Published in final edited form as:

Amyloid. 2013 March ; 20(1): 21–26. doi:10.3109/13506129.2012.757216.

A novel method for quantifying peripheral tissue amyloid load by using the radiolabeled amyloidophilic peptide, p5

Jonathan S. Wall^{1,2}, Tina Richey¹, Sally Macy¹, Eric Heide³, Craig Wooliver¹, and Stephen J. Kennel^{1,2}

¹Department of Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN, 37922. USA

²Department of Radiology, University of Tennessee Graduate School of Medicine, Knoxville, TN, 37922. USA

³Department of Graduate School of Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN, 37922. USA

Abstract

Quantitation of peripheral amyloid deposits by non-invasive molecular imaging can be useful for diagnosis, prognostication and monitoring response to therapy. In order to obtain reliable quantitative data, it is necessary to show a linear positive correlation between the uptake of the molecular probe and the tissue amyloid load. The transgenic H-2/IL-6 mouse model of AA amyloidosis was used to generate animals with varied stages of visceral amyloid disease. The mice were injected with ¹²⁵I-labeled peptide p5 and tissues analyzed 2 h post-injection using Congo red staining, radioisotope biodistribution and micro-autoradiography. Micro-autoradiography confirmed that ¹²⁵I-p5 was deposited at all amyloid deposits and sites of Congophilia but not at amyloid-free sites within the tissues evaluated. Furthermore, biodistribution studies revealed that the amount of ¹²⁵I deposited in liver and spleen correlated with the amount of Congo red birefringence (expressed as 0–4+ or as tissue area [μm^2]) in these tissues with correlation coefficients of $r > 0.7$ ($p < 10^{-6}$). Deposition of ¹²⁵I-p5 is a quantitative measure of the amount of AA amyloid in liver and spleen in this mouse model. The p5 peptide has potential as a quantitative amyloid imaging agent in human disease.

Keywords

AA amyloid; Peptides; Tissue amyloid; Biodistribution; Congo red

Introduction

The visceral amyloidoses comprise a group of protein misfolding disorders wherein insoluble fibrils are deposited in a number of tissues causing malfunction and ultimately death [1, 2]. There is a critical need to be able quantify the amyloid load in patients with these diseases. Such information can assist with prognostication and provide a longitudinal, noninvasive method to monitor regression or progression of the disease in response to

Corresponding author: Jonathan Wall, University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway, Knoxville, TN 37920, jwall@utmck.edu, Tel: (865) 305 5447.

Declaration of Interests

JSW, TR and SJK are co-founders of Solex LLC. that has licensed intellectual property related to the use of p5 peptide for imaging amyloid. JSW and SJK have intellectual property relating to the use of peptide p5.

treatment. In addition, when used during drug discovery, the ability to quantify amyloid in preclinical animal models of disease can be of importance in defining the efficacy of novel treatments and exploratory therapeutic regimens. Similarly, rapid, quantitative assessment of therapeutic efficacy could provide quick feedback regarding the efficacy of the treatment in patients and allow the physician to assess alternatives rapidly if no response is observed.

One non-invasive method for the detection of amyloid deposits is molecular imaging (positron emission [PET] or single photon emission [SPECT] tomographic imaging) using radiolabelled probes [3–7]. Imaging provides an estimate of amyloid load and also can inform the organ distribution of the deposits which heavily influences prognoses [4, 8]. Whole body imaging has obvious advantages over localized tissue biopsy techniques, but it also has drawbacks with respect to the levels of detection and ability to quantify. While advances in the physics of imaging and image analyses have successfully addressed some of these problems [9–11], it is still essential to demonstrate that the amount of target-bound radioactive probe is directly related to the concentration of the target; in this case, the amount of amyloid deposited in the organ is considered the target.

Amyloid fibrils can be made of over 25 different misfolded proteins; although, the most common are immunoglobulin light chains (AL), serum amyloid protein A (AA) and transthyretin (ATTR). Amyloid deposits, in addition to insoluble fibrils, contain a significant amount of carbohydrate including heparan sulfate (HS). HS proteoglycans (HSPGs) are a ubiquitous component of all types of extracellular amyloid deposits and can be distinguished from HSPGs in normal tissue due to their hypersulfation [12–14]. Recently, we have identified several heparin-binding peptides that bind with high affinity to amyloid deposits [7]. These peptides specifically recognize amyloid deposits in animal models of disease and in human amyloid laden formalin-fixed paraffin-embedded tissue [7, 15].

