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Invited Perspective

"Kit like" ^{18}F labeling method for synthesis of RGD peptide-based PET probes

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Abstract: Positron Emission Tomography (PET) has become a popular imaging technique widely used for diagnostic purposes. To date, much attention has been devoted to ^{18}F-fluoride because of the characteristics of its nuclear decay, as well as its relative ease of preparation from ^{18}O-water. However, with a half-life of 110 minutes, swift and efficient incorporation of ^{18}F-fluorine into biomolecules is necessary to minimize loss of activity. Therefore, the discovery of rapid and reliable incorporation of ^{18}F-fluorine atoms into biomolecules would be highly beneficial, especially if these protocols can be carried out directly in irradiated ^{18}O-water. In the study published in the American Journal of Nuclear Medicine and Molecular Imaging, cyclo-RGD-^{18}F-aryltrifluoroborate conjugates were prepared based on one-step and one-pot-two-step methods. This paper represents recent efforts on the design and development of novel PET tracers based on the "Kit like" ^{18}F labeling method.

Keywords: Positron emission tomography (PET), ^{18}F, RGD, integrin $\alpha_v\beta_3$, molecular imaging

Introduction

Positron emission tomography (PET) is a non-invasive, quantitative, repeatable and highly sensitive imaging modality that provides *in vivo* radiolabeled biomolecule distribution information [1]. Unlike anatomical imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI), the functional information provided by PET imaging allows an earlier diagnosis of the disease state, which is crucial to provide reliable prognosis and therapeutic intervention. For example, the success of 2-deoxy-2-^{18}F-fluoro-D-glucose (^{18}F-FDG) has made PET a routine clinical practice in cancer diagnosis, patient stratification, and monitoring the treatment of cancer patients [2]. Various positron-emitting radionuclides, including ^{11}C, ^{18}F, ^{64}Cu, ^{68}Ga, and ^{125}I, have been employed in PET probe designs and syntheses. Among them, ^{18}F-fluroide ($t_{1/2}$ = 110 min; β^+, 99%) is an ideal short-lived PET isotope produced in small biomedical cyclotrons. Its half-life is long enough to allow syntheses, transportation, and imaging procedures to be extended over the course of hours, yet with reduced radiation doses for patients in contrast to long-lived radioisotopes. Furthermore, the low positron energy of ^{18}F decay results in a short positron range in tissue, leading to high resolution PET imaging.

^{18}F labeling of RGD peptides using traditional methods

Direct incorporation of ^{18}F into biomolecules, like proteins and peptides, at high specific activity is challenging due to the limited functional groups for nucleophilic ^{18}F-fluorination. Moreover, harsh radiofluorination conditions (i.e., high temperature, strongly basic) cause denaturation and decomposition of sensitive biomolecules. In order to circumvent this obstacle, peptides and proteins are generally labeled with ^{18}F using fluorinated prosthetic groups (also referred as bifunctional labeling agents) [3]. For example, various RGD peptides (with high affinity towards integrin $\alpha_v\beta_3$) have been labeled with ^{18}F and extensively studied in animal and clinical research. The vitronectin receptor integrin $\alpha_v\beta_3$ has been the focus of intense research because of its major role in several

