A kit method for direct radiolabeling of anti-amyloid peptide AT-01 with technetium-99m (^{99m}Tc) for the detection of cardiac amyloidosis by SPECT/CT

Stephen J. Kennel, Joseph W. Jackson, Alan Stuckey, Tina Richey, Jonathan Wall
University of Tennessee Graduate School of Medicine, Knoxville TN, 37920, USA

BACKGROUND: Amyloid deposition in the heart is one possible cause of restrictive cardiomyopathy. In a Phase 1/2 imaging study (NCT03678259) the radioiodinated (I-124) amyloid-binding peptide AT-01 (formerly p5+14) was shown to be an effective radiotracer for the detection of cardiac amyloid by PET/CT imaging with high sensitivity. This amyloid-reactive peptide has previously been evaluated as a Tc-99m-labeled reagent in preclinical studies (1). A facile synthesis of ^{99m}Tc-AT-01 for use in SPECT/CT or planar gamma scintigraphic imaging would provide cardiologists with an easily accessible and sensitive imaging agent that binds directly to amyloid deposits, for the detection of cardiac amyloidosis of any type.

OBJECTIVE: Commercial radiopharmacies generally do not employ complex chemical syntheses to generate clinical radiotracers, but rather rely on kit methods for production, especially for Tc-99m-labeled probes. A radiolabeling kit has been developed for the facile synthesis of ^{99m}Tc-AT-01.

METHODS: Peptide labeling kits were prepared using adaptations of the reagents and concentrations previously published (1). The kits contain 20 µg of tin chloride ($SnCl_2$) and 100 µg peptide in 0.03 N NaOH. The radiolabeled peptide product was generated, for research evaluation, by addition of 1-2 mCi of pertechnetate. The resulting ^{99m}Tc-AT-01 was purified and evaluated for radiochemical purity, radiochemical yield, and bioactivity. The storage conditions and stability of the kit formulation were evaluated over a 3-month period. Amyloid reactivity of ^{99m}Tc-AT-01 (100 - 200 µCi) was evaluated *in vivo* using a murine model of systemic AA amyloidosis, by SPECT/CT imaging and tissue biodistribution measurements. In additional studies, ^{99m}Tc-PyP was used as a comparator. Human heart and liver tissue sections containing AL and ATTR amyloid deposits were used in overlay autoradiography studies to demonstrate binding of the probe to human amyloid.

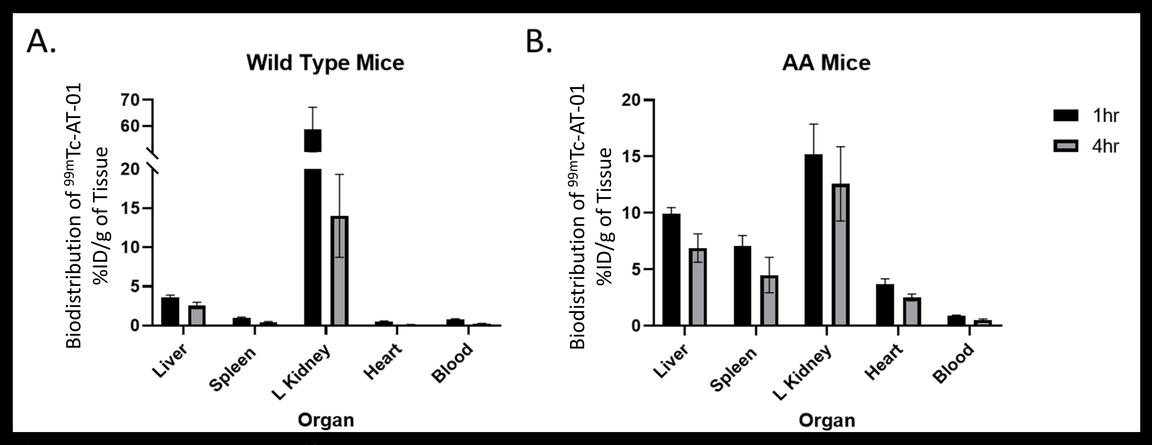

Figure 3 Biodistribution of ^{99m}Tc-AT-01 in AA and wild type mice. The tissue distribution of radiotracer was quantified in organs harvested post-mortem and expressed in % injected dose per gram (%ID/g) after correction for isotopic decay.