We have developed a model of AA amyloidosis, the H-2/IL-6 transgenic mice [16, 17], wherein SAA is deposited as AA amyloid in numerous visceral organs. Although these mice develop spontaneous disease as they age, AA amyloid deposition can be accelerated and synchronized by injection of 8 wk-old mice with amyloid enhancing factor (AEF). Amyloid development after AEF injection is progressive, and AA loads can be predicted with some accuracy. We have used the radiolabeled heparin-binding peptide p5 as a probe for PET and SPECT imaging of AA amyloid in the H-2/IL-6 mouse model. Image analyses have demonstrated that organs having AA deposits readily accumulate radioactive peptide [7]. To relate these data back to the amyloid load in these organs, it was necessary to demonstrate that the amount of probe deposited correlated with the amount of amyloid in that organ based on Congo red birefringence. In this paper, we have used organ biodistribution and micro-autoradiography to document that the uptake and tissue retention of radioiodinated peptide p5 is quantitative and correlates linearly with hepatosplenic amyloid burden.

Methods

Mouse model

The transgenic mouse model H-2/IL-6 constitutively expressed on a Balb/c background has been described previously [16]. Briefly, mice were bred, weaned and genotyped in the UT Medical Center vivarium in accordance with the Institutional Animal Care and Use protocol #1651. Mating wild type females with heterozygotic males yielded approximately 50% transgenic progeny. At 6–8 wk of age, mice were injected iv with 0 μ g, 1 μ g or, 10 μ g AEF derived from murine AA spleens [18, 19] suspended in 100 μ L of sterile phosphate-buffered saline (PBS). In order to sample a variety of amyloid burden, mice were analyzed 2–5 wk post-AEF for amyloid quantitation. All animal manipulations were performed in accordance

with the Guide for the Care and Use of Laboratory Animals (8th ed.) and under the auspices of Institutional Animal Care and Use Committee-approved protocols.

Radioiodination

Peptide p5 was obtained from W. M. Keck Biotechnology (New Haven, CT) and further purified by using a C3 reverse phase chromatography with a 20% - 70% acetonitrile gradient with 0.1% trifluoroacetic acid. The sequence integrity was confirmed by mass spectrometry: Peptide p5 sequence: GGGYS KAKQA QAKQA KQAQK AKAQA AKQAK Q. Radioiodination and purification of the peptide has been described [7, 20]. Briefly, 50 µg of p5 was radioiodinated in 0.5 M sodium phosphate buffer pH 7.6 with 1 mCi of ¹²⁵I-iodide (Perkin Elmer) using 20 µg chloramine T (Sigma). The reaction was quenched with a stoichiometric amount of sodium metabisulfate and the product diluted into PBS containing 0.1 mg/mL gelatin as carrier protein. The probe was purified by gel filtration using Sephadex G-25 resin (PD10; GE healthcare, Pittsburgh, PA) using PBS/gelatin solution as the mobile phase. Fractions containing radioiodinated peptide were pooled and tested for radiochemical purity in SDS-PAGE followed by phosphorimage analyses of the gels. Radiolabeled peptide had >95% radiopurity and a specific activity of ~10 µCi/µg.

Samples of ¹²⁵I-p5 were diluted in PBS/gelatin to 150 - 175 µCi/mL for iv injection (200 µL). At 2 h post-injection, mice were euthanized by isoflurane inhalation overdose. Necropsy was performed harvesting 11 tissues with one piece of each tissue placed in tared vials for biodistribution determination and another piece fixed in 10% buffered-formalin. Formalin-fixed tissues were sectioned from paraffin blocks and sequential sections used for micro-autoradiography [20] or for quantitative evaluation by Congo red staining.

Biodistribution

Biodistribution data were calculated from the net weight of tissue sample and the ¹²⁵I content measured on a Wizard gamma counter (Perkin Elmer, Santa Clara, CA). Data were expressed as the % injected dose recovered/g tissue (%ID/g).