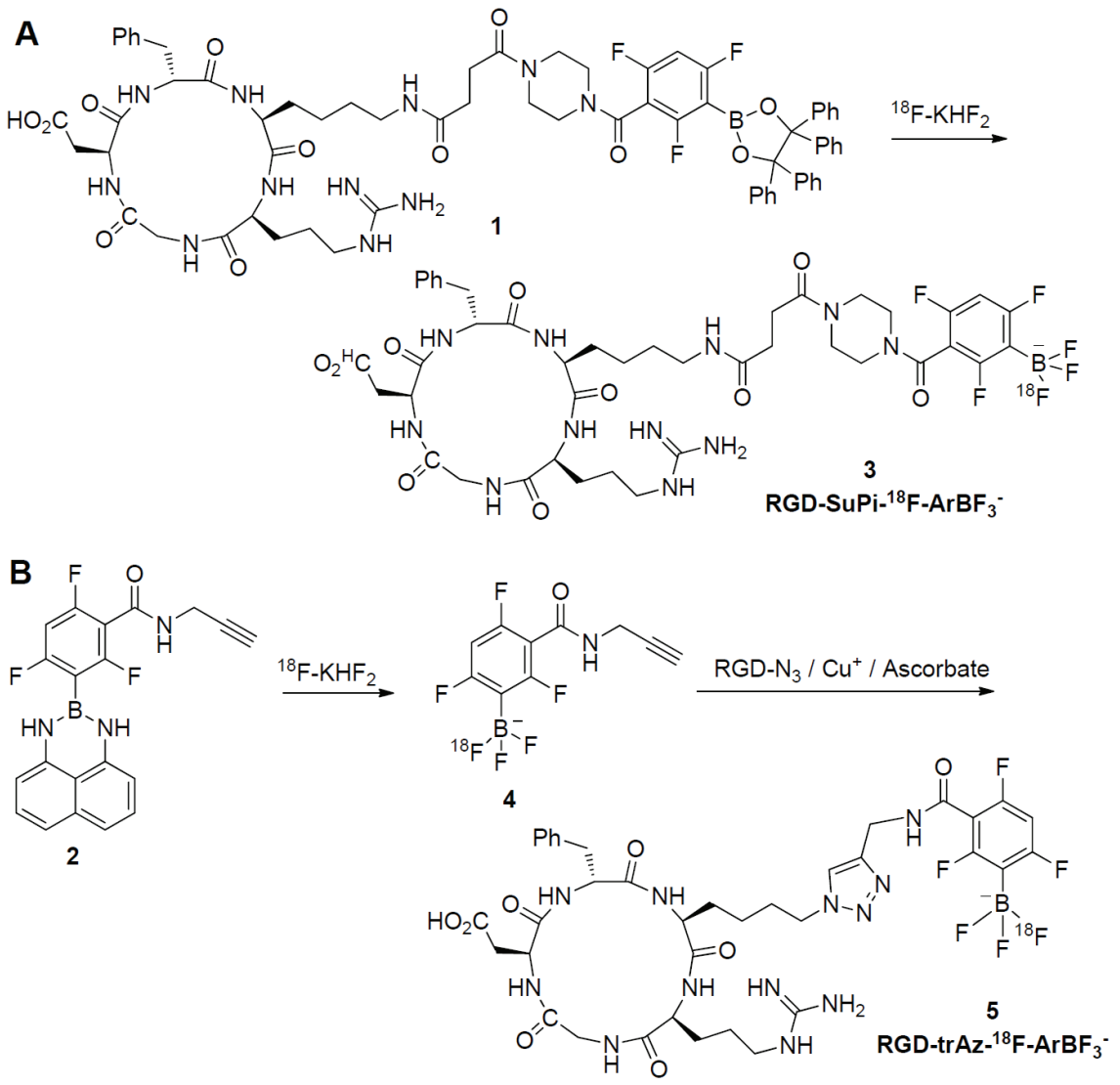

Figure 1. Synthetic scheme for the preparation of RGD-SuPi-^{18}F-ArBF$_3^-$ and RGD-trAz-^{18}F-ArBF$_3^-$: A. a tetraphenylpinacolateboronate ester conjugate to RGD is directly converted to the RGD-SuPi-^{18}F-ArBF$_3^-$. B. The alkyne borimidine 2 is converted first to the corresponding ^{18}F-ArBF$_3^-$ alkyne 4 in situ, which is then directly conjugated to the RGD-azide to provide RGD-trAz-^{18}F-ArBF$_3^-$.

distinct processes, particularly osteoclast mediated bone resorption, angiogenesis, pathological neovascularization, and tumor metastasis [4]. In 2001, Galacto-RGD was labeled with 4-nitrophenyl 2-^{18}F-fluoropropionate (^{18}F-NPFP), which successfully imaged integrin $\alpha_v\beta_3$ expression *in vivo*. The clinical PET studies of ^{18}F-Galacto-RGD show highly favorable biodistribution in humans and visualization of $\alpha_v\beta_3$ expression with high contrast [5, 6]. Although promising results were obtained, the overall radiochemical yield for ^{18}F-Galacto-RGD was 29 ± 5% with a total reaction time of 200 ± 18 min, including final HPLC preparation [7]. *N*-succinimidyl 4-^{18}F-fluorobenzoate (^{18}F-SFB) was also used to label monomeric, dimeric and tetrameric RGD peptides through the side-chain ε-amino group of the lysine residue [8-10]. The radiotracers were evaluated *in vivo* and demonstrated favorable tumor targeting efficacy and pharmacokinetics. The radiolabeling was finished in approximately 2 h with 20–25% decay-corrected yields and specific activity of 230 GBq/μmol (6.2 Ci/μmol) at end of synthesis. Other reactions that have been used for ^{18}F labeling of RGD peptide include

"click" chemistry, tetrazine–*trans*-cyclooctene ligation, thiol/maleimide and oxime/aldehyde chemistry (^{18}F-AH111585) [11-16].

