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PET Imaging of Angiogenesis after Myocardial Infarction/ Reperfusion using a One-Step Labeled Integrin Targeted Tracer ^{18}F -AIF-NOTA-PRGD2

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Abstract

Objective—The $\alpha_v\beta_3$ integrin represents a potential target for noninvasive imaging of angiogenesis. The purpose of this study was to evaluate a novel one-step labeled integrin $\alpha_v\beta_3$ targeting PET probe, ^{18}F -AIF-NOTA-PRGD2, for angiogenesis imaging in a myocardial infarct/reperfusion (MI/R) animal model.

Methods—Male SD rats underwent 45 min transient left coronary artery occlusion followed by reperfusion. The myocardial infarction was confirmed by ECG, ^{18}F -FDG imaging and cardiac ultrasound. *In vivo* PET imaging were used to determine myocardial uptake of ^{18}F -AIF-NOTA-PRGD2 at different time points following reperfusion. The control peptide RAD was labeled with a similar procedure and used to confirm the specificity. *Ex vivo* autoradiographic analysis and CD31/CD61 double immunofluorescence staining were performed to validate the PET results.

Results—Myocardial origin of the ^{18}F -AIF-NOTA-PRGD2 accumulation was confirmed by ^{18}F -FDG and autoradiography. PET imaging demonstrated increased focal accumulation of ^{18}F -AIF-NOTA-PRGD2 in the infarcted area started at day 3 (0.28 ± 0.03 %ID/g, $p < 0.05$), peaked between 1 and 3 weeks (0.59 ± 0.16 and 0.55 ± 0.13 %ID/g, respectively). The focal accumulation decreased but still kept at a higher level than the sham group after 4 months of reperfusion (0.31 ± 0.01 %ID/g, $p < 0.05$). Pretreatment with unlabeled RGD peptide significantly decreased tracer uptake, indicating integrin specificity of this tracer. At 1 week after MI/R, uptake of the control tracer ^{18}F -AIF-NOTA-RAD that does not bind to integrin, in the infarcted area, was only 0.21 ± 0.01 %ID/g. Autoradiographic imaging showed the same trend of uptake in myocardial infarction area. The time course of focal tracer uptake was consistent with the pattern of vascular density and integrin β_3 expression as measured by CD31 and CD61 immunostaining analysis.

Conclusion—PET imaging using one-step labeled ^{18}F -AIF-NOTA-PRGD2 allows noninvasive visualization of ischemia-reperfusion induced myocardial angiogenesis longitudinally. The favorable *in vivo* kinetics and easy production method of this integrin targeted PET tracer facilitates its future clinical translation for lesion evaluation and therapy response monitoring in patients with occlusive cardiovascular diseases.

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Keywords

PET imaging; angiogenesis; myocardial infarction; integrin; RGD peptide

Introduction

After myocardial ischemia/reperfusion (MI/R) injury occurs, new blood vessels form primarily from the ischemic border zone into the infarcted tissue *via* either angiogenesis or vasculogenesis [1]. The process will restore oxygen and nutrient supply to the infarcted tissue and is considered to be a key factor for post-ischemic repair [2]. Accumulating data support that imaging of post-MI/R angiogenesis may provide additional *in vivo* insight into the time course, magnitude, heterogeneity, and localization of angiogenesis related proteins and genes [3]. Integrins are a family of proteins that facilitate cellular adhesion and migration to extracellular matrix proteins found in intercellular spaces and basement membranes, and regulate cellular entry and withdrawal from the cell cycles [4]. Especially, $\alpha_v\beta_3$ integrin has a key role in endothelial cell survival and migration during angiogenesis [5, 6]. $\alpha_v\beta_3$ integrin is highly expressed on activated neovascular endothelial cells during angiogenesis [6]. In contrast, expression of integrin $\alpha_v\beta_3$ is low in resting endothelial cells and most normal organ systems [7]. Therefore, integrin $\alpha_v\beta_3$ is a promising target for angiogenesis imaging with the specific probes including Arginine-Glycine-Aspartic acid (RGD) containing peptides [8].

Besides intensive tumor angiogenesis imaging with RGD-based imaging probes [9], integrin $\alpha_v\beta_3$ expression in the heart after myocardial ischemia-reperfusion injury has been noninvasively investigated by a number of isotopes including ^{99m}Tc , ^{111}In , ^{123}I for single-photon emission computed tomography (SPECT) imaging [10–12] and ^{18}F for positron emission tomography (PET) imaging [13, 14]. Compared with SPECT, PET offers high sensitivity, reasonable spatial resolution, and permits more accurate attenuation correction.

