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Dual-Energy SPECT and the Development of Peptide p5+14 for Imaging Amyloidosis

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Abstract

Amyloidosis is associated with a number of rare diseases and is characterized by the deposition, in abdominothoracic organs and peripheral nerves, of extracellular protein fibrils, which leads to dysfunction and severe morbidity. Effective clinical evaluation and management of patients with systemic amyloidosis are hampered by the lack of a noninvasive, quantitative method for detecting whole-body amyloid load. We have used a battery of assays including dual-energy SPECT imaging and comparative effectiveness studies in support of translation of a synthetic polybasic peptide, p5+14, as a novel radiotracer for visualization of amyloidosis by molecular imaging. These data provide support for a phase 1 positron emission tomography/computed tomography imaging trial of this reagent, labeled with iodine-124, in patients with all forms of systemic amyloidosis.

Keywords

advances in PET/SPECT probes, animal models of disease, animal imaging, in vivo multimodality imaging of mice, SPECT/CT

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Background

Amyloid is a complex pathology composed principally of protease-resistant protein fibrils and hypersulfated heparan sulfate (HHS) proteoglycans.[1,2] The deposition of extracellular amyloid in abdominothoracic organs and peripheral nerves is associated with ~30 rare, but often fatal, disorders. The most common forms of systemic amyloidosis are associated with the deposition of monoclonal immunoglobulin light chains (AL), transthyretin (ATTR), or leukocyte chemotactic factor-2 (ALect2). Developing reagents to accurately diagnose, quantitatively monitor, and effectively treat the amyloid in these patients remains an area of clinical importance. Presently, in the United States, diagnosis and assessment of the distribution of systemic amyloid are routinely achieved by a pathologist following the evaluation of biopsies that are stained with the cotton dye Congo red.

Although there are no molecular imaging agents approved in the United States for imaging systemic amyloid, positron emission tomography (PET) agents for the detection of Aβ amyloid in the brains of patients with Alzheimer disease have proven useful for detecting cardiac amyloidosis in patients with AL and ATTR.[3,4] However, appropriate clinical trials have not been performed to determine whether these reagents are capable of imaging all amyloid in all organs and, therefore, may suffer from the limitations of bone-seeking agents such as ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid[5,6] and ^{99m}Tc-pyrophosphate[7] that serendipitously bind only ATTR cardiac amyloid deposits. In Europe, the amyloidophilic protein, serum amyloid P (SAP) component, labeled with iodine-123 (^{123}I), is widely employed for monitoring amyloid load and distribution by gamma scintigraphy.[8,9] However, SAP is not approved by the Food and Drug Administration and does not routinely image cardiac amyloid disease.

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Rationale

To address the clinical need for an imaging agent capable of specifically and, ideally, quantitatively detecting the whole-body amyloid load, we have developed a polybasic peptide, designated p5+14, and undertaken exhaustive preclinical validation studies to support translation to a clinical trial.[10,11] Our strategy was borne from the hypothesis that systemic amyloid deposits might be specifically targeted by a reagent that binds the highly electronegative polymer heparin due to the ubiquitous presence of HHS in amyloid. Both fibrils and HHS are charged linear arrays capable of binding such a reagent.[11] There are numerous examples of small polybasic peptides, such as protamine, that bind with high affinity to heparin. We, therefore, considered that synthetic polybasic peptides containing a high content of appropriately spaced lysine residues might serve as effective specific amyloid-targeting agents.[12]

Radioiodination is an effective method of labeling peptides that does not require conjugation of chelators to lysine side chains, which are required for p5+14 interactions with heparin and amyloid. Furthermore, given that amyloid deposits are extracellular, dehalogenation of the bound radioiodinated peptide will likely be slow in contrast to an internalized peptide that would be exposed to intracellular deiodinases. Additionally, iodination with iodine-125 (^{125}I) is suitable for studying tracer biodistribution in preclinical, experimental murine models by single photon emission computed tomography (SPECT) imaging. These data would support the use of iodine-124 (^{124}I) and iodine-123 (^{123}I) for PET and SPECT imaging, respectively, in the clinical arena, thereby providing consistency and flexibility in the translation of the peptide.

Experimental Analyses

Using a panel of in vitro binding assays, including pull-down studies with synthetic amyloid fibrils and human amyloid extracts derived from patients with AL and ATTR as substrates, as well as europium-linked immunosorbent assays and surface plasmon resonance studies, we demonstrated that peptide p5+14 exhibited nanomolar affinity for both fibrils and extracts.[11] Due to the lack of experimental animal models that recapitulate the 3 most common forms of systemic amyloidosis in the United States, namely AL, ATTR, and ALect2, immunohistochemical techniques with biotinylated peptide were used to demonstrate the pan-amyloid reactivity of peptide p5+14 with tissue amyloid in situ (Figure 1A). Binding to all forms of amyloid was predicted based on the ubiquitous presence of HHS proteoglycans in all amyloid deposits, which serve as a target for binding p5+14. We confirmed, using molecular dynamics simulations, that binding of the peptide to a model fibril was dominated largely by electrostatic interactions involving the lysine side chains that align on one face of the helical peptide (Figure 1B).[11]