Micro-autoradiography

Micro-autoradiography was performed using 6 µm-thick formalin-fixed paraffin-embedded tissue sections. Slides were dipped in photographic emulsion (Eastman Kodak, Atlanta, GA) and exposed for 12 d before being developed and counter-stained with hematoxylin and eosin.

Tissue Evaluation

Congo red-stained sections were evaluated by visual examination at 10× magnification by 2 observers blinded to the sample history. Basic criteria were used for qualitative scoring on a scale of 0 – 4+ including the extent of staining and the distribution of stain within the tissue. Quantitative estimation of tissue amyloid burden (µm²) was made by measuring the surface area of Congo red birefringence in the entire organ tissue section on digital images taken from 1 slide.

Statistical Methods

Descriptive and frequency statistics were run on the data to correct for data entry errors, check for outliers and assess normality. Pearson correlations were utilized to analyze the associations between the tissue samples. Statistical significance was assumed at a *p* < .05 level, and all analyses were conducted using SPSS Version 19 (SPSS Inc, Chicago, IL).

Results

Peptide p5 was readily radioiodinated with ^{125}I and yielded a single species when resolved using SDS gel electrophoresis, indicating the absence of high molecular weight SDS-stable aggregates. Although further examination of the aggregation state of the peptide was not performed, if aggregates were present in the injected preparation they were not large enough to be sequestered by cells of the reticuloendothelial system and were amyloid-reactive. The peptide was purified from free radioiodide by gel filtration and injected iv into H-2/IL-6 mice that had been primed with AEF for 2–5 wk with consequently varying amounts of visceral amyloidosis. The mice developed AA deposits in multiple organs with increasing severity according to the amount of time post-AEF. Two hours after injection of ^{125}I -p5, the mice were euthanized and the organs harvested and analyzed for radioactivity (biodistribution) or histologic evaluation (Congo red staining or micro-autoradiography). Precise co-localization of the radioiodinated p5 with amyloid deposits was confirmed microscopically by comparing the distribution of peptide in micro-autoradiographs with the presence of amyloid revealed by Congo red birefringence (Fig. 1). In every tissue evaluated, the appearance of black silver grains in the autoradiographs, indicative of the presence of ^{125}I -p5, correlated precisely with the presence of Congo red birefringent amyloid in consecutive tissue sections (Fig. 1). No silver grains were seen in areas of tissue devoid of Congo red birefringence. Furthermore, no significant peptide uptake was observed in organs or tissues where amyloid was absent (e.g., skin, lung, etc.) or in healthy mice with no amyloid by using small animal single photon emission computed tomographic (SPECT) imaging of ^{125}I -labeled p5 [7]. These data demonstrated that ^{125}I -p5 injected iv localized at sites of amyloid deposition and not in normal, amyloid-free tissue.

To assess the correlation between ^{125}I -p5 uptake and amyloid burden, we focused our attention on the liver and spleen. Amyloid load in these tissues was analyzed quantitatively by digital evaluation as well as qualitatively (observer rated 0 – 4+) after Congo red staining of tissue sections and compared with biodistribution data (%ID/g). Figure 2 shows representative examples of data for spleens from 4 animals with increasing amyloid burden.

Micro-autoradiography (ARG) revealed the perifollicular distribution of the ^{125}I -p5 peptide which correlated with Congo red (CR) birefringence (Fig. 2). Quantitation of the amyloid burden (Fig. 2: CRq – false colored red) performed by image analysis was deemed visually accurate and correlated also with the Congo red birefringence.

The correlations between amyloid load based on histochemical analyses and ^{125}I -p5 uptake (% ID/g) are plotted in Fig 3. In all cases, there was a significant linear correlation between the uptake of peptide within the liver or spleen and the amyloid burden (Table 1). Correlation coefficients were all >0.7 with those for the liver somewhat higher than for the spleen ($p = 10^{-5}$ to 6×10^{-12} , for spleen and liver, respectively).

