positions to enable labeling via a bifunctional ligand. On the basis of the chemical character-

Box 1.

Key features of antimicrobial peptides

- Antimicrobial peptides usually contain < 50 amino acids with a net positive charge owing to an excess of basic residues, such as lysine and arginine, and ~ 50% hydrophobic amino acids.
- The majority of antimicrobial peptides are derived from larger precursors harboring a signal sequence, whereas other peptides are generated by proteolysis from larger proteins (such as lactoferricin).
- Antimicrobial peptides are part of the chemical barrier against the constant microbial assault found at various anatomical sites, such as epithelia [17].
- The expression of antimicrobial peptides by cells may be constitutive or induced upon contact with microorganisms or their products, like LPS, or pro-inflammatory cytokines.
- Most antimicrobial peptides can affect both planktonic bacteria and those residing in biofilms [18, 19], viruses such as HIV [20], and fungi [21] *in vitro* [22-25] and in laboratory animals [26,28].
- In addition to their antimicrobial actions, antimicrobial peptides such as α- and β-defensins, cathelicidins (LL-37), and lactoferrin-derived peptides, participate at the interface of innate and adaptive immunity by modulating cytokine and chemokine production by a range of cell types, chemoattracting various immune effector cells [29] and mesenchymal stem cells [30], regulating autophagy in conjunction with vitamin D [31], modulating the differentiation of monocytes to macrophages [32] and dendritic cells [33] and stimulating angiogenesis and wound healing [34].

istics (amino acid sequence and secondary structure), and the biological features of domains present in natural antimicrobial peptides/proteins, various potential domains within intact antimicrobial proteins, such as human lactoferrin (hLF) and ubiquicidin (UBI), have been identified [22, 38-40]. Promising candidates for fungal infection detection were selected on the basis of *in vitro* binding studies showing a preferential binding of these peptides to microorganisms over activated human leukocytes, detection of infectious over inflammatory processes in mice, and favorable pharmacokinetics. Further investigations were performed regarding other properties, like immunological adverse effects, toxicology, and antimicrobial activity [41].

To overcome the disadvantage that UBI-derived and lactoferrin-derived peptides (as well as other antimicrobial peptides) exerting their antimicrobial activities and thus destroying their targets for infection imaging, these peptides were used for scintigraphic studies at doses lacking microbicidal activity [42].

Fluconazole

Fluconazole is the most frequently employed among the triazole antifungal agents in treating *Candida* infections in individuals with severe immunodeficiency. It inhibits the ergosterol biosynthesis pathway and, in particular, the cytochrome P450-dependent lanosterol 14α-demethylase, encoded by the *ERG11* gene. Drug-treated cells display a reduction in ergosterol content, the major component of fungal membrane, and accumulation of methylated sterol precursors, such as lanosterol. Such change in sterol composition disturbs membrane function and results in growth inhibition and death of the fungal cells. Indeed, ergosterol contributes to a variety of cellular functions, including fluidity and integrity of the membrane and the proper function of membrane-bound enzymes, such as proteins associated with nutrient transport and chitin synthesis. Ergosterol is also a major component of secretory vesicles in *Saccharomyces cerevisiae*, and has an important role in mitochondrial respiration. Indeed, mutants defective in ergosterol biosynthesis and yeasts treated with azole compounds are induced to a respiratory deficient "petite" status at a high frequency [43]. A further role of ergosterol can be found in mating conditions as demonstrated by mutations in *ERG4*, encoding the enzyme that catalyzes the last step of ergosterol biosynthesis that impair both shmoo formation and cell fusion [44].

