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Kit-like ^{18}F-labeling of RGD-^{19}F-Arytrifluroborate in high yield and at extraordinarily high specific activity with preliminary *in vivo* tumor imaging

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ABSTRACT

Introduction: Positron Emission Tomography (PET) is a rapidly expanding, cutting edge technology for preclinical evaluation, cancer diagnosis and staging, and patient management. A one-step aqueous ^{18}F-labeling method, which can be applied to peptides to provide functional *in vivo* images, has been a long-standing challenge in PET imaging. Over the past few years, we have sought a rapid and mild radiolabeling method based on the aqueous radiosynthesis of *in vivo* stable aryltrifluoroborate ($ArBF_3^-$) conjugates. Recent access to production levels of ^{18}F-Fluoride led to a fluorescent-^{18}F-$ArBF_3^-$ at unprecedentedly high specific activities of 15 Ci/μmol. However, extending this method to labeling peptides as imaging agents has not been explored.

Methods: In order to extend these results to a peptide of clinical interest in the context of production-level radiosynthesis, we applied this new technology for labeling RGD, measured its specific activity by standard curve analysis, and carried out a preliminary evaluation of its imaging properties.

Results: RGD was labeled in excellent radiochemical yields at exceptionally high specific activity (~14 Ci/μmol) (n = 3). Preliminary tumor-specific images corroborated by *ex vivo* biodistribution data with blocking controls show statistically significant albeit relatively low tumor uptake along with reasonably high tumor: blood ratios (n = 3).

Conclusions: Isotope exchange on a clinically useful ^{18}F-$ArBF_3^-$ radiotracer leads to excellent radiochemical yields and exceptionally high specific activities while the anionic nature of the aryltrifluoroborate prosthetic results in very rapid clearance. Since rapid clearance of the radioactive tracer is generally desirable for tracer development, these results suggest new directions for varying linker arm composition to slightly retard clearance rather than enhancing it.

Advances in Knowledge and Implications for patient Care: This work is the first to use production levels of ^{18}F-activity to directly label RGD at specific activities that are an order of magnitude higher than most reports and thereby increases the distribution window for radiotracer production and delivery.

1. Introduction

Positron Emission Tomography (PET) Imaging is a powerful technique for visualizing and quantifying the dynamic distribution of target-specific ligands, and is particularly useful for imaging solid cancers in regards to specific cellular targets [1–6]. Peptides as potential imaging agents are relatively large water-soluble ligands that exhibit high target selectivity yet clear rapidly so as to ensure high tumor-to-blood and tumor-to-muscle (T:NT) ratios. Once a peptide sequence has been identified and optimized for target binding and specificity, usually a site (e.g. N-terminus, C-terminus, pendant lysine) can also be identified for appending a suitable linker arm without reducing affinity or target specificity. This linker arm is then conjugated to a suitable radio-prosthetic that, with a robust labeling strategy, provides high specific activities. Of several available β^+-isotopes, ^{18}F-fluorine is often the isotope of choice owing to its excellent nuclear properties and on-demand production at Curie levels in hospital cyclotrons [7].

Although anionic ^{18}F-fluoride is routinely produced at high specific activity, its lack of reactivity in water [8], along with a relatively short half-life (109.8 min), makes single-step ^{18}F-labeling of peptides

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challenging. Generally, ^{18}F-labeling of peptides usually proceeds via the radiosynthesis of an ^{18}F-labeled prosthetic that is conjugated to the peptide in a second step, typically via acylation, oxime ligation, thiol-alkylation [9], or various bio-orthogonal click chemistries, which have been touted in terms of a general ease of labeling [10–14]. Nevertheless, most two-step methods still suffer from relatively long synthesis times (100–180 min) [15–17] that further erode specific activities to ~1 Ci/μmol [9–11,15,16,18]. In contrast, methods for direct labeling on carbon [19], silicon [14,20–28], boron [29–31], and aluminum chelates [32–37], which have been recently reviewed [25,27,38,39], are now receiving increasing attention owing to the inherent radiosynthetic simplicity of a single step. Such reactivity would enable the use of radiolabeling kits containing aliquots of precursors for on-demand labeling that ideally would obviate the need for very dry ^{18}F-fluoride while requiring little in the way of radiosynthetic skill.

Irrespective of isotope, labeling procedure, or prosthetic composition, the specific activity of a radiotracer, defined as Ci/μmol radiotracer, represents an impartial measure of radiotracer quality with important repercussions for imaging low-abundance targets as well as meeting regulations for microdosing (*vide infra*) [40,41]. While the specific activity of carrier-free ^{18}F-fluorine is 1720 Ci/μmol, the specific activity of no-carrier added (NCA) ^{18}F-fluoride ion obtained directly following bombardment falls in the range of 15–30 Ci/μmol [40,42,43]. Anion exchange trapping, which is often used to concentrate the fluoride, and which may be required to remove contaminating radioactive metals that cannot be injected into patients, further erodes the specific activity to approximately <10 Ci/μmol. Hence, most small molecule tracers as well as most radio-prosthetics are labeled at specific activities of 8 Ci/μmol, or less [13,44–48]. For ^{18}F-labeling of peptides, which often requires two steps, there are few examples where specific activities exceed 2 Ci/μmol [19,47,49–51] and to the best of our knowledge there is no example of routine peptide labeling at >10 Ci/μmol. Corroboration of this assertion is found in a literature survey, where to date, a value of 1 Ci/μmol is often described as "high" [9,48,52] in contrast to more standard values of 0.5 Ci/μmol [53–56] and even much lower values of <0.25 Ci/μmol, which nevertheless have been sufficient for the publication of animal and human PET images [57–59].