Finally, since the deposition of AA amyloid in these mice (as well as other models) begins within the spleen before involving the liver and other tissues, we compared the amyloid burden in liver and spleen using Congo red data and ^{125}I -p5 peptide accumulation (Fig. 4). Regardless of the method for monitoring amyloid burden, there was a significant linear correlation between amyloid load in the liver and spleen (Table 1) suggesting that AA amyloid deposition occurs in parallel in these two organs.

Discussion

Molecular imaging is now a critical method to determine localization and extent of disease, especially in oncology. Recently, probes have been developed for the detection of A β amyloid in the brains of patients with Alzheimer's disease (AD), mild cognitive impairment

and otherwise healthy subjects [21]. A β amyloid burden, measured by using Pittsburgh compound B (PIB) [22] or florbetapir [23] PET imaging, correlates with the early phases of cognitive impairment. Most studies indicate that A β deposition in the brain is apparent before symptoms occur but that levels in brain reach a plateau even as the patient's mental status continues to deteriorate [24–26]. Thus, the amount of amyloid present may not correlate linearly with symptomology, especially in the late stages of disease.

Peripheral amyloidosis can be evaluated clinically by using planar scintigraphy and, recently, SPECT imaging with ^{123}I -labeled serum amyloid P component (SAP) [3, 4]. This method has been effective in detecting amyloid in several, but not all, peripheral organs and is available only in the UK and Europe. In the US, the amyloid-reactive therapeutic antibody 11–1F4, when labeled with ^{124}I , has been used to monitor whole-body amyloid burden by using PET/CT imaging [6]. However, only ~60% of patients with biopsy-proven AL amyloid were successfully imaged, and not all clinically relevant deposits within an individual were imaged; notably, renal and cardiac amyloidosis was rarely detected in the PET images. In an attempt to develop alternative imaging agents for quantitative, whole-body detection of visceral amyloidosis for patients in the US, we have evaluated a number of heparin-reactive peptides that bind amyloid deposits *in vitro* and *in vivo* [7, 15]. One peptide (p5) bound specifically to murine AA amyloid laden tissues *in vivo* but not to normal tissue even though there were high levels of circulating SAA (~ 2 mg/mL) in the serum and healthy tissue contains many HSPGs [7]. Additionally, we have shown by using immunohistochemistry that the biotinylated p5 peptide binds other forms of amyloid; notably, AL κ , AL λ , ATTR, human AA and human A β [7]. The selectivity and specificity of this probe for many forms of amyloid suggests it may be of use as a molecular imaging agent for peripheral amyloid disease in the US.

Quantitation of amyloid deposits or any pathology by using molecular imaging is difficult [27]. There are numerous barriers that complicate the quantitative nature of imaging. Parameters such as the rate of clearance of unbound radiotracer competing for binding, the ability to effectively penetrate blood vessels and access the extravascular space, as well as target retention (a function of avidity and many other factors) must be optimized to generate accurate images that can be confidently quantified. Peptides have several advantageous properties for imaging. They are relatively small which allows for fast clearance of unbound reagent as well as relatively rapid penetration of blood vessel walls and extravascular amyloid architecture. While peptides may be superior to larger imaging agents, absolute control of all the parameters that can affect imaging quality is impossible. To validate quantitative studies, it is useful to relate the final probe deposition a priori with amounts of amyloid in a model system.

We have used a model system in which transgenic mice express the H-2/IL-6 gene product constitutively resulting in a chronic state of inflammation. These mice spontaneously develop AA at ~5 mos of age, but the disease process can be accelerated by injection of a preparation of AA fibrils (AEF) that results in significant AA deposition within weeks. The disease process recapitulates the human condition in many regards. In particular, deposits in liver and spleen develop rapidly, and there is significant amyloid in the renal papilla, interstitium and glomerulae. Indeed, renal papillary necrosis is likely the major cause of morbidity and mortality in these animals and is seen in other cases of naturally occurring AA [28, 29]. These and other properties make this model useful and representative of human disease.