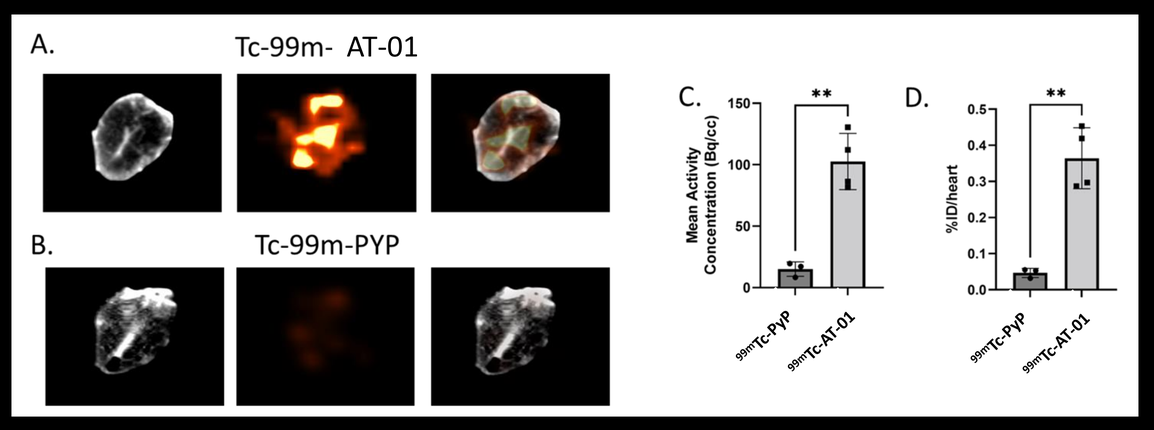

Figure 4 *Ex vivo* SPECT/CT imaging of murine cardiac tissue. AA mice (n=3), at 6 wk post AEF, were administered ~200 µCi of either ^{99m}Tc-AT-01 (A) or ^{99m}Tc-PYP (B) and hearts were excised 1 h thereafter, perfused with CT contrast agent diluted 1:1 (v/v) in PBS and imaged. Statistical significance (C & D) was determined by student's t-test **p≤0.01.

Experimental variation	Radiochemical yield[1]	Bioactivity% bound[2]
Standard kit formulation	85	92
Kit with no peptide	4	ND
Standard kit formulation	78	88
Kit with 4x peptide	80	81
Standard kit formulation	77	88
Ambiopharm peptide	92	96
Standard chemistry	82	96
Standard chemistry	86	95

Table 1 Variation of kit components. [1] % of initial. [2] % bound in rVλ6Wil fibril pulldown assay. ND, not determined

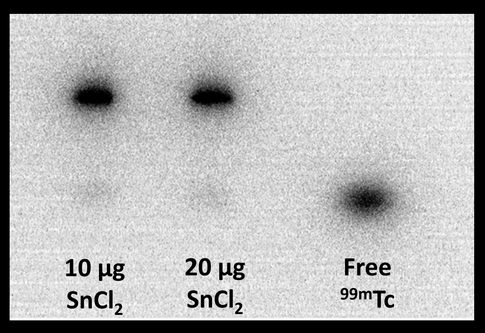

Figure 1. Evaluation of radiopurity. AT-01 peptide was labelled using the standard kit containing either 10 µg or 20 µg of $SnCL_2$ and the radiopurity assessed by SDS-PAGE. The mobility of free ^{99m}Tc is shown in the right lane.

Storage(°C)	Kit age (days)	RC yield (%)	RC purity (%)	Bioactivity (%)
-20	2	86.8	91.4	93.8
-80	2	84.2	90.7	93.6
-20	16	84.5	83.5	92.7
-80	16	82.8	85.7	93.6
-20	89	67	68.7	86.4
-80	89	84	86.5	92.6
-20	131	64	62	85.7
-80	131	72	81	91.9

Table 2: Effects of storage condition on kit performance based on characteristics of the ^{99m}Tc-peptide product

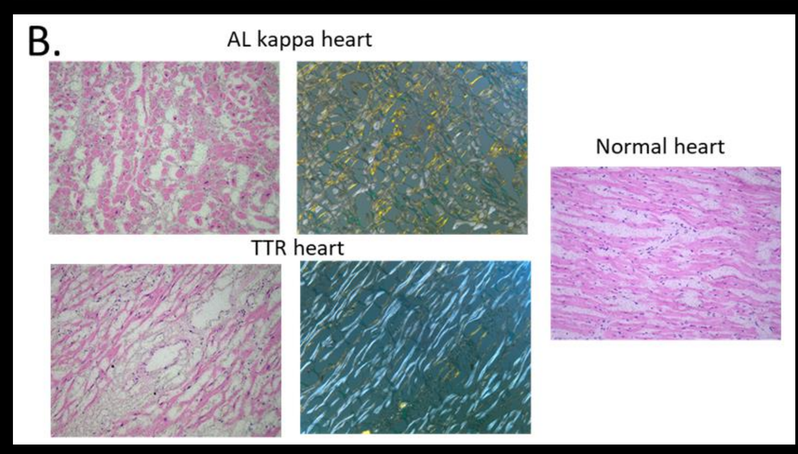

Figure 5 Phosphorimaging and microautoradiography of human tissue. Deparaffinized sections of human tissues were overlayed with ^{99m}Tc-AT-01 (~60 nCi, ~7 ng peptide/ 10 µL), incubated, washed as and evaluated by phosphorimaging (A). Tissue sections were then processed for autoradiography and counterstained with H&E (B).

