

Hypersulfated heparan sulfate glycosaminoglycans – Novel cerebral and visceral amyloid-associated biomarkers in mouse and man that can be targeted with the heparin-binding peptide p5

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Introduction

Heparan sulfate proteoglycans (HSPG) are ubiquitous components of pathologic amyloid deposits in the organs of patients with disorders such as Alzheimer's disease, systemic light chain (AL), and reactive (AA) amyloidosis. Although recent advances have been made in the translation of novel tracers for imaging Aβ amyloid in patients with Alzheimer's disease, molecular imaging methods for the early detection of visceral amyloidosis (e.g., AL and AA) are limited and generally unavailable outside the U.K. Therefore, there remains a continued need to develop novel, specific, amyloidophilic imaging radiotracers to assist in diagnosis, disease staging and monitoring response to therapy.

Amyloid-associated HSPG has been differentiated from that found in surrounding healthy cells and tissues by the preferential binding of certain HS-reactive scFv antibodies. One of these scFvs, for which the chemical target was well defined, was used to show that HSPG in amyloid was likely hypersulfated and, therefore, electrochemically similar to heparin. This unique property renders HSPG a potential novel biomarker in amyloid that might be targeted specifically with appropriate reagents

Disease	Organ Involved	Protein
Alzheimer's Disease	Brain	Aβ (fragments)
AL amyloidosis; Multiple myeloma	Heart, Liver, Spleen, Kidney, (all)	Ig light chain (fragments)
ATTR amyloidosis; Senile systemic amyloidosis	Heart, Nerve	Transthyretin (mutant and WT)
AA amyloidosis; Familial Mediterranean Fever; Rheumatoid Arthritis	Spleen, Kidney, (all)	serum apolipoprotein A
Type 2 diabetes	Pancreatic Islets	Islet amyloid polypeptide (amylin)

Table 1. Partial List of Amyloid-Associated Diseases

GGGYS KAQKA QAKQA KQAQK AQAQA AKQAK Q

Peptide p5	
MW ¹	3,303.7
Theoretical pI ¹	10.31
Net charge	+8
Source	chemically synthesized
Target in amyloid	heparan sulfate proteoglycan
Catabolism	kidney

Figure 1. Primary Structure of peptide p5 and table of physical properties.
¹Calculated using, www.expasy.org/cgi-bin/protparam

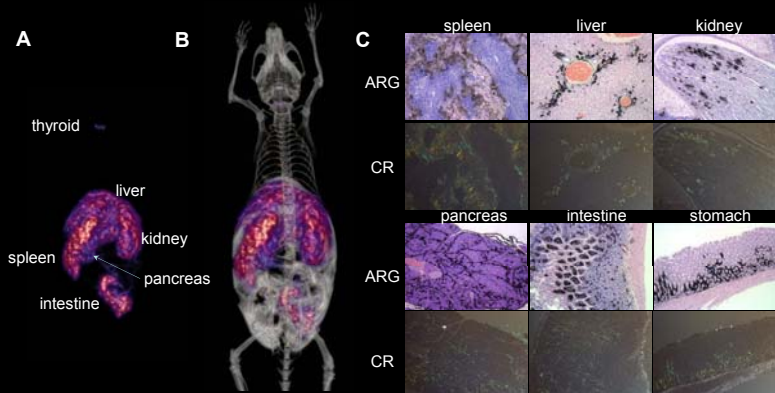


Figure 2. Peptide p5 specifically binds AA amyloid in vivo using a mouse model of the disease. (A) SPECT image of ¹²⁵I-p5 peptide in mouse with systemic AA amyloidosis at 2 h post-injection. (B) Co-registered SPECT and contrast-enhanced CT image of the same mouse in (A). (C) Micro-autoradiography of selected tissues from a mouse injected with ¹²⁵I-p5 peptide. The black deposits associated with ¹²⁵I-p5 correlate with amyloid deposits as seen by Congo red (CR) birefringence in consecutive tissue sections.