We have shown that when peptide p5 is injected into wild type mice with no amyloid, the peptide is taken up in kidney tubules within about 7 min. The radioiodine is released from the peptide back into the circulation where it transits through halide symporters in the

stomach or is organified in the thyroid. In contrast, when radioiodinated peptide is bound to amyloid deposits, e.g. in the spleen or liver, loss of radioiodine does not occur, and radioiodinated peptide can be found in the amyloid of these tissues as late as 72 h post injection [30]. For the purposes of these studies we chose a time of 2 h post injection for analyses of tissues.

The data in Fig. 1 show that ^{125}I peptide p5 binds only at sites that exhibit Congo red birefringence and, therefore, contain amyloid. This was determined by autoradiographic studies with high ($\sim 10\ \mu\text{m}$) resolution. In all organs tested, amyloid deposits also contained silver grains representative of ^{125}I -p5 deposition, including the heart and kidneys. Correlation with both liver and spleen were highly significant indicating that accumulation of ^{125}I -p5 binding increased linearly with the amount of amyloid present in the tissues. This was apparent even in mice that had very large amyloid deposits estimated to be as much as 100-fold more than the amount of probe added. It should be noted that correlations were better for amyloid in the liver as compared to the spleen. This might be due to several factors including the accessibility of the amyloid to the peptide in circulation. Since the circulation time of the peptide is very short ($t_{1/2} \sim 7\ \text{min}$), it is likely that amyloid close to blood vessels would preferentially bind the probe. Thus, less-dense amyloid might be expected to accumulate blood-borne peptide tracer more effectively as compared to the more dense amyloid material. Even with these considerations, we found a good correlation between the amount of radiolabeled p5 peptide deposited in liver and spleen with the volume of amyloid seen in these animals.

Conclusions

The data indicate that deposition of ^{125}I -labeled p5 peptide correlates with amyloid load and physical location in the transgenic mouse model of AA amyloidosis. Thus, SPECT images with this probe can be reliably used to estimate the extent of disease. Furthermore, labeling of the peptide using the same chemistry with the positron emitter, ^{124}I , will enable tomographic, whole body, quantitative imaging by using PET/CT in patients with visceral amyloidosis.

Acknowledgments

We thank Emily Martin for reviewing the manuscript. This work was supported by Award Number R01DK079984 from the National Institute Of Diabetes And Digestive And Kidney Diseases and a Collaborative Research Agreement with Elan/Neotope Biosciences.

Abbreviations

AEF	amyloid enhancing factor
CR	Congo red
HSPG	heparan sulfate proteoglycan
PET	positron emission tomography
SAA	serum amyloid protein A
SPECT	single photon emission computed tomography

References

1. Lachmann HJ, Hawkins PN. Systemic amyloidosis. *Curr Opin Pharmacol*. 2006; 6:214–220. [PubMed: 16483845]
2. Pepys MB. Amyloidosis. *Annu Rev Med*. 2006; 57:223–241. [PubMed: 16409147]