With almost 100% positron efficiency, low β^+ energy (0.64 MeV) and relatively short physical half-life ($t_{1/2} = 109.7$ min), F-18 is the most widely used positron emitting radioisotope for PET imaging, and its physical properties are ideally suited for RGD peptide-based PET imaging probes. The majority of methods used in labeling RGD peptides with ^{18}F use a prosthetic group, such as *N*-succinimidyl 4- ^{18}F fluorobenzoate (^{18}F SFB) [15] or 4-nitrophenyl 2- ^{18}F fluoropropionate (^{18}F NPFP) [16]. The time-consuming multiple-step synthetic procedure required for preparation may hinder the widespread use of these ^{18}F -labeled RGD tracers. Efforts have been made with some success to simplify the labeling procedure by direct labeling of RGD peptides with a pre-attached functional group with a proper leaving group for fluoride displacement [17]. On the other hand, labeling radioactive metal ions through a pre-attached chelator on RGD peptides has been blessed with a much simpler procedure [18–20]. Application of chelation chemistry has led to recent discovery and development of an ^{18}F fluoride-aluminum complex to radiolabel peptides, which provides a strategy to simplify the labeling procedure [21–23]. We also performed a comparison study and found that ^{18}F -AIF-NOTA-PRGD2 has imaging properties and pharmacokinetics comparable to those of ^{18}F -FPPRGD2 in tumor xenograft models. Considering the ease of preparation and good imaging qualities, ^{18}F -AIF-NOTA-PRGD2 is a promising alternative to ^{18}F -FPPRGD2 for PET imaging of integrin $\alpha_v\beta_3$ expression [24].

In this study, we established a rat model of experimental MI/R and performed longitudinal PET imaging using ^{18}F -AIF-NOTA-PRGD2. With PET imaging, we successfully visualized and quantified neo-angiogenesis after MI/R non-invasively.

Materials and methods

General materials

All reagents were of analytical grade and were obtained from commercial sources. ^{18}F - F^- radionuclide was obtained from the NIH Clinical Center's cyclotron facility by proton irradiation of ^{18}O -enriched water. ^{18}F -FDG was purchased from the Nuclear Pharmacy of Cardinal Health and reconstituted with sterile saline. PRGD2 was obtained from CS Bio Company, Inc. (Menlo Park, CA).

Radiosynthesis of ^{18}F -AIF-NOTA-PRGD2

The synthesis of ^{18}F -AIF-NOTA-PRGD2 was performed according to the procedure reported previously [24]. In brief, 1 mL V-vial containing 0.1 mL of aqueous [^{18}F]fluoride (0.37 GBq) was added 3 μL of 2 mM aluminum chloride in 0.2 M pH 4 sodium acetate buffer and heated at 100 C for 10 min to form the aluminum-fluoride complex. Then 6 μL of 2 mM NOTA-PRGD2 in 0.2 M pH 4 sodium acetate buffer was added and heated at 100 C for another 10 min. The reaction mixture was directly loaded on the C_{18} cartridge without HPLC purification and was eluted off with 0.3 mL of 1 mM HCl ethanol solution. The final product was buffered in PBS solution for *in vivo* study. ^{18}F -AIF-NOTA-RAD was synthesized by a similar procedure.

Animal model of coronary occlusion and reperfusion

All animal studies were conducted in accordance with the principles and procedures outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Clinical Center, NIH. Adult male Sprague–Dawley rats weighing from 250 to 300 g were used for this study. Anesthesia was induced with 5% isoflurane and maintained with 2% isoflurane. After the animals were intubated for mechanical ventilation, MI/R was induced by ligation of the left anterior descending coronary artery 2–3 mm from the tip of the left auricle with a 6-0 polypropylene suture. Forty-five minutes after the coronary occlusion, the suture was removed to obtain reperfusion. In the sham-operated animals, a suture was placed in the myocardium (without ligating the left coronary artery). The occlusion and reperfusion were confirmed by ST-segment elevation on an electrocardiogram monitor (EC-60 model; Silogic) and ^{18}F -FDG imaging at 2 days after surgery.

