

Stable and rapid binding of ^{124}I -labeled peptide p5 to visceral amyloid *in vivo* as evidenced by dynamic PET imaging

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Introduction

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

We have demonstrated that amyloid deposits are preferentially targeted *in vivo* by scFv antibodies reactive with heparin-like HS. In addition, a synthetic, heparin-binding peptide, designated p5, when radiolabeled with ^{125}I , was used to image AA amyloid in mice by SPECT/CT. We examined the dynamic behavior (binding and clearance) of ^{124}I -p5 peptide by using PET/CT in diseased and healthy mice.

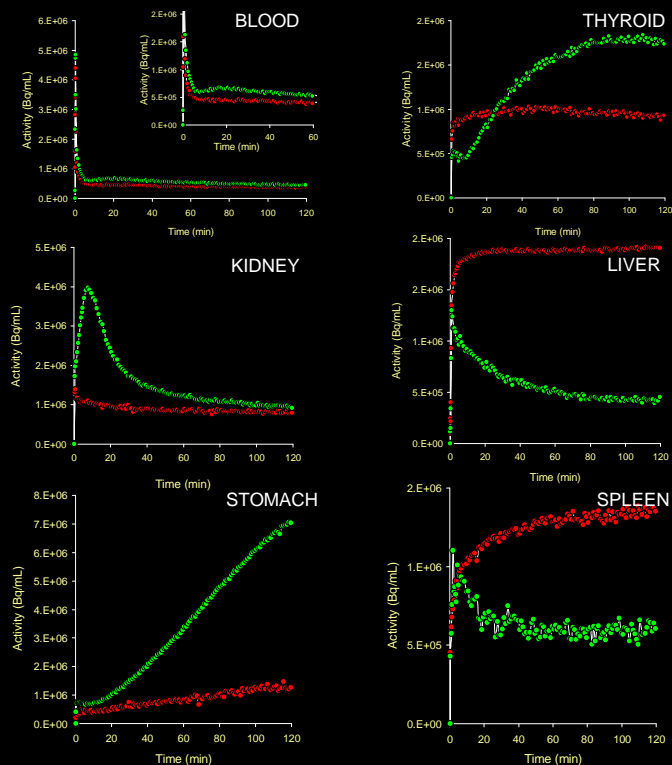


Figure 1. Peptide ^{124}I -p5 is rapidly cleared by the kidneys and dehalogenated in wild type (WT; green) mice but not mice with AA amyloidosis (red). This is evidenced in PET/CT time activity curves by a loss of radioiodine signal from the blood and kidneys concordant with an increase in radioactivity in the stomach and thyroid – sites of free iodide sequestration in mammals. In contrast, mice with AA amyloid have accumulation of radiotracer in the liver and spleen over 2 hours with dramatically less free iodide liberated indicating less catabolism. Opposite is a schematic describing this model. Notably, unbound ^{124}I -p5 peptide is rapidly dehalogenated during catabolism, but amyloid bound material is not.

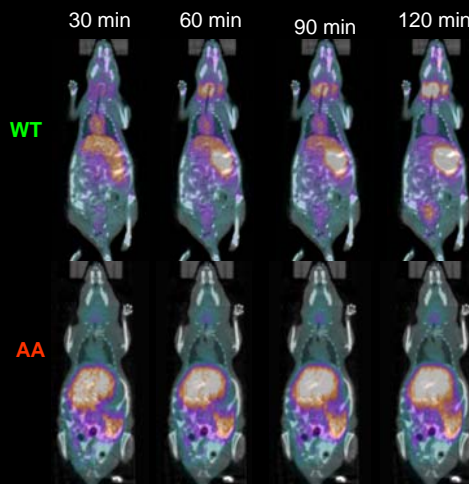


Figure 2. Dynamic distribution of ^{124}I -p5 peptide in WT and AA mice. Coronal PET/CT images of mice at 30, 60, 90, and 120 min post injection of radiotracer. Note the slow loss of activity from the blood and accumulation of free iodide in the stomach and thyroid. In contrast amyloid in the liver, pancreas, and intestines sequester ^{124}I -p5 in AA mice.

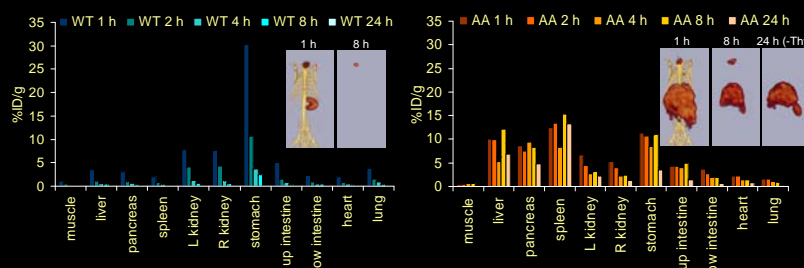


Figure 3. Long term kinetic analysis of ^{125}I -p5 peptide in healthy WT mice and those with AA amyloidosis. The biodistribution of ^{125}I -p5 was monitored in WT and AA mice at 1, 2, 4, 8, and 24 h post injection. Notably the radiolabeled peptide remained present at 24 h post injection in organs with high amyloid content – the liver, spleen, pancreas, intestines and kidneys. This activity was imaged by SPECT/CT in AA mice

Results and Discussion

In healthy, amyloid-free mice, ^{124}I -p5 peptide was rapidly cleared from the blood pool with a $K_{\text{fast}} = 1.8 \pm 0.06$ and $K_{\text{slow}} = 0.02 \pm 0.004 \text{ min}^{-1}$, respectively. The peptide appeared rapidly in the kidneys with a peak uptake time of ~ 7 min followed by a rapid bi-exponential loss of radioactivity with a K_{deg} of $0.08 \pm 0.003 \text{ min}^{-1}$. The decrease in radioactivity from the kidney coincided with its reappearance in the blood pool and subsequent uptake by the stomach at a rate of 64 kBq/mL/min . These data indicated that rapid dehalogenation of the peptide was occurring in the kidneys. The free ^{124}I -iodide then returned into the circulation as evidenced by its uptake in the stomach and, to lesser degree, the thyroid. No accumulation of radioactivity was observed in the spleen or liver.

In AA mice, however, there was rapid binding of ^{124}I -p5 peptide in the liver ($K_{\text{deg}} = 1.96 \pm 0.09 \text{ min}^{-1}$) which reached a plateau at ~ 25 min post injection and did not decay over 2 h. In mice with AA, blood pool clearance of the tracer was essentially equivalent to that seen in healthy mice; however, only modest dehalogenation of the peptide occurred in AA animals as evidenced by the scant uptake of ^{124}I -iodide in the stomach.

Peptide p5 is rapidly and stably accumulated in AA amyloid deposits in the liver (and spleen) up to 2 h post injection – a desirable property in an amyloid imaging agent. Furthermore, when not bound to amyloid, the radio-iodinated p5 peptide was rapidly dehalogenated in the kidneys, and the liberated iodide was sequestered by the stomach and thyroid. This fortuitous process led to enhanced signal-to-noise ratios in the kidney, a major site of visceral amyloid deposition in man, when imaging with radioiodide-conjugated p5 peptide.