3. Hawkins PN, Pepys MB. Imaging amyloidosis with radiolabelled SAP. *Eur J Nucl Med*. 1995; 22:595–599. [PubMed: 7498219]
4. Hazenberg BP, van Rijswijk MH, Piers DA, Lub-de Hooge MN, Vellenga E, Haagsma EB, Hawkins PN, et al. Diagnostic performance of ^{123}I -labeled serum amyloid P component scintigraphy in patients with amyloidosis. *Am J Med*. 2006; 119:e315–e324. 355.
5. Wall JS, Kennel SJ, Paulus MJ, Gleason S, Gregor J, Baba J, Schell M. Quantitative High-Resolution Microradiographic Imaging of Amyloid Deposits in a Novel Murine Model of AA-Amyloidosis. *Amyloid: The Journal of Protein Folding Disorders*. 2005; 12:146–151.
6. Wall JS, Kennel SJ, Stuckey AC, Long MJ, Townsend DW, Smith GT, Wells KJ, et al. Radioimmunodetection of amyloid deposits in patients with AL amyloidosis. *Blood*. 2010; 116:2241–2244. [PubMed: 20522711]
7. Wall JS, Richey T, Stuckey A, Donnell R, Macy S, Martin EB, Williams A, et al. In vivo molecular imaging of peripheral amyloidosis using heparin-binding peptides. *Proc Natl Acad Sci U S A*. 2011; 108:E586–E594. [PubMed: 21807994]
8. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with ^{123}I -labeled serum amyloid P component. *N Engl J Med*. 1990; 323:508–513. [PubMed: 2377176]
9. Townsend DW. Positron emission tomography/computed tomography. *Semin Nucl Med*. 2008; 38:152–166. Epub 2008/04/09. [PubMed: 18396176]
10. Townsend DW. Multimodality imaging of structure and function. *Phys Med Biol*. 2008; 53:R1–R39. Epub 2008/02/12. [PubMed: 18263942]
11. Lois C, Jakoby BW, Long MJ, Hubner KF, Barker DW, Casey ME, Conti M, et al. An assessment of the impact of incorporating time-of-flight information into clinical PET/CT imaging. *J Nucl Med*. 2010; 51:237–245. Epub 2010/01/19. [PubMed: 20080882]
12. Lindahl B, Lindahl U. Amyloid-specific heparan sulfate from human liver and spleen. *J Biol Chem*. 1997; 272:26091–26094. [PubMed: 9334172]
13. Smits NC, Kurup S, Rops AL, ten Dam GB, Massuger LF, Hafmans T, Turnbull JE, et al. The heparan sulfate motif (GlcNS6S-IdoA2S)₃, common in heparin, has a strict topography and is involved in cell behavior and disease. *J Biol Chem*. 2010; 285:41143–41151. [PubMed: 20837479]
14. Zhang X, Li JP. Heparan sulfate proteoglycans in amyloidosis. *Prog Mol Biol Transl Sci*. 2010; 93:309–334. [PubMed: 20807650]
15. Wall JS, Richey T, Williams A, Stuckey A, Osborne D, Martin E, Kennel SJ. Comparative analysis of peptide p5 and serum amyloid P component for imaging AA amyloid in mice using dual-isotope SPECT. *Mol Imaging Biol*. 2012; 14:402–407. Epub 2011/11/02. [PubMed: 22042488]
16. Solomon A, Weiss DT, Schell M, Hrcic R, Murphy CL, Wall J, McGavin MD, et al. Transgenic mouse model of AA amyloidosis. *Am J Pathol*. 1999; 154:1267–1272. Epub 1999/05/11. [PubMed: 10233864]
17. Wall JS, Richey T, Allen A, Donnell R, Kennel SJ, Solomon A. Quantitative tomography of early-onset spontaneous AA amyloidosis in interleukin 6 transgenic mice. *Comp Med*. 2008; 58:542–550. [PubMed: 19149411]
18. Ailles L, Kisilevsky R, Young ID. Induction of perlecan gene expression precedes amyloid formation during experimental murine AA amyloidogenesis. *Lab Invest*. 1993; 69:443–448. Epub 1993/10/01. [PubMed: 8231112]
19. Axelrad MA, Kisilevsky R, Willmer J, Chen SJ, Skinner M. Further characterization of amyloid-enhancing factor. *Lab Invest*. 1982; 47:139–146. Epub 1982/08/01. [PubMed: 7109539]
20. Wall JS, Paulus MJ, Gleason S, Gregor J, Solomon A, Kennel SJ. Micro- Imaging of Amyloid in Mice. *Methods in Enzymology*. 2006; 412:161–182. [PubMed: 17046658]
21. Villemagne VL, Rowe CC. Amyloid imaging. *Int Psychogeriatr*. 2011; 23(Suppl 2):S41–S49. Epub 2011/07/07. [PubMed: 21729418]
22. Mathis CA, Mason NS, Lopresti BJ, Klunk WE. Development of Positron Emission Tomography beta-Amyloid Plaque Imaging Agents. *Semin Nucl Med*. 2012; 42:423–432. Epub 2012/10/03. [PubMed: 23026364]