Assessment of LV contractility with echocardiography

Cardiac function was assessed as previously described [25]. Briefly, 7 days after MI/R, the rats received isoflurane (3%) for general anesthesia and were placed on the scanning table. Echocardiographic images were obtained using a dedicated small-animal high-resolution-imaging unit and a 30-MHz linear transducer (Vevo 770; VisualSonics) [26]. Using the parasternal short-axis view, LV end-diastolic and LV end-systolic diameters (LVEDD and LVESD, respectively) were measured, and LV fractional shortening was calculated as $(\text{LVEDD} - \text{LVESD})/\text{LVEDD} \times 100$. All measurements were averaged on 3 consecutive cardiac cycles.

Small animal PET scan

At 3 days, 1 week, 3 weeks and 4 months after MI/R, PET scans were performed using an Inveon microPET scanner (Siemens Preclinical Solutions) [27]. Animals were anesthetized with isoflurane (3%) and approximately 13 MBq (350 μCi) of ^{18}F -AIF-NOTA-PRGD2 or ^{18}F -AIF-NOTA-RAD was administered via tail vein injection. Ten-minute static PET images were acquired at 1 h after injection of the tracer. The images were reconstructed using a two-dimensional ordered-subset expectation maximum (2D OSEM) algorithm, and

no correction was applied for attenuation or scatter. For each scan, regions of interest (ROIs) were drawn using vendor software (ASI Pro 5.2.4.0) on decay-corrected whole-body coronal images. The radioactivity concentrations (accumulation) within the heart were obtained from mean pixel values within the multiple ROI volume and then converted to MBq per milliliter. These values were then divided by the administered activity to obtain (assuming a tissue density of 1 g/ml) an image-ROI-derived percent injected dose per gram (%ID/g). In addition, signal from the infarcted area was compared with the contralateral myocardium (at the septal level), which was taken as background signal, and expressed as the signal-to-background ratio. To assess the specificity of tracer uptake, 18 mg/kg of cyclic RGD peptide dimer E[c(RGDyK)]₂ was injected 10 min before ¹⁸F-AIF-NOTA-PRGD2 (18.5 MBq) administration in rats at 1 week after MI/R (n = 3).

Immediately after the ¹⁸F-AIF-NOTA-PRGD2 scan, animals were kept on the scanning gantry (for image co-registration) and injected with 18.5 MBq (500 µCi) of ¹⁸F-FDG. One hour after, 10-min static acquisitions were performed with the mid thorax in the center of the FOV, and images were reconstructed and analyzed with the same procedure described above.

Autoradiography

At 3 days, 1 week, 3 weeks and 4 months after MI/R, in parallel with PET imaging, the same amount of ¹⁸F-AIF-NOTA-RGD2 was administered to rats. At 1 h after the administration, the heart of each rat was excised, embedded and frozen in CRYO-OCT compound (Tissue-Tek). Serial short axis sections in 1 mm intervals (15 µm thickness each), covering the entire heart were obtained using a cryostat (Ultrapro5000, Vibratome, St. Louis, MO). The autoradiographic exposure was performed for 12 h and then developed in a Cyclone Plus Storage Phosphor System (PerkinElmer, US). The acquired photos were analyzed with Optiquant Acquisition and Analysis Software V5.0 (Perkin Elmer). ROI was manually defined for a focal myocardial tracer uptake region and a contra-lateral normal region on a mid-myocardial section. If no focal myocardial tracer accumulation was observed, ROI were placed over the anterolateral wall. Radioactivity values of each ROI were measured in digital light units (DLU) /per area (mm²). DLU is an arbitrary linear unit that describes the intensity of photon emissions released during the scan. ¹⁸F-AIF-NOTA-PRGD2 uptake ratio was calculated by dividing the ROI value of the focal tracer uptake region with that of the contra-lateral normal area.

HE and TTC staining

Slices with fifteen µm thickness adjacent to those used for autoradiography were selected for haematoxylin and eosin (HE) by use of standard techniques. Two mm thick slices adjacent to those used for autoradiography and HE imaging were also prepared and stained with a buffered solution of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) at 38 °C to identify myocardial infarction.