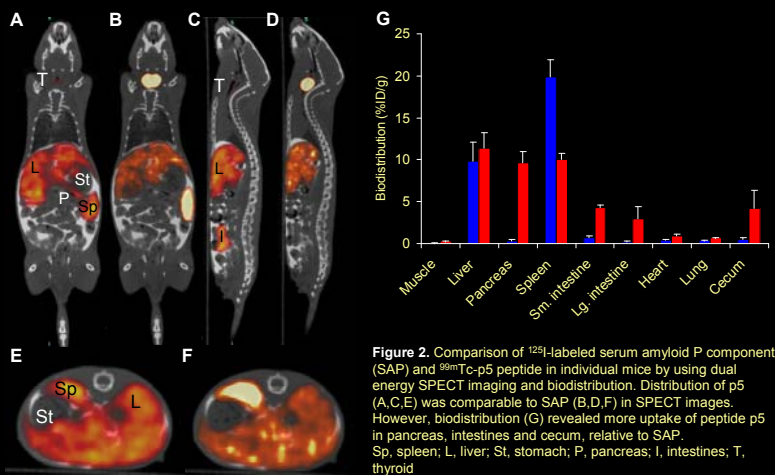


Figure 2. Comparison of ¹²⁵I-labeled serum amyloid P component (SAP) and ^{99m}Tc-p5 peptide in individual mice by using dual energy SPECT imaging and biodistribution. Distribution of p5 (A,C,E) was comparable to SAP (B,D,F) in SPECT images. However, biodistribution (G) revealed more uptake of peptide p5 in pancreas, intestines and cecum, relative to SAP. Sp, spleen; L, liver; St, stomach; P, pancreas; I, intestines; T, thyroid

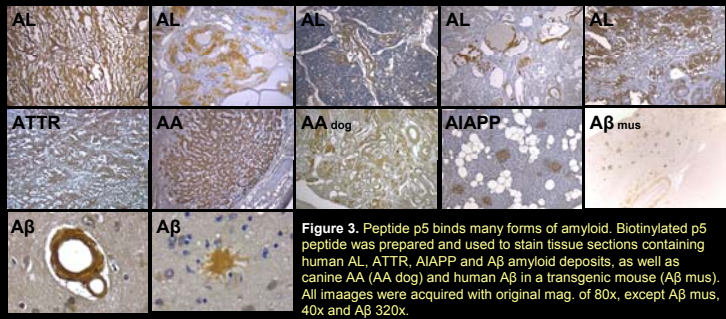


Figure 3. Peptide p5 binds many forms of amyloid. Biotinylated p5 peptide was prepared and used to stain tissue sections containing human AL, ATTR, AIAPP and Aβ amyloid deposits, as well as canine AA (AA dog) and human Aβ in a transgenic mouse (Aβ mus). All images were acquired with original mag. of 80x, except Aβ mus, 40x and Aβ 320x.

Results and Discussion

Using a transgenic murine model of visceral AA amyloidosis, we have examined the amyloid reactivity of 7 heparin-binding peptides in vivo by using SPECT/CT imaging, micro-autoradiography and tissue biodistribution measurements. All of the peptides bound amyloid deposits within 1 h post-injection, but the degree of reactivity differed widely as evidenced by SPECT images, biodistribution measurements and the grain density seen in autoradiographs.

One radiolabeled peptide, designated p5, bound rapidly and specifically to murine AA amyloid in the liver, spleen, kidney, intestines and pancreas with sufficient avidity to be observed in SPECT images within 1 h and as late as 24 h post-injection. To generalize these findings, a biotinylated form of this peptide was generated and shown histochemically to bind human AA, ALx, ALx, ATTR, and Aβ amyloid deposits in formalin-fixed paraffin-embedded tissue sections. This basic, heparin-binding peptide selectively recognized the HSPG in murine, canine, feline and human amyloid deposits in vivo and in ex vivo tissues and, therefore, has potential as a radiotracer for the non-invasive molecular imaging of amyloid deposits in patients with these devastating diseases.