Radiolabeling of antimicrobial peptides and fluconazole

Obligatory conditions for adequate radiolabeling include the firm attachment of radionuclide or its incorporation into the antimicrobial peptide/antifungal molecule. Furthermore the labeling yield and the stability of the tracer should be high to allow the visualisation of the infection. The chosen labeling conditions should not affect the binding activity of the peptide/antifungal to the microorganism. The radiolabel is also very important. Technetium-99m is the radiolabel of choice since it is readily available from a molybdenum generator as ^{99m}Tc-pertechnetate, at low cost. In addition, its relatively short half-life (6 hours) results in low radiation burden for the patient. Various methods of labeling peptides with ^{99m}Tc, such as the indirect labeling using the preformed chelate approach or bifunctional chelating agents and the direct labeling method, have been extensively discussed [11, 45]. The radiolabeled tracer obtained with the preformed chelate approach is chemically well-defined as the ^{99m}Tc is bound to the specific chelating moiety before incubation with the peptide, thereby not exposing the compound to harsh labeling conditions. The drawbacks of this method are that it is complex and not well-suited for kit formulation. The use of bifunctional chelating agents incorporated into the peptide molecules, like HYNIC, N_3S, DADT, amongst others, is chemically well-defined. However, introduction of a chelator may alter the lipophilicity of the peptide and thus its pharmacokinetics. Moreover, the large amounts of these chelating agents used to achieve high peptide-chelator conjugation yields require a time-consuming

purification step. The direct labeling method is, however, a simple procedure that has been used to successfully label an array of peptides and antibiotics, e.g. ciprofloxacin [46] and fluconazole [47], while keeping their biological functions intact [48]. This labeling method is performed under reducing conditions at a final pH 5-6 and requires a relatively short labeling time of approximately 10-20 min, resulting in a high labeling yield over 95% [41]. The stability of the ^{99m}Tc-peptide/fluconazole complex in diluted human serum was excellent, and the peptides/anti-infectives retained their antimicrobial activity towards microorganisms in *in vitro* killing assays [41, 42, 49, 50]. In the reaction mixtures no colloids or free radioactive pertechnetate exceeding 5% of the total ^{99m}Tc-activity were observed [41].

In vitro binding studies and scintigraphic detection of infections by ^{99m}Tc-labeled antimicrobial peptides/fluconazole

A first selection of peptides displaying a preferential binding to fungi and bacteria over activated human leukocytes was performed by testing *in vitro* an array of ^{99m}Tc-labeled synthetic peptides derived from natural human antimicrobial peptides, e.g. UBI and hLF [40, 49, 50]. The most promising peptides were ^{99m}Tc-UBI29-41 and ^{99m}Tc-hLF1-11. In an attempt to fulfill all the previously mentioned criteria ^{99m}Tc-labeled fluconazole has also been considered for fungal infection imaging [47]. The results of *in vitro* binding studies of the selected peptides and ^{99m}Tc-fluconazole to *Aspergillus fumigatus*, *Candida albicans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and activated leukocytes are shown in Table 1.

Following these investigations, ^{99m}Tc-peptides and ^{99m}Tc-fluconazole were injected into mice with a bacterial or fungal infection or sterile inflammatory process in order to study whether they could discriminate infections from sterile inflammatory lesions using scintigraphic techniques. Scintigraphic analysis revealed that these ^{99m}Tc-tracers accumulated at sites of infection, but not inflammation (Figure 1). The results are expressed as the ratio between

Table 1. In vitro binding studies of ^{99m}Tc-compounds to microorganisms and activated leukocytes[a]

Binding per 10^7 cells (% of added radioactivity)					
Compound	*Aspergillus fumigatus*	*Candida albicans*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	Activated leukocytes
^{99m}Tc-hLF 1-11	55±6	26±2	20±4	2±1	10±1
^{99m}Tc-UBI 29-41	52±3	17±1	38±4	11±3	2±0.4
^{99m}Tc-fluconazole	18±2	38±3	3±0.4	6±0.1	13±5
^{99m}Tc-IgG	17±1	17±1	58±2	36±1	8±1