Over the past few years, we have sought to exploit the well-known fluorophilicity of boron to capture aqueous ^{18}F-fluoride in one step to provide an *in vivo* stable ^{18}F-ArBF$_3^-$ conjugate. This method was used to directly label biotin [30], Lymphoseek [60], Marimastat [31], and RGD [61], while a one-pot-two-step copper click labeling was used to label and image bombesin [62]. In these examples, specific activities were calculated to be 0.16–0.5 Ci/μmol yet were never measured. Both the relatively low specific activities that were calculated along with the lack of concrete measurement thereof raised concerns over the utility, if not the validity, of this approach. In light of these concerns, we improved this method to demonstrate extraordinarily high specific activities (~15 Ci/μmol) in good-to-excellent radiochemical yields at record synthetic times of 15 min [63,64]. In order to directly measure such values, we converted a boronate ester/borimidine to the corresponding ^{18}F-ArBF$_3^-$ using ~10 mCi NCA ^{18}F-fluoride (specific activity of which was measured independently at 5 Ci/μmol) and then click-conjugated it to a fluorophore, which provided an unambiguous and direct measurement of 3-fold higher specific activity [64]. Notably, calculated values approximated measured values, within experimental error. In order to label with production levels of fluoride e.g. 400–1000 mCi, we featured ^{19}F-^{18}F isotope exchange (IEX) [63], which was first elegantly disclosed by Schirrmacher et al. [24], for use with silylfluorides and later by Li et al. [65], who provided an ^{18}F-ArBF$_3^-$, albeit at very low specific activities in the absence of any peptide labeling or tumor targeting. Likewise, the fluorophore provided unambiguous proof that the specific activities were as high as 15 Ci/μmol when IEX was performed.

To test whether this IEX method could be extended to peptides of clinical relevance, here we focus our efforts on RGD, which was chosen on two accounts: i) its clinical relevance [66] to human and animal images of the $\alpha_v\beta_3$ integrin receptor, a well-defined prognostic indicator for several different types of cancers [10,19,67–69]; and ii) the identical RGD-^{18}F-ArBF$_3^-$ bioconjugate (Fig. 1, below) had been previously imaged at low specific activity (0.06–0.16 Ci/μmol) following both one-step and one-pot-two-step click labeling to provide apparent tumor uptake values of ~2% ID in the raw image [61]. Here, using IEX on the same RGD-tracer, we demonstrate radiochemical yields in excess of 50% at specific activities that are 100 fold higher than we previously reported and 14 fold higher than values normally described as "high". This method affords easy operation in fully shielded hot cells with up to 1 Ci ^{18}F-activity, which should be of immediate interest for use in production labs. Moreover this work demonstrates that IEX labeling can easily be extended to peptides of clinical relevance while preliminary *in vivo* data with blocking controls show statistically significant specific tumor uptake with good tumor:blood ratios. The potential advantages of routine labeling at 14 Ci/μmol are discussed.

2. Material and methods

2.1. General information

Amino acids and resin for the solid-phase synthesis of RGD were obtained from Novabiochem, KHF_2 was obtained from Acros, Tetraphenylpinacol, piperazine, and succinic anhydride were obtained from Alfa-Aesar, Butyl-lithium, 4 M HCl in dioxane, trimethoxyborane, and HFIP were obtained from Sigma-Aldrich. Trifluorobenzene was obtained from Oakwood Products Inc. ^{18}F Trap & Release Columns were purchased from ORTG Inc. (Oakdale, TN) and C18 Sep-Pak cartridge (Vac 1 cc, 50 mg) was obtained from Waters. Deuterated solvents were purchased from Cambridge Isotope Laboratories.

3. HPLC methods

Unless otherwise stated, all samples were resolved on a Phenomenex Jupiter 10 μ C18 300 Å 4.6 × 250 mm analytical column. Gradients for purification are listed below: Gradient A: Solvent A: 0.1% TFA water; solvent B: 0.05% TFA MeCN; 0 to 6 min: 10% to 10% B, 6 to 10 min, 10% to 15% B, 10 to 13 min: 15% to 100% B, 13 to 15 min: 100% to 10% B, 15 to 16 min: 10% to 10% B. Flow rate: 1 mL/min, column temperature: 19 to 21 °C; Gradient B: Solvent A: 0.04 M ammonium formate pH 6.8; solvent B: MeCN; 0 to 5 min: 0% to 5% B, 5 to 10 min: 5% to 35% B, 10 to 20 min, 35% to 45% B, 20 to 22 min: 45% to 100% B, 22 to 28 min: 100% to 100% B, 28 to 30 min: 100% to 20% B, 30 to 33 min: 20% to 5% B; flow rate: (3 mL/min for a semi-preparative column, 1 mL/min for an analytical column), column temperature: 19 to 21 °C; Gradient C: Solvent A: 0.04 M ammonium formate pH 6.8; solvent B: MeCN; 0 to 5 min: 0% to 5% B, 5 to 10 min: 5% to 35% B, 10 to 20 min, 35% to

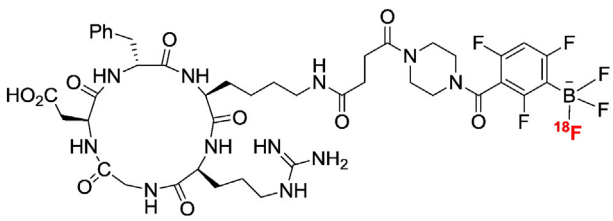

Fig. 1. Structure of RGD-^{18}F-ArBF$_3^-$ labeled in one step by IEX at very high specific activity.