Despite compelling positive in vitro data, off-target reactivity and utility as a clinical imaging agent can only be validated using in vivo models of disease that provide the requisite biological complexity for determining specific tracer–target

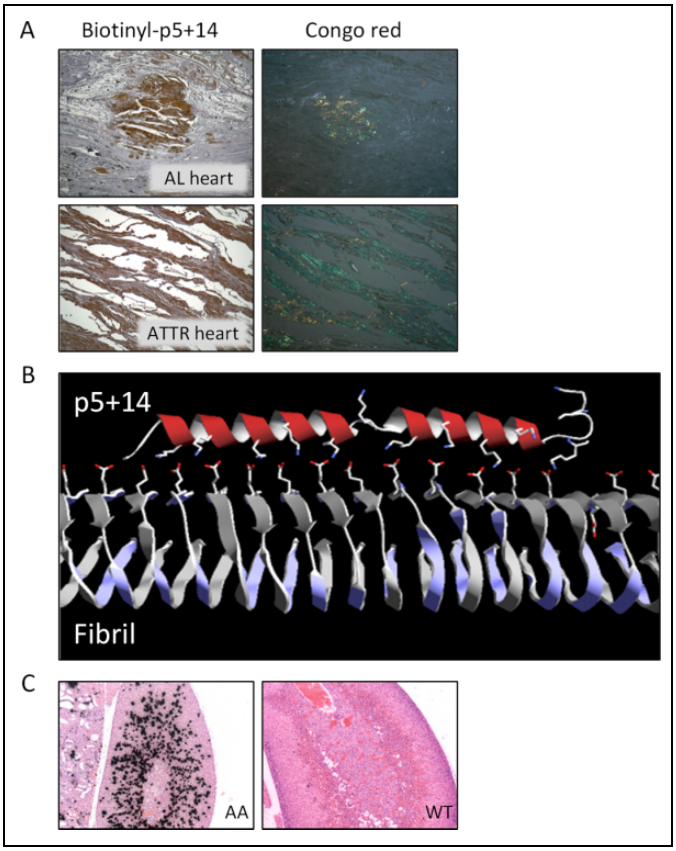

Figure 1. Peptide p5+14—A polybasic pan-amyloid reactive reagent that specifically targets amyloid. A, Biotinylated p5+14 binds AL and ATTR amyloid in formalin-fixed tissue sections as evidenced by the specific binding to areas coincident with green-blue birefringent amyloid seen in Congo red-stained tissue sections. B, Molecular dynamics simulations predicted p5+14 interactions with amyloid fibrils, here a model of an Aβ(17-42) fibril (PDB #1BEG), via electrostatic interactions with the peptide's lysine side chains. C, Specific binding of ^{125}I-p5+14 with AA amyloid deposits in vivo (eg, mouse adrenal) was demonstrated microautoradiographically, where black silver grains indicate the presence of the peptide coincident with amyloid deposits. No peptide binding was observed in amyloid-free, WT tissues. WT indicates wild-type.

interactions. When ^{125}I-labeled p5+14 was administered intravenously to H2/interleukin 6 mice, an exemplary model of severe systemic amyloidosis in which serum amyloid protein A (AA)-associated amyloid develops,[13] the tracer rapidly accumulated in organs and tissues associated with amyloid deposition as evidenced by small animal SPECT/computed tomography (CT) imaging. Microautoradiography, which affords cellular resolution biodistribution of the radioiodinated peptide, was used to show precise localization of ^{125}I-p5+14 within amyloid deposits in these organs.[11]

Importantly, similar studies in healthy mice indicated that little or no ^{125}I-p5+14 was retained by amyloid-free tissues (Figure 1C). Furthermore, the rapid dehalogenation of unbound peptide during catabolism in the renal proximal tubules, and redistribution of the radioiodide to the circulation, provided the opportunity for accurate renal amyloid

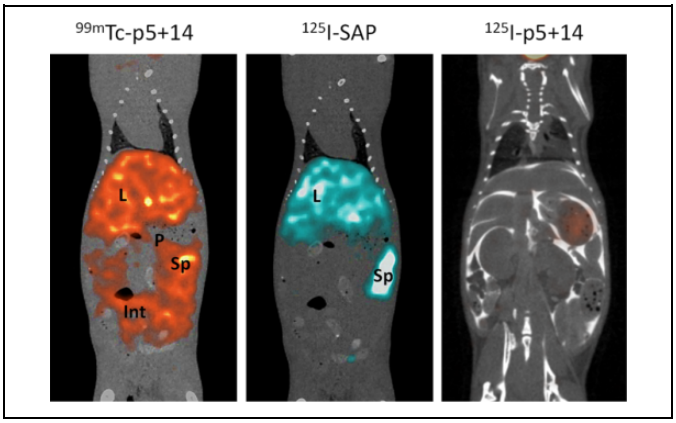

Figure 2. Comparative effectiveness evaluation of ^{125}I-SAP and ^{99m}Tc-p5+14 in a single mouse by using dual-energy SPECT imaging. The binding of peptide p5+14 to abdominothoracic amyloid was more extensive, as seen in SPECT/CT images, as compared to serum amyloid P (SAP). No retention of the peptide was observed in amyloid-free organs—only free radioiodide in the mouse stomach was observed in a WT animal in a contrast-enhanced SPECT/CT image (^{125}I-p5+14). CT indicates computed tomography; Int, intestine; L, liver; P, pancreas; Sp, spleen; WT, wild-type.

detection when imaging was performed at later time points postinjection.

