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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Early Development and Pre-Clinical Evaluation of a Fluorine-18 Labeled Peptide p5+14 for PET/CT Imaging of Cardiac Amyloidosis

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*Background:*Systemic amyloidosis is a rare protein misfolding disorder, where patients can present with varied symptoms due to the diverse organ involvement. This is particularly true of patients with light chain associated (AL) amyloidosis, which makes early and accurate diagnosis challenging. Currently there are no FDA-approved methods that allow visualization of amyloidosis in patients. Nuclear imaging is a promising method for early detection of amyloid. We have developed a novel amyloid-reactive peptide, designated p5+14, capable of binding AL, as well as other types of amyloid, via multivalent electrostatic interactions. Peptide p5+14 has been successfully radiolabeled with radioiodine and technetium-99m and evaluated clinically, labeled with iodine-124, in a PET/CT imaging study of patients with amyloidosis (NCT03678259). While there are numerous advantages to using I-124 as the radionuclide for amyloid imaging, fluorine is a viable alternative for detecting cardiac amyloidosis. Fluorine-18 has a shorter half-life (108 min as compared to 4.2 days) thus reducing the internal radiation dose to patients. Moreover, F-18 is the most common nuclide used in PET imaging. We have previously generated a ^{18}F-labeled variant of p5+14; however, the reaction was inefficient and not translatable. Here we present a novel development strategy for generating ^{18}F labeled p5+14 using a commercially available precursor that can bind AL amyloid and perform initial characterization of the reagent in a murine model of inflammation-associated systemic AA amyloidosis.
Methods: Isotopic exchange chemistry using a silicon-fluoride-acceptor (SiFA) method was used to label peptide p5+14 with F-18. The peptide precursor was purchased from Almac Life Sciences (Penicuik, UK). ^{18}F was purchased from PetNet (Siemens Healthineers, Knoxville, TN) and prepared for reaction according to standard methods. After drying, F-18 (1000 - 1900 MBq) was dissolved in anhydrous acetonitrile and the pH neutralized before the addition of peptide precursor. Radiolabeling was performed at room temperature for 5 min. After standard purification of the product, radiochemical purity studies demonstrated that it was stable, with no significant loss of F-18 for at least 5 hours. Radiofluorinated p5+14 was assessed in bioactivity "pulldown" assays to ensure binding to synthetic AL amyloid-like fibrils and human AL amyloid extracts. The radiolabeled peptide was also diluted in PBS and injected IV in the lateral tail vein of mice with severe systemic AA amyloidosis. After 1 h, mice were euthanized small animal PET/CT imaging performed and tissue biodistribution measurements were conducted using an automated Wizard 3 gamma counter (1480 Wallac Gamma Counter, Perkin-Elmer).
Results: Using optimized synthesis conditions, the non-decay corrected radiochemical yield was 55±4.5%. Radiochemical purity was 99±1% at end of synthesis. Peptide purity was shown to be 93.7±4.9% by assessed using HPLC. Radiochemical stability was similarly evaluated using an HLPC method and remained >97.5% radiochemical purity at 5 hours after synthesis. The binding of ^{18}F-p5+14 to synthetic AL amyloid like fibrils composed of a l6 variable domain was 96.2%, mimicking data using the radioiodinated peptide, and 87.2% to human AL amyloid extracts. Background binding to control beads was 17.4%. In mice, accumulation of ^{18}F-peptide in the liver and spleen, the organs with most AA amyloid deposits in this mouse model, was readily evident in PET images taken 1 h post injection. There was also evidence of hepatic clearance of the peptide, manifest as a prominent gall bladder in the images. There was no accumulation of F-18 in bone, indicating positive *in vivo* stability. Imaging of amyloid-free wild type mice showed rapid clearance with radioactivity in the urinary bladder, gall bladder and gastrointestinal tract with little or no hepatosplenic radiotracer retention.
Conclusion: Radiofluorination of peptides, such as p5+14, has been made more efficient through the use of novel precursors amenable to isotopic exchange. ^{18}F-peptide binds amyloid effectively and may serve as a next generation radiotracer for the detection of cardiac amyloid by PET/CT imaging.
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