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Probing the structural requirements of polybasic peptides for effective and specific amyloid reactivity

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Background

Polybasic peptides, such as peptide p5, with a predicted α -helical secondary structure bind human amyloid extracts and synthetic amyloid fibrils [1]. When radiolabeled, peptide p5 and similar reagents, such as p5 + 14 [2], have been shown to bind peripheral amyloid specifically *in vivo* thereby allowing imaging of the disease. At present, there are no FDA-approved clinical techniques for whole body imaging of systemic amyloidosis in patients.

We hypothesize that understanding the structural features that govern amyloid binding by these small polybasic peptides will result in increased understanding of the structural requirements that are critical for specific amyloid binding. Further, this may result in the generation of enhanced amyloid-targeting agents for imaging and potentially therapeutic applications. The structural requirements for effective binding to synthetic amyloid fibrils have been studied *in vitro* [3], and the biodistribution in healthy wild-type (WT) mice and those with systemic deposits of inflammation-associated (AA) amyloid has been interrogated by using small animal SPECT/CT imaging.

Materials and methods

Based on the structure of peptide p5, the following synthetic peptide homologs were evaluated: peptides with disrupted α -helices [p5_(coil) and p5_(Pro3)]; a peptide with a propensity to form a β -sheet [p5_(sheet)]; and an all D-enantiomer variant of p5 [p5_(D)]. All peptides were synthesized and purchased from Anaspec (Fremont, CA) and further purified by reverse phase HPLC. Peptide–ligand interactions were studied using solution-phase “pull-down” binding assays using radioiodinated (¹²⁵I) peptides, as previously described [3]. *In vivo* biodistribution measurements in healthy wild-type (WT) mice and those with AEF-induced systemic AA amyloidosis was assessed by small animal SPECT imaging at 24

h post injection of ^{125}I -labeled peptide. Molecular dynamics simulations over 20 ns were used to study the interaction of peptide p5 and p5_(sheet) with a model of A β amyloid fibril (PDB# 1BEG), as previously described [4] using the Titan supercomputer at Oak Ridge National Laboratory.

Results

All the p5-related peptides, when radiolabeled, bound synthetic fibrils composed of rV λ 6Wil (Figure 1A) and A β (1–40) (Figure 1B) in PBS, pH 7.2. However, peptide binding in 1 M NaCl, pH 7.2 disrupted the binding of peptides [p5_(coil) and p5_(Pro3)] whereas the other three peptides still bound the fibrils (Figure 1A and B). Notably, the all-D enantiomer and the β -sheet peptide bound the synthetic fibrils as well as, or better than, p5 (Figure 1A and B). When radiolabeled with ^{125}I and injected IV into healthy mice, peptide p5(D) was seen localized to the same extent in the liver and kidneys of both WT (Figure 1C) and AA (Figure 1D) In contrast, the β -sheet peptide, p5_(sheet), was cleared rapidly from healthy WT mice (Figure 1C) but was observed specifically associated in organs known to contain AA amyloid (notably the liver and spleen – Figure 1D). The interaction of α -helical and β -sheet structured p5 peptides with a model A β fibril was simulated and predicted to involve principally ionic interactions between the peptide lysyl side chains and an arrayed glutamate side chain on the A β fibril (Figure 1E and F).

Discussion and conclusions

The α -helical secondary structure of peptide p5 is important for heparan sulfate/heparin and amyloid fibril binding; however, helicity is not an absolute requirement for polybasic peptide-amyloid interactions as evidenced by the comparable binding of a β -sheet peptide variant with the same net positive charge, both *in vitro* and *in vivo*. The all-D enantiomer of p5, despite binding amyloid effectively *in vitro*, exhibited significant off-target reactivity *in vivo* and was, therefore, deemed unsuitable as an amyloid imaging agent. The polybasic β -sheet motif is a novel model structure for developing improved amyloid targeting reagents *in vivo*.

Acknowledgments

Declaration of interest

JSW and SJK have patent rights associated with the use of peptides to target amyloid.

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Figure 1.

Functional activity of structurally modified variants of peptide p5. Binding of ^{125}I -labeled peptides to rV 6W11 (A) and Ab(1–40) (B) fibrils in PBS (red; left bar for all pairs) and 1 M NaCl (green). SPECT imaging of the biodistribution of ^{125}I -p5_(D) and p5_(sheet) in WT (C) and AA amyloid-laden (D) mice at 24 h pi. Molecular dynamics simulation of the interaction of helical peptide p5 (E) and p5_(sheet) (F) with a model Aβ fibril indicate similar electrostatic interactions govern the binding.