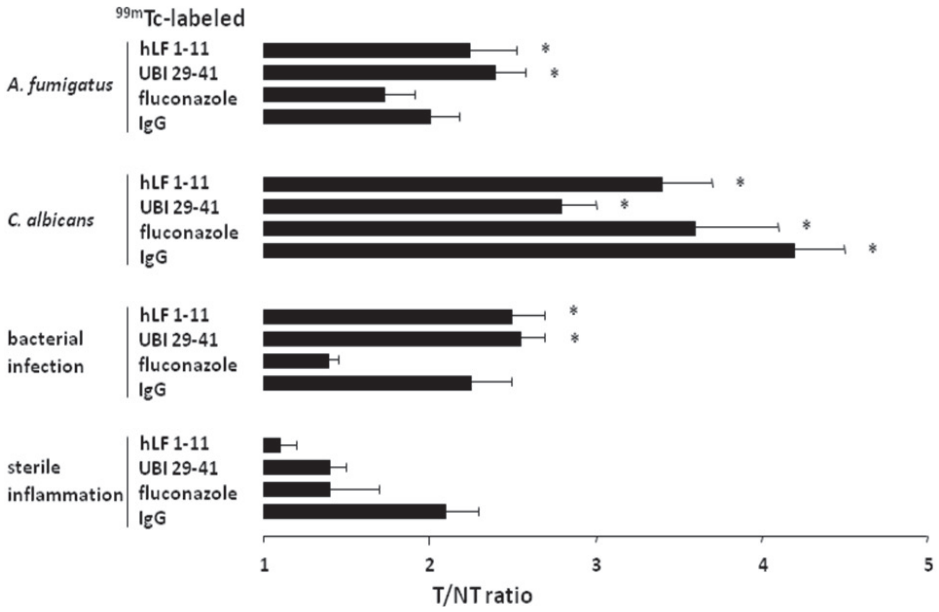
In vitro binding of ^{99m}Tc-labeled compounds to 10^7 CFU of *Aspergillus fumigatus*, *C. albicans*, *S. aureus*, *K. pneumoniae* and activated leukocytes is expressed as the percentage of added radioactivity. Activation of human leukocytes was accomplished after incubation of the cells with a combination of LPS and formyl-Met-Leu-Phe for 1 h at 37°C [Welling 04]. Values are means±SEM of at least eight observations.

[a]From Lupetti *et al.* [Current Drug Targets]

the amount of radioactivity in the infected or inflamed (target) and the non-infected or non-inflamed (non-target) thighs, further referred as to target-to-non-target (T/NT) ratio. ^{99m}Tc-human polyclonal IgG, which accumulates nonspecifically at sites of both infection and inflammation, was used as positive control. The radiolabeled antimicrobial peptides accumulated rapidly (within 30 min) in the target (infected) tissues (1-2% of the injected dose) with little accumulation at sites of sterile inflammation, indicating that fungal and bacterial infections can be distinguished from sterile inflammation by these tracers [47, 50]. After killing the animals, the number of viable microorganisms was assessed in homogenized infected thigh muscles using microbiological techniques. Target-to-non-target ratios for the different tracers are shown in Figure 1.

In contrast to ^{99m}Tc-peptides, which detected both *C. albicans* and bacterial infections in immunocompetent mice, and *A. fumigatus* in immunocompromised mice, ^{99m}Tc-fluconazole accumulated poorly in bacterial and *A. fumigatus* infected thigh muscles [47]. In contrast, a correlation (R^2=0.864, P<0.05; n=12) was found between T/NT ratios at 2 hours after injection of ^{99m}Tc-fluconazole and the number of viable *C. albicans* present in the infected thigh muscles [47]. In previous studies a correlation was seen between the accumulation of ^{99m}Tc-UBI peptides in *C. albicans*-infected thigh muscles in mice and the number of viable yeasts. In

Figure 1. Mean T/NT ratio of infected/inflamed thigh muscles after injection of ^{99m}Tc-labeled compounds.



Legend: Accumulation of ^{99m}Tc-labeled compounds in thigh muscles infected with *A. fumigatus*, *C. albicans*, bacteria (*S. aureus* and *K. pneumoniae*), or inflamed with lipopolysaccharide (LPS). Values represent mean±SEM observed at 30, 60, and 120 min after injection of tracer. * T/NT is significantly higher (P<0.05) than T/NT in mice injected with LPS.

addition, the possibility that ^{99m}Tc-UBI peptides can monitor the efficacy of antifungal therapy against *C. albicans* infection was investigated. The effect of the various concentrations of fluconazole on the accumulation of ^{99m}Tc-UBI29-41 in a fluconazole-sensitive *C. albicans* thigh muscle infection was quantified [51]. The results revealed decreasing amounts of ^{99m}Tc-UBI29-41 at the site of infection with increasing doses of this antifungal agent (R= -0.605; $P< 0.017$). As expected, an inverse correlation (R= -0.788; $P<0.001$) between the number of viable *C. albicans* and the concentration of fluconazole was observed. These data indicate that ^{99m}Tc-UBI29-41 scintigraphy can successfully monitor the efficacy of fluconazole in mice with a disseminated *C. albicans* infection, the lower detection limit being 10^3 colony forming unit (CFU).

