

Imaging the biodistribution of systemic AA amyloidosis in mice using ¹²⁵I-labeled protamine

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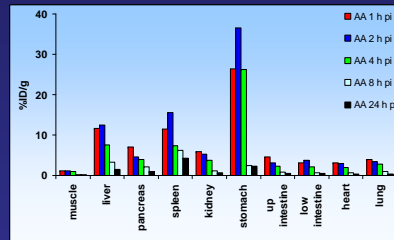
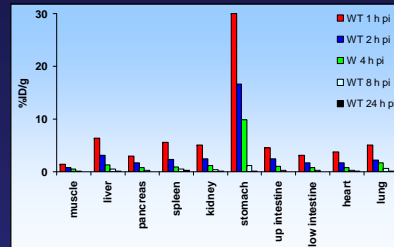
Introduction

Heparan sulfate (HS) is a major component of amyloid deposits in the brain and peripheral organs. Data suggest that the chemical structure of amyloid-associated heparan sulfate differs from that found ubiquitously in the extra-cellular matrix of essentially all normal tissues rendering it a potential new biomarker for these pathologic deposits. Therefore, we have studied the biodistribution of the heparin-binding peptide protamine as a novel agent for amyloid imaging.

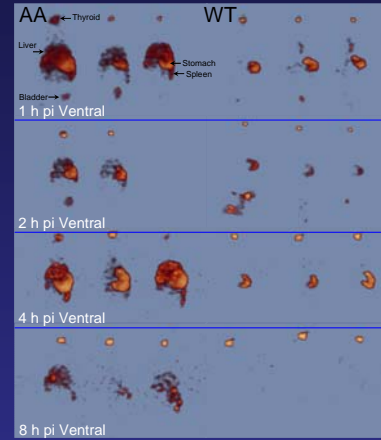
Methods

Synthetic protamine was purified by reverse phase HPLC and the amino acid sequence confirmed by MS. Following radioiodination, ¹²⁵I-labeled protamine (¹²⁵I-protamine) was administered to mice with severe systemic inflammation-associated AA amyloidosis or to amyloid-free animals and allowed to circulate for 1, 2, 4, 8 or 24 h. At each time point microSPECT/CT images were acquired and the tissue distribution of the radioactivity determined by gamma counting. Finally, 6 μm-thick tissue sections were prepared for autoradiography and histological evaluation of the micro-distribution of the radiolabeled peptides and amyloid deposits, respectively.

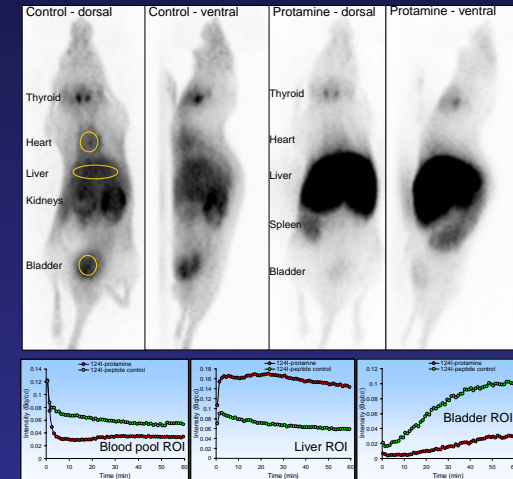
Tissue Retention of ¹²⁵I-protamine is greater in AA mice as compared to WT animals



SPECT imaging of ¹²⁵I-protamine in AA mice and WT mice



AA amyloidosis evidenced by difference in uptake and clearance kinetics of ¹²⁴I-labeled protamine or control peptide in AA mice up to 1 h pi by PET imaging



Autoradiography (ARG) of ¹²⁵I-protamine in WT and AA mice at 1 h pi correlates with Congo red (CR)-stained amyloid



Results and Conclusion

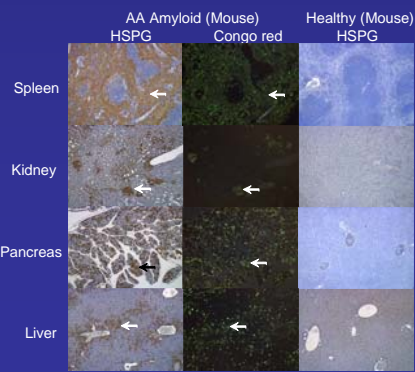
In disease-free mice the ¹²⁵I-protamine was rapidly dehalogenated and ¹²⁵I-iodide sequestered by the stomach which, with the thyroid was visible in SPECT images up to 8 h post-injection. In contrast, ¹²⁵I-protamine when injected into AA mice was observed by SPECT and autoradiography within the liver, kidney, pancreas and spleen, as well as free iodide in the stomach and thyroid. Tissue-to-muscle ratios for the liver, pancreas, spleen and kidney (all sites of AA) were at least 2-fold greater in mice with amyloid as compared to the WT animals at every time point studied, indicating specific retention of the protamine peptide by AA deposits. Micro-autoradiography further confirmed that ¹²⁵I-protamine preferentially associated with the AA amyloid deposits in these organs. In amyloid-free mice there was little or no specific accumulation of ¹²⁵I-protamine in any organ or tissue.

These data indicate that although HS is expressed by normal tissue, the heparin-reactive peptide protamine is a promising new reagent for imaging amyloidosis.

Acknowledgements

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Immunostaining of HSPG in Mice



Tissue:Muscle ratios of ¹²⁵I-protamine are higher in amyloidotic organs of AA mice as compared to WT

	Tissue:Muscle ratio (AA)				
	Time (h pi)				
	1	2	4	8	24
muscle	1.00	1.00	1.00	0.99	1.00
liver	10.02	10.78	8.32	19.55	12.17
pancreas	6.10	3.95	4.33	12.45	9.41
spleen	9.67	13.57	8.13	36.98	34.20
kidney	5.08	4.53	4.05	6.95	5.48
stomach	22.76	31.74	28.76	14.71	23.28
up intestine	3.93	2.67	2.46	5.07	5.43
low intestine	2.66	3.22	2.29	3.99	3.83
heart	2.67	2.58	2.16	3.69	2.62
lung	3.37	2.98	3.05	5.86	2.81

	Tissue:Muscle ratio (WT)				
	Time (h pi)				
	1	2	4	8	24
muscle	1.00	1.00	0.99	0.99	0.99
liver	4.58	4.27	2.79	4.71	5.12
pancreas	2.12	2.29	1.69	2.04	2.29
spleen	4.00	3.12	1.97	4.37	10.74
kidney	3.63	3.28	2.54	4.02	5.19
stomach	21.54	22.43	20.96	11.08	7.78
up intestine	3.25	3.34	2.08	2.83	2.59
low intestine	2.25	2.34	1.59	2.55	2.05
heart	2.66	2.24	1.79	2.82	3.45
lung	3.64	3.06	3.68	6.04	7.21