23. Kung MP, Weng CC, Lin KJ, Hsiao IT, Yen TC, Wey SP. Amyloid plaque imaging from IMPY/SPECT to AV-45/PET. *Chang Gung Med J.* 2012; 35:211–218. Epub 2012/06/28. [PubMed: 22735052]
24. Doraiswamy PM, Sperling RA, Coleman RE, Johnson KA, Reiman EM, Davis MD, Grundman M, et al. Amyloid-beta assessed by florbetapir F 18 PET and 18-month cognitive decline: A multicenter study. *Neurology.* 2012; 79:1636–1644. Epub 2012/07/13. [PubMed: 22786606]
25. Newberg AB, Arnold SE, Wintering N, Rovner BW, Alavi A. Initial clinical comparison of ¹⁸F-florbetapir and ¹⁸F-FDG PET in patients with Alzheimer disease and controls. *J Nucl Med.* 2012; 53:902–907. Epub 2012/05/12. [PubMed: 22577238]
26. Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, Pontecorvo MJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA.* 2011; 305:275–283. Epub 2011/01/20. [PubMed: 21245183]
27. Basu S, Kwee TC, Surti S, Akin EA, Yoo D, Alavi A. Fundamentals of PET and PET/CT imaging. *Ann N Y Acad Sci.* 2011; 1228:1–18. Epub 2011/07/02. [PubMed: 21718318]
28. Newkirk KM, Newman SJ, White LA, Rohrbach BW, Ramsay EC. Renal lesions of nondomestic felids. *Vet Pathol.* 2011; 48:698–705. Epub 2010/09/30. [PubMed: 20876911]
29. Chew DJ, DiBartola SP, Boyce JT, Gasper PW. Renal amyloidosis in related Abyssinian cats. *J Am Vet Med Assoc.* 1982; 181:139–142. Epub 1982/07/15. [PubMed: 7118693]
30. Martin E, Kennel S, Richey T, Stuckey A, Osborne D, Wall J. The peptide p5 binds rapidly to visceral amyloid *in vivo* and persists for more than 72 h rendering it suitable for PET/CT imaging using I¹²⁴. *J Nuc Med.* 2012; 53:336P.

Figure 1.

Radiolabeled peptide p5 binds AA amyloid *in vivo*. The reactivity of the ^{125}I -p5 peptide in stomach liver and kidney was evidenced by the appearance of black deposits in micro-autoradiographs (ARG) that correlated with the distribution of Congo red (CR)-birefringent amyloid in consecutive tissue sections.

Figure 2. Quantification and ^{125}I -p5 labeling in splenic AA amyloid in mice. ^{125}I -p5 was seen in micro-autoradiographs (ARG) to bind weak (1+) and intense (4+) splenic AA amyloid. The ^{125}I -p5 distribution correlated with Congo red (CR)-birefringent amyloid that was then quantitatively measured by image analysis (CRq – area detected by algorithm is false colored red).

Figure 3.

The binding and retention of ¹²⁵I-p5 peptide in the liver and spleen correlates with amyloid load. Linear regression analysis with 95% confidence lines (dotted) or ¹²⁵I-p5 (% injected dose/gram tissue) and Congo red scoring (0 – 4+: CR_{sq}) and Congo red area on the stained tissue (CR_q).

Figure 4. Splenic AA amyloid load correlates positively with deposits in the liver in H-2/IL-6 transgenic mice. Amyloid burden was measured by $^{125}\text{I-p5}$ peptide retention in vivo (%ID/g), Congo red scoring (0 – 4+: CR_{sq}), and measuring Congo red-birefringent area (μm^2 : CR_q).

Table 1

Correlation analysis between different measures of amyloid burden in the liver and spleen of AA mice.

Organ Correlation	R ² of linear regression	Pearson correlation coefficient	Pearson correlation <i>P</i> value
L p5 vs L CR _{sq}	0.9	0.94	6×10^{-12}
L p5 vs L CR _q	0.9	0.95	2×10^{-8}
Sp p5 vs Sp CR _{sq}	0.8	0.87	1×10^{-8}
Sp p5 vs Sp CR _q	0.8	0.87	1×10^{-5}
L p5 vs Sp p5	0.7	0.82	4×10^{-7}
L CR _{sq} vs Sp CR _{sq}	0.8	0.89	3×10^{-9}
L CR _q vs Sp CR _q	0.8	0.91	2×10^{-6}

Where: L, liver; Sp, spleen; CR_q, quantitative measure of Congo red birefringence (μm^2); CR_{sq}, semi-quantitative method of Congo red birefringence (0 – 4+).