Validation

In a recent report, we performed comparative effectiveness (CE) studies of p5+14 in mice with AA amyloidosis using, as a gold standard, human SAP, which is routinely employed clinically for amyloid imaging in Europe. Although CE research, defined as "*the generation and synthesis of evidence that compares the benefits and harms of alternative methods to prevent, diagnose, treat and monitor a clinical condition,*"[14(p203)] is generally considered the domain of clinical studies, we maintain that preclinical investigations of this kind provide compelling rationale for the translation of novel radiotracers to the clinic. Comparative analysis of 2 reagents usually requires the use of 2 (or more) large cohorts of study subjects—due to inherent variabilities in the population and the pathology. As an alternative, dual-energy SPECT imaging and tissue biodistribution measurements can provide a unique opportunity to evaluate 2 reagents within a single subject, given that the tracers can be independently labeled with high- and low-energy γ-emitting radionuclides. In our experiments, this approach minimized variability associated with amyloid load, anatomic site involvement, and physiological activity or dysfunction. Using this technique, ^{125}I-SAP and ^{99m}Tc-p5+14 were evaluated qualitatively by SPECT imaging and quantitatively by crossover-corrected tissue biodistribution measurements, in mice with systemic AA amyloidosis.[10] Such an approach is amenable to highly powered, within-subject statistical analysis and requires only small cohorts of experimental animals. These studies demonstrated that, with the exception of the spleen and liver, peptide p5+14 was accumulated in anatomic sites associated with amyloid deposition in significantly greater amounts, as compared to SAP. Examination of the SPECT images indicated that the p5+14 peptide bound amyloid in more anatomic sites as compared to SAP (Figure 2). This would be advantageous in the clinical setting where the goal is to provide images of whole-body amyloid burden.

In this study, our use of ^{99m}Tc-labeled p5+14 prevented accurate evaluation of renal amyloid due to retention of the ^{99m}Tc in the renal cortex during catabolism of unbound peptide. We have recently developed methods for performing dual-energy studies using ^{125}I- and ^{123}I-labeled peptides that circumvent the confounding issues associated with low-energy X-ray emission from the latter.[15]

With respect to the relatively greater hepatic and splenic uptake of SAP in the AA mice, we consider that these 2 highly vascularized, heavily amyloid-laden organs in the mouse sequestered the SAP, perhaps in a first-pass manor, and given that SAP irreversibly binds amyloid in the mouse, this prevented uptake in less accessible anatomic sites such as the intestines, pancreas, and likely the heart. This was not the case for p5+14. We have also speculated that amyloid deposits in different anatomic sites may be phenotypically distinct and, therefore, exhibit nonuniform radiotracer uptake. Amyloid heterogeneity may arise from differences in the HHS content or the biochemical and electrochemical structure of the HHS—since it is contributed by organ-specific cells that likely serve as a substratum for the formation of amyloid fibrils. Additionally, the ultrastructural morphology of the fibrils deposited in each tissue may vary. This is analogous to solid tumor cancers, where phenotyping studies have demonstrated heterogeneity not only between the primary lesion and the distant metastases but also between metastases in different anatomic locations.[16] Although conjectural, if true, phenotypic diversity of amyloid introduces additional challenges to the development of an effective pan-amyloid radiotracer and may partially explain why other amyloid-targeting agents appear selective for amyloid in particular organs and incapable of visualizing whole-body amyloid load.

Potential Impact

Given the fact that p5+14 binds the 2 major components of amyloid deposits, HHS and fibrils, and exhibits pan-amyloid reactivity, we anticipate that it may prove to be a valuable clinical tool in the management of patients with amyloidosis. In light of the compelling preclinical data, we successfully applied for assistance from the National Heart, Lung and Blood Institute's SMARTT program to provide services in support of an investigational new drug filing for a first-in-man phase 1 PET/CT imaging trial of ^{124}I-p5+14 in patients with systemic amyloidosis. At present, human grade peptide has been synthetized for the requisite stability testing as well as toxicologic and pharmacologic evaluation in rodents at 50× and 100× the human equivalent dose. Preliminary data from a dose-range finding study indicated no acute toxicity associated with the

peptide. We anticipate initiation of the phase 1 trial in late 2017. The CE studies we performed in mice with systemic amyloidosis have provided compelling support for the utility of p5+14 as an imaging agent in patients and heightened enthusiasm for its translation to a phase 1 clinical trial in patients.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: J.S.W. and S.J.K. are inventors on a US patent (# 8.808 666) that describes the use of p5+14 as an imaging agent for amyloidosis. J.S.W., S.J.K. and E.B.M. are owners of Solex LLC, which sublicensed rights to intellectual property from the University of Tennessee.

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