In vivo molecular imaging of peripheral amyloidosis using heparin-binding peptides

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AUTHOR SUMMARY

Peripheral amyloidoses are relatively uncommon diseases, with ~5,000 new cases reported annually in the United States. The disease is caused by the insidious accumulation of amyloid deposits of protein fibrils and heparan sulfate proteoglycan (HSPG) in vital organs and tissues, which can eventually lead to organ dysfunction and death. Amyloid may accumulate within a single organ such as the brain (i.e., nonperipheral amyloidosis) or pancreas, or it may systematically involve many, if not all, visceral tissues in the human body (1). The development of amyloid may be sporadic or heritable and is associated with a growing number of diseases that include Alzheimer’s disease (Aβ), light chain (AL) amyloidosis, familial Mediterranean fever (AA), and senile systemic amyloidosis (ATTR).

Current methods for diagnosing peripheral organ amyloidoses rely on the histochemical evaluation of biopsy-obtained tissue samples, whereas methods to monitor disease progression are limited to evaluating surrogate markers of organ function and whole-body scintigraphic imaging with iodine 123 (123I)-labeled serum amyloid P component, which is performed only in Europe (2). Routine anatomic imaging techniques [e.g., computed tomography (CT), MRI, and ultrasound] are widely used to assess the extent of amyloid deposition, particularly within the heart, but these methods are not amyloid-specific. Furthermore, the amyloid pathology in these diseases is rarely visualized using standard nuclear medicine agents. Because of the diverse clinical pictures presented by patients with visceral amyloidosis, noninvasive methods for detecting amyloid deposits are needed to aid diagnosis and help stage and monitor the progression or regression of disease. To this end, we have identified a heparin-binding peptide, designated p5, that reacts rapidly and specifically in vivo with murine AA amyloid in the liver, spleen, kidney, adrenal, heart, intestines, and pancreas of mice, which is evidenced in single photon emission computed tomographic (SPECT) images, radiotracer biodistribution measurements, and autoradiography (Fig. P1 A–E). This peptide was also shown histochemically to bind several types of human amyloid deposits (e.g., AA, ALκ, ALβ2, ATTR, and Ap) present in patient-derived tissue samples (Fig. P1 E and F). Our findings, therefore, show the potential use of the peptide p5 to bind and thereby, image human amyloid in patients.

Amyloid-associated HSPG attracted our interest as a potential imaging target, because it is biochemically distinct from the HSPG found in healthy tissues, which is evidenced by the finding that human AA amyloid-derived HS exhibited a shift in the ratio of disaccharides relative to HS from healthy tissue (3). Accordingly, we focused on identifying reagents that specifically target amyloid to develop amyloid imaging radiotracers for eventual evaluation in patients. Herein, we report the results of an in vivo screening study in which we evaluated seven small (~30 aa) radiolabeled peptide probes for their ability to bind amyloid. In vivo binding was shown by injecting each of the peptides radiolabeled with 125I i.v. into mice with experimentally induced severe systemic AA amyloidosis or healthy control animals. At 1 and 4 h postinjection, SPECT and X-ray (CT) images were acquired using a small animal imaging platform (4), and organs were harvested (Fig. P1 A and B). The amount of radioactivity in each tissue was quantified using standard techniques (Fig. P1E). Microautoradiography of formalin-fixed, paraffin-embedded tissue sections revealed that the radiolabeled peptide specifically associated...
ciated with tissue amyloid deposits, which were visualized by Congo red staining of consecutive tissue sections (Fig. P1 C and D). Based on these studies, a single peptide, designated p5, was identified that bound rapidly (within 1 h of injection) and specifically to amyloid in vivo, with >10% injected dose/g radiotracer at 1 h postinjection in the liver, spleen, and pancreas, which represent the major sites of amyloidosis in these mice. Additional studies revealed that this peptide was cleared rapidly or dehalogenated in healthy mice, with an effective half-life of ∼1 h, but that ∼50% of the injected dose was retained by the amyloid-laden mice for up to 24 h in sufficient amounts to be readily imaged by SPECT. In vivo reactivity of 125I-labeled p5 peptide with amyloid in the liver and spleen, which was measured by the percent injected dose per g tissue, correlated positively and significantly with the amyloid load, which was estimated qualitatively by scoring (0–4+) Congo red-stained tissue sections. To determine the distribution of the ligand recognized by the p5 peptide and deduce the potential use of this radiolabeled reagent for imaging human amyloid, we assessed in vitro the reactivity of biotinylated p5 peptide histochemically with human brain tissue from patients with Alzheimer's disease as well as diseased tissue samples obtained from patients with the three most common forms of peripheral amyloidosis, namely AL, AA, and ATTR. In each case, the peptide was shown to colocalize specifically with the amyloid, indicating its potential use as an amyloid imaging agent for patients with these diseases.

We have shown that the 125I-labeled heparin-binding peptide p5 bound specifically to amyloid in vivo, but it was not observed in significant amounts by SPECT imaging or autoradiography in healthy organs. Although we used a murine model of AA amyloidosis to select this amyloid imaging peptide from a group of seven heparin-binding reagents, we also showed the reactivity in vitro of p5 with the most common forms of human amyloid using formalin-fixed, paraffin-embedded tissue sections. Histochemical staining does not recapitulate the complicated binding conditions involved with in vivo imaging; however, this technique is widely used as a means to predict the binding specificity of radiotracers with their target ligands.

The ability to noninvasively image the extent of peripheral amyloidosis provides additional tools for the diagnosis of these diseases and affords methods for staging and monitoring response to therapy. We have identified a heparin-binding peptide that, because of its electrochemical and structural properties, bound amyloid-associated HSPG specifically and not the HSPG found ubiquitously in the extracellular matrix and plasma membranes of all healthy tissues. When radioiodinated, the p5 peptide could be used to document the presence of amyloid in visceral organs by using small animal SPECT imaging up to 24 h postinjection. Use of the 31-aa peptide p5 for imaging amyloid offers several potential advantages over large multimeric proteins such as serum amyloid P component or antibodies. Notably, small peptides are generally less immunogenic, can be chemically synthesized to high purity, and are relatively inexpensive to generate for human use compared with other biological reagents. Furthermore, this small peptide radiotracer can access amyloid in tissues with tight junction endothelia, which may hinder the penetration of larger biological radiotracers. Small peptides are also more rapidly cleared from the circulation, providing improved signal to noise ratios that will facilitate correct interpretation of subject images and result in decreased radiation exposure. The fact that 125I-p5 is retained at amyloid deposits for up to 24 h postinjection while being cleared in hours from normal tissue affords the opportunity for imaging at long time points and the potential for using long-lived radionuclides such as 124I for high-resolution, quantitative PET/CT imaging of human patients. The possibility that p5 peptide may bind many types of extracellular amyloid deposits because of the universal presence of HSPG renders it a potential pan-amyloid imaging agent for rapid and noninvasive detection of amyloidosis as well as a tool for monitoring disease progression.