"Kit like" ^{18}F labeling of RGD peptides

Although encouraging results were obtained for ^{18}F-labeled RGD probes, most of the above methods used the conventional formation of a C-^{18}F bond. The main shortcomings of this approach include multistep synthetic pathways, time-consuming procedures, and most notably the need for specially trained radiochemist. To overcome these problems, "Kit Formulation" for ^{18}F-labeling has been proposed. The basic idea is to introduce an atom to the target compound, which could form a stable bond with F^-. In an ideal situation, the labeled compound could be obtained by simply mixing the $^{18}F^-$ with the modified precursor. The "Kit like" ^{18}F labeling approach is appealing because time-consuming synthetic steps can be carried out before introduction of the short-lived ^{18}F radionuclide. However, this field of research remains relatively underdeveloped with only a few types of captors investigated thus far, which include boron fluoride acceptor, silicon fluoride acceptor, phosphorous-^{18}F compounds, and NOTA-Al-fluoride complex [17-24]. In this study published in the American Journal of Nuclear Medicine and Molecular Imaging, Perrin et al reported an elegant approach to synthesize ^{18}F labeled RGD peptide based on arylboronic compound featuring electron-withdrawing substituents [25]. Because the arylboronic compounds quickly react with fluoride ions to form the corresponding arylfluoroborates, a simple one-step or one-reactor RGD labeling could be accomplished in aqueous media, which lead to two RGD probes: RGD-SuPi-^{18}F-$ArBF_3^-$ and RGD-trAz-^{18}F-$ArBF_3^-$ (**Figure 1**) [25].

In the synthesis of RGD-SuPi-^{18}F-$ArBF_3^-$, the protocol mixed the RGD borate precursor (compound 1, **Figure 1**) and the pre-concentrated ^{18}F-fluoride together, followed by incubation at room temperature for 1 h. The crude mixture was subjected to HPLC purification to obtain the final product. The total synthetic time was 2 h with approximately 4% yield (non-decay corrected yield). In the synthesis of RGD-trAz-^{18}F-$ArBF_3^-$, the borate precursor equipped with alkyne functional group (compound 2, **Figure 1**) was mixed with concentrated ^{18}F for fluorination, followed by addition of RGD-N_3 peptide to the same reactor. After purification with HPLC and C18 cartridge, RGD-trAz-^{18}F-$ArBF_3^-$ was obtained with the non-decay corrected yield of 6.8% (total synthetic time is 3.7 hours). These two tracers were studied with microPET using U87MG tumor models. The tumor uptakes of RGD-SuPi-^{18}F-$ArBF_3^-$ and RGD-trAz-^{18}F-$ArBF_3^-$ have no significant difference at time points examined and were comparable to other publications on ^{18}F labeled RGD peptides [8, 26]. Furthermore, the authors claimed the bone uptake for both probes were minimum based on the microPET analysis, which indicated negligible defluorination. Clearly, the preliminary data warrant further evaluation and improvement of these newly developed ^{18}F labeling methods.

We would also like to point out that the labeling of RGD-SuPi-^{18}F-$ArBF_3^-$ was performed in strong acidic conditions. Although it is acceptable for the labeling of RGD peptides, other ligands (such as proteins and antibodies) may be sensitive to acidic conditions. In addition, both reactions are carrier-added (KHF_2), and relatively low specific activities for the products were obtained. This could be a limitation for the syntheses of PET probes with high toxicity or when the target has low density *in vivo*. Further modification of the labeling protocol may be necessary to obtain higher specific activity in those cases. Although the Log(P_{ow}) of RGD-trAz-^{18}F-$ArBF_3^-$ was -3.8 in terms of octanol-water partition, substantial uptake in the liver, spleen, and gallbladder was observed after injection. This indicates the pharmacokinetic properties of these peptides could be further improved to achieve increased tumor-to-background contrasts and reduced radiation exposure to normal organs. The Galacto- or PEG (polyethylene glycol)-linker could be employed to improve imaging quality as demonstrated previously [7, 27].

In summary, much effort has been made to investigate ^{18}F-labeling chemistry for biologically important peptides. Traditional labeling tactics such as ^{18}F-SFB and ^{18}F-NPFP have greatly advanced the PET imaging field. However, the preparation of these labeling moieties often requires multiple step procedures, sophisticated synthetic modules, and skilled radiochemists. The disadvantages severely hamper the use of these labeling approaches

in routine clinical production of peptide-based PET probes. Therefore, the discovery of general and reliable protocols for the rapid incorporation of ^{18}F-fluorine atoms into biomolecules would be highly beneficial, especially if these protocols can be carried out directly in the irradiated ^{18}O-water. In the study published in the American Journal of Nuclear Medicine and Molecular Imaging, cyclo-RGD-^{18}F-aryltrifluoroborate conjugates were prepared based on one-step and one-pot-two-step methods. This paper represents recent efforts on the design and development of novel PET tracers based on "Kit like" ^{18}F labeling method. Certainly, continued efforts are needed to further optimize these methods in order to establish ideal "Kit-labeling" procedures of ^{18}F-labeled peptides for clinical applications.

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