Biodistribution

Kinetic studies using intravenously injected ^{99m}Tc-hLF1-11, ^{99m}Tc-UBI29-41, and ^{99m}Tc-fluconazole into *C. albicans* infected mice revealed that the former highly accumulates in the gall bladder and intestines, making it unfavorable for infection detection, whereas ^{99m}Tc-UBI29-41 and ^{99m}Tc-fluconazole are rapidly cleared from the circulation (half-life approximately 30-60 min) via kidneys and urinary tract with low activity in the liver and no intestinal deposits (Table 2). That the activity is mainly cleared through the kidneys is an advantage over hepatobiliary clearance because high activity in the liver and especially in the bowel would have made ^{99m}Tc-tracers less suitable for the imaging of infectious foci in the abdomen (intra-

Table 2. Biodistribution of ^{99m}Tc-labeled compounds in mice infected with *C. albicans.*

Compound	Injected radioactivity (% injected dose)								
	Bladder			*Kidneys*			*Liver*		
Time:	15 min	60 min	240 min	15 min	60 min	240 min	15 min	60 min	240 min
^{99m}Tc-hLF 1-11	12±2	18±3	27±3	15±3	15±2	19±2	24±2	26±2	38±2
^{99m}Tc-UBI 29-41	23±3	32±5	17±3	19±2	22±2	12±2	17±2	14±2	10±1
^{99m}Tc-fluconazole	29±3	34±2	29±7	24±2	22±7	22±4	19±2	10±2	8±7
^{99m}Tc-IgG	17±3	47±2	7±3	14±7	20±2	18±2	17±2	14±2	10±1

Values are means±SD of at least four observations.

From Lupetti *et al.* [02]

abdominal abscesses). In agreement, a previous study which reported on fungal imaging with ^{18}F-fluconazole and PET suffered from rather poor accumulation at sites of infection and high amounts of radioactivity were deposited in the liver [52]. Due to the very different labeling methods ^{18}F-fluconazole is more lipophilic than ^{99m}Tc-fluconazole, and thus is more readily taken up by the liver [53].

Clinical trials with ^{99m}Tc-labeled antimicrobial peptide UBI29-41

Unfortunately, to our knowledge no clinical studies that investigated imaging of fungal infections in patients by radiolabeled tracers have been reported. However, scintigraphic studies in a small series of 18 patients with suspected bacterial bone or soft-tissue infections of the limbs (including prosthesis) showed that ^{99m}Tc-UBI29-41 discriminates between bacterial infections and sterile inflammations in patients and confirmed that this tracer is without adverse effects. Human biodistribution studies showed rapid clearance of ^{99m}Tc-UBI29-41 through the kidneys with gradual accumulation in the urinary bladder in time [54, 55]. Approximately 85% of the injected activity was eliminated by renal clearance 24 hours after injection of the tracer. Accumulation at the site of infection of ^{99m}Tc-UBI29-41 reached the maximum value already from 30 min [55] to 2 hours [56] after intravenous injection. Both in bone and soft tissue infections [55] and in suspected mediastinitis after cardiac surgery [56] the specificity and sensitivity resulted to be around 80% and 100%, respectively. No accumulation of ^{99m}Tc-UBI29-41 was in agreement with negative microbiological results in culture. On the other hand, in most cases, the positive ^{99m}Tc-UBI29-41 scintigraphic images were beneficial for determining the presence of infection and confirmed in microbiological culture. Overall, ^{99m}Tc-UBI29-41 is a highly sensitive and specific agent for localizing bacterial infected foci in various body districts and discriminating them from sterile inflammatory processes.

