Available online at www.sciencedirect.com
ScienceDirect
Nuclear Medicine and Biology 36 (2009) 259–266
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Gamma scintigraphy imaging of murine invasive pulmonary aspergillosis with a ^{111}In-labeled cyclic peptide☆

Zhi Yang[a,1,2], Dimitrios P. Kontoyiannis[b,2], Xiaoxia Wen[a], Chiyi Xiong[a], Rui Zhang[a], Nathaniel D. Albert[b], Chun Li[a,*]

[a]*Department of Experimental Diagnostic Imaging, Infection Control and Employee Health, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA*

[b]*Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA*

Received 14 August 2008; received in revised form 9 November 2008; accepted 8 December 2008

Abstract

Introduction: Invasive pulmonary aspergillosis (IPA) is a leading cause of infection-associated death in immunosuppressed patients. Early detection and early administration of antifungal therapy are critical factors in improving outcome for patients with IPA. Here, we evaluated the imaging properties of a ^{111}In-labeled cyclic peptide targeted to *Aspergillus fumigatus* in an immunosuppressed murine model of IPA.
Methods: A cyclic peptide c(CGGRLGPFC)-NH_2 was labeled with ^{111}In by means of diethylenetriaminepentaacetic acid (DTPA). Two days after intranasal inoculation of 17.5×10^6 conidia of *A. fumigatus*, mice were injected ^{111}In-DTPA-c(CGGRLGPFC)-NH_2 intravenously. Biodistribution data were obtained at 2 h, and γ-images were acquired at 10 min and 2 h after radiotracer injection. Healthy mice were used as controls. In addition, a group of infected mice were co-injected with the radiotracer and unlabeled c(CGGRLGPFC)-NH_2 to evaluate the inhibition of radiotracer's binding to infected lungs. Autoradiographs of lungs from infected and healthy mice were compared with corresponding photographs of transaxial sections of the lung tissues stained for *A. fumigatus* hyphae.
Results: The labeling efficiency was >98%, with specific radioactivity of up to 74 MBq/nmol peptide. Significantly higher uptake of ^{111}In-DTPA-c(CGGRLGPFC)-NH_2 was observed in the lungs of mice infected with *A. fumigatus* than in those of healthy mice (0.37±0.06 %ID/g vs. 0.14±0.02 %ID/g, P=.00044). Simultaneous injection with unlabeled peptide reduced radioactivity in the infected lungs by 41% (P=.0037). Increased radioactivity in the lungs of infected mice was visible in γ images at both 10 min and 2 h after radiotracer injection. Moreover, autoradiography confirmed radiotracer uptake in infected lungs, but not in the lungs of healthy mice or infected mice co-injected with unlabeled peptide.
Conclusions: γ-Imaging with ^{111}In-DTPA-c(CGGRLGPFC)-NH_2 clearly delineated experimental IPA in mice. Peptides directly targeting fungi therefore may be valuable agents for noninvasive detection of opportunistic mycoses.

Keywords: Aspergillosis; Fungal infection; Gamma scintigraphy; Cyclic peptide; Indium-111

1. Introduction

Invasive pulmonary aspergillosis (IPA) is an important cause of morbidity and mortality in immunocompromised patients [1–4]. As the pathogenesis of IPA involves inhalation of airborne conidia by susceptible hosts, pneumonia is the most common clinical manifestation of IPA [5,6]. In high-risk patients (e.g., those with acute leukemia or those who have received a bone marrow transplant), IPA is associated with a mortality rate of 42–83%, despite the administration of systemic antifungal therapy [7,8]. The high mortality rate associated with IPA can be attributed to the

☆ This research was supported in part by John S. Dunn Foundation.
* Corresponding author. Tel.: +1 713 792 5182; fax: +1 713 794 5456.
E-mail addresses: cli@mdanderson.org, cli@di.mdacc.tmc.edu (C. Li).
[1] On leave from the Department of Nuclear Medicine, Peking University School of Oncology, Beijing Cancer Hospital, Beijing 100142, China, and from the Isotope Department, China Institute of Atomic Energy, Beijing 102413, China.
[2] These authors contributed equally to this work.

0969-8051/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2008.12.004

immunosuppression of the affected patients, the typically late diagnosis and the suboptimal in vivo efficacy of antifungal agents against *Aspergillus* species. Thus, early detection and early administration of antifungal agents, i.e., when the tissue fungal burden is relatively low, may help improve the outcomes of patients with IPA. Because of the high mortality rate of *Aspergillus* infection, there is an urgent need to develop new strategies for the early diagnosis of IPA.