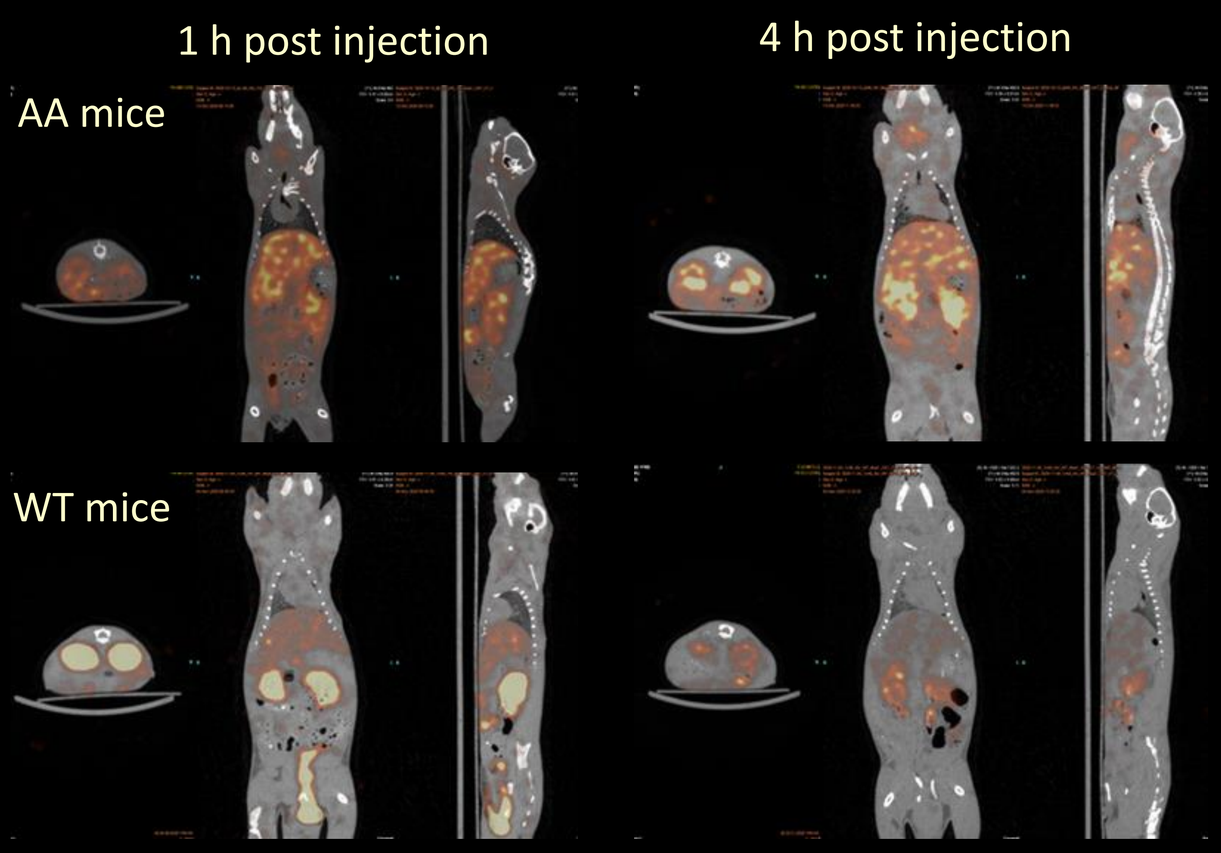

Figure 2 SPECT/CT imaging of ^{99m}Tc-AT-01 in AA and wil mice. Mice were administered 200 µCi of radiotracer and small animal imaging performed at 1 or 4 h post injection. At both time points the radiotracer was seen in the kidneys of WT mice, as compared to the AA mice where uptake in the hepatosplenic amyloid is also observed.

RESULTS: The ^{99m}Tc-AT-01 tracer was produced with >75% yield, >85% radiopurity and >90% bioactivity in a fibril pulldown assay. The labeling kits were stable for more than 3 months when stored at -80°C, with no significant decrease in production efficiency. When injected into mice, ^{99m}Tc-AT-01 detected hepatosplenic AA amyloid, the major sites of amyloid deposition in this model, as well as the scant deposits in the hearts of AA mice using ex vivo SPECT/CT imaging of isolated heart (Fig. 4). ^{99m}Tc-PYP has been shown to bind AA amyloid in the heart (*Eur. J. Nuc. Med.* (1990), **16**, 663-670); however, there was significantly more uptake of ^{99m}Tc-AT-01 as compared to ^{99m}Tc-PYP in the mouse heart. in the The distribution of the radiotracer in peripheral amyloid deposits in the AA mouse model were consistent with the findings using the I-124 radiolabeled tracer. Micro-autoradiography studies demonstrated that ^{99m}Tc-AT-01 effectively and specifically bound human amyloid deposits in the liver with AL and in both AL- and ATTR-containing cardiac tissue sections (Fig. 5).

SUMMARY: This kit method for production of ^{99m}Tc-AT-01 would provide a facile method for generating an easily accessible, highly effective, amyloid-binding radiotracer. This reagent could serve as next generation agent for the detection and diagnosis of cardiac amyloidosis by SPECT or scintigraphic imaging.

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