Newer radioligands

In the attempt to develop new tracers that specifically detect fungal infections, components of fungal cell wall have been considered highly selective targets. Since chitin is a component of fungal cell wall, which is absent in mammalian cells, a radiolabeled marker for chitine, ^{123}I-chitinase was developed in order to bind specifically to fungal cells. The results revealed that this radioiodine labeled enzyme accumulates in *C. albicans* and *A. fumigatus* infections in mice; these infections can be visualized at 24 hours after injection of the tracer and its accumulation correlates with the number of viable fungal cells [57] without visualizing bacterial infections or sterile inflammations. Since radioiodinated peptides are rapidly dehalogenated *in vivo*, ^{123}I was rapidly taken up by thyroid and stomach, resulting in quite disturbed scintigraphic imaging. Furthermore chitinase, which is a protein of ~60 kDa, is not retained in kidneys. To this respect a smaller fragment like the chitin binding domain of this protein

could show better characteristics for *in vivo* imaging, such as rapid uptake, faster blood clearance and therefore faster visualization of fungal infection. Thus, the chitin-binding protein (CBP21; 21kDa) produced by *Serratia marcescens*, which binds chitin with high affinity, has been labeled with ^{99m}Tc via the bifunctional chelating agent HYNIC as a novel radiopharmaceutical for fungal infection imaging [58]. The maximum uptake of ^{99m}Tc-HYNIC-CBP21 was found between 5 and 7 hours postinjection of the tracer. T/NT ratios for *A. fumigatus* were significantly higher than T/NT ratios for *C. albicans*, maybe related to a different chitin percentage in their cell wall or difference in the accessibility of the chitin. Similar results were found in *in vitro* binding studies. T/NT ratios for fungal infection were higher than T/NT ratios for bacterial infections and sterile inflammation between 5 and 7 hours postinjection but not earlier. Clearance was via kidneys and urinary bladder but the uptake in stomach was high up till 7 hours after injection of the tracer. Since it is generally understood that stomach uptake is due to ^{99m}Tc-O_4^- and the tracer was > 97% pure, these data point to instability of the tracer *in vivo* [58]. Potential limitations of these newer tracers are the following. First, since chitin is an abundant substance in nature, chitinase or a chitin binding protein might be non specific for fungal cells. In addition, Guan *et al.* have been recently demonstrated that the chitinase BjCHI1, identified to possess two chitin-binding domains, inhibits not only the growth of fungi but also the growth of gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) more effectively than Gram-positive bacteria (*Micrococcus luteus*, *Bacillus megaterium*) through its agglutination activity [59]. Therefore, depending on the bacteria causing the infection, ^{123}I-chitinase as well as ^{99m}Tc-HYNIC-CBP21 could result in non specific tracers for fungal infections. Second, it has been recently demonstrated that acidic mammalian chitinase is produced by lung epithelial cells, macrophages and eosinophils at sites of Th2 inflammation [60], being expressed in an exaggerated fashion in epithelial and inflammatory cells in tissues from patients with moderate-severe asthma. Furthermore, the serum level of the chitinase-like protein YKL-40, that was elevated in patients with asthma, was correlated with severity, thickening of the subepithelial basement membrane, and pulmonary function [61]. Therefore, injection of these tracers in humans could have important immunological side effects.

Conclusions

Newer tracers, i.e. radiolabeled antimicrobial peptides, fluconazole and agents targeting chitin, may be useful for imaging of fungal infections. The main limitation of radiolabeled antimicrobial peptides - that discriminate between infections and sterile inflammatory processes - is that they can not distinguish fungal infections from bacterial infections. However, radiolabeled antimicrobial peptides were successful in monitoring antifungal therapy in *C. albicans*-infected mice. Moreover, radiolabeled fluconazole distinguished *C. albicans*

infections from bacterial infections/sterile inflammatory processes, but failed to image *A. fumigatus* infections. In addition, ^{99m}Tc-UBI29-41 and ^{99m}Tc-fluconazole are eliminated from the circulation mainly via the urinary tract, thus showing a favorable pharmacokinetics. Finally, radiolabeled agents that target chitin may be an interesting option for fungal infection imaging at later time points. Further studies exploring novel radioligands able to distinguish invasive fungal infections from bacterial infections as well as from sterile inflammatory processes are needed.

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