In cases of suspected pulmonary infection, computed tomography (CT) provides high-quality anatomic information, but in the absence of structural morphological changes in the lungs, it is difficult to diagnose a pulmonary infection in its early stages by CT alone. Other diagnostic methods, e.g., fungal culturing and non–culture-based methods such as serodiagnosis or polymerase chain reaction, may also be used [5,9–11]. The sensitivity and specificity of these methods, however, remain suboptimal. Nuclear imaging techniques may be a valuable alternative in the diagnosis of IPA. Several nuclear imaging techniques have been evaluated for use in the diagnosis of fungal infections. In one approach, diagnosis is made based on structural and physiological changes in the lung induced by the invading microorganisms. An example of this approach is the injection and scanning of ^{67}Ga-citrate, which binds to circulating transferrin and extravasates at the site of infection because of the increased vascular permeability [12]. Radiolabeled white blood cells (WBC) that migrate toward and infiltrate the inflammatory and infectious lesions have also been used for imaging fungal infections. WBC can be labeled either ex vivo [13,14] or in vivo [15,16]. In vivo labeling of WBC can be achieved using ^{99m}Tc-labeled antigranulocyte antibody tracers [17,18]; ^{99m}Tc-labeled interleukin-8 (IL-8), which binds to IL-8 receptors [15]; or ^{111}In-labeled leukotriene B4 (LTB4) antagonist, which targets LTB4 receptors [16]. However, because in vivo cell labeling depends on radiolabeled materials' attaching to receptors expressed on neutrophils, this approach might have limited utility in the diagnosis of IPA in severely neutropenic patients. [^{18}F]Fluoro-2-deoxy-D-glucose (^{18}F-FDG) accumulates avidly in metabolically active inflammatory cells [19]. Recent evidence suggests that ^{18}F-FDG positron emission tomography (PET) imaging could be a useful tool in the diagnosis and management of opportunistic infections, including fungal infections in immunocompromised patients [20,21]. However, diagnosis of fungal infection with ^{18}F-FDG PET is not specific and is prone to errors (i.e., patients are often misdiagnosed as having a malignancy) [22].

An alternative approach for nuclear imaging of fungal infection, which may offer better detection specificity as compared to the methods described above, exploits the differences between fungi and normal host tissues or with bacteria. In this study, we investigated a ^{111}In-labeled cyclic peptide that directly targets *A. fumigatus* in a clinically relevant model of IPA. The cyclic peptide c(CGGRLGPFC)-NH_2 was identified through bacteriophage display technology and was found to bind in vitro to the surface of conidia and hyphae of *A. fumigatus* [23]. Radiolabeled peptides are promising nuclear imaging agents because of their pharmacokinetic properties, rapid binding and relatively low immunogenicity [24]. Our data suggest that ^{111}In-labeled c(CGGRLGPFC)-NH_2 can selectively accumulate in infected lungs; therefore it may facilitate diagnostic imaging of *A. fumigatus* infection.

2. Materials and methods

2.1. Materials

All *N*α-Fmoc amino acids, 1-hydroxybenzotriazole (HOBt), diisopropylcarbodiimide (DIC), triisopropylsilane (TIS) and Fmoc-Rink linker, were purchased from Novabiochem (San Diego, CA, USA). *N,N*-Diisopropylethylamine, trifluoroacetic acid (TFA), ethylenediaminetetraacetic acid (EDTA), cyclophosphamide and cortisone acetate were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Aminobenzyl diethylenetriaminepentaacetic acid (DTPA-Bz-NH_2) was obtained from Macrocyclics (Dallas, TX, USA). Indium-111 chloride ($^{111}InCl_3$) was purchased from Iso-Tex Diagnostics (Houston, TX, USA). All solvents were also obtained from Sigma-Aldrich Chemical.

2.2. Peptide synthesis

The cyclic peptide c(Cys-Gly-Gly-Arg-Leu-Gly-Pro-Phe-Cys)-NH_2 [c(CGGRLGPFC)-NH_2] was synthesized by standard solid-phase techniques using a manual synthesizer (Torviq; Granger, IN, USA) with *N*α-Fmoc/*tert*-butyl chemistry. Rink amide resin was swollen in dimethylformamide (DMF) for 1 h. The resin was washed with DMF, and *N*α-Fmoc protecting group on the resin was removed with 20% piperidine in DMF (2×20 min). The resin was washed again with DMF, and the next *N*α-Fmoc amino acid was coupled using amino acid preactivated with 0.3 M HOBt in DMF (3 eq of *N*α-Fmoc amino acid, 3 eq of HOBt and 3 eq of DIC). The resin slurry was stirred for 2 h until the Kaiser test became negative. When the coupling reaction was finished, the resin was washed with DMF; the same procedure was then repeated for the next amino acid until all the amino acids in the sequence were attached. Side chain deprotection with concomitant cleavage of the product from the resin was achieved by treating the resin with TFA/H_2O/TIS (95:2.5:2.5, v/v/v) for 3 h. The resin was removed by filtration, and 8 volumes of cold ether was added to the filtrate containing the peptide. The precipitate was collected. After several washes with ether, the crude product was dissolved in 0.1 M NH_4HCO_3, and the pH of the solution was adjusted to 8.5 using 1N NaOH. The peptide was cyclized through the disulfide bond of cysteines by passing air through the reaction mixture at room temperature for 2 h. The reaction's progress was monitored by liquid chromatography-mass spectrometry (LC-MS). Once the cyclization reaction was complete, the solution was lyophilized. The