Immunofluorescence staining

Myocardial frozen tissue slices (5-µm thick) were fixed with ice-cold acetone for 10 min and dried in the air for 30 min. The slices were rinsed with phosphate-buffered saline for 2 min and blocked with 10% donkey serum for 30 min at room temperature. For staining of CD31 and CD61, the slices were incubated with mouse anti-rat CD31 (1:20, BD Pharmingen™) and hamster anti-rat CD61 (1:20, BD Pharmingen™) antibody. The slices were incubated with Cy3 conjugated donkey anti-mouse and Dylight488-conjugated goat anti-hamster secondary antibody (1:100; Jackson ImmunoResearch Laboratories, Inc.). Fluorescence images were acquired with an epifluorescence microscope (Olympus, X81).

Statistical Analysis

Data are expressed as mean $\pm$ SD. Statistical analysis used the unpaired Student t test with unequal variance (for comparison between sham-operated and MI animals) and the paired Student t test for comparison before and after MI. A p value < 0.05 was considered statistically significant.

Results

Chemistry and radiochemistry

The structure of ^{18}F -AIF-NOTA-PPRGD2 is shown in Figure 1a. The labeling efficiency for ^{18}F -AIF-NOTA-PPRGD2 varied between 5 and 25% depending on the reaction volumes. When the reaction volume was kept between 50 and 100 μl , the yield was consistently between 20–25%. The total synthesis time was about 25 min without HPLC purification. The radiochemical purity was over 97% (Figure 1b).

Characterization of MI/R model

The MI/R procedure had a mortality of 15–20%, similar to that reported by other investigators [25]. The success of MI/R model was confirmed by ST-T segment elevation using ECG during surgery (Fig. 2a). There was no difference in weight or heart rate between sham ($n = 3$) and MI/R ($n = 8$) animals (data not shown). Three days after surgery, obvious uptake defect of ^{18}F -FDG in the anterolateral wall of left ventricle was observed with ^{18}F -FDG PET (Fig. 2b). On day 7 after MI/R, akinesis of the anterolateral wall with a significant decrease in cardiac function was identified by M-mode high-resolution ultrasound (Fig. 2c). Ejection function (EF) value decreased from 50% to 30% in rats after ischemia-reperfusion. In contrast, no uptake defect or compromised cardiac function was observed with ^{18}F -FDG PET and M-mode high-resolution ultrasound in sham-operated animals

In vivo PET imaging

Due to the high metabolic rate of myocardium, heart is one of the major organs for ^{18}F -FDG uptake [16]. Thus, we expected that ^{18}F -FDG PET could provide anatomical reference and reflect the metabolism of the infarcted area. To facilitate image coregistration, the animals were kept at the exactly same position on the scanning gantry during the tracer injection and the imaging process. Representative myocardial short-axis serial slices from the same animal following *in vivo* PET imaging of ^{18}F -AIF-NOTA-PRGD2 and ^{18}F -FDG are presented in Figure 3. High level of ^{18}F -FDG accumulation was visualized homogenously in normal heart while almost no ^{18}F -AIF-NOTA-PRGD2 uptake was observed in the heart region. Compared with that in sham-operated rats, the heart in MI/R rats still showed decreased ^{18}F -FDG uptake in the anterolateral wall of left ventricle at day 7 after surgery. At the same time, localized ^{18}F -AIF-NOTA-PRGD2 uptake was observed in rats after MI/R. Moreover, the localization matched perfectly with the areas of infarcted myocardium, as evidenced by the decrease of ^{18}F -FDG uptake.

To evaluate angiogenesis after MI/R, longitudinal PET imaging was performed at 3 days, 1 week, 3 weeks and 4 months after surgery using ^{18}F -AIF-NOTA-PRGD2 as the imaging probe. Figure 4a shows representative transaxial PET images with ^{18}F -AIF-NOTA-PRGD2. Compared with the control group, uptake increase was observed as early as day 3 after MI/R and reached the maximum between 1 week and 3 weeks after MI/R. The signal then decreased after 4 months. Animals from both groups showed increased uptake of ^{18}F -AIF-NOTA-PRGD2 at the region of the surgical wound, reflecting an angiogenic response during the wound healing process.

The quantitative results based on PET imaging are presented in Figure 4b. The myocardial uptake of ^{18}F -AIF-NOTA-PRGD2 in the sham group at 1 week after surgery was determined to be 0.15 ± 0.04 %ID/g. MI/R induction was associated with a significant increase in uptake of ^{18}F -AIF-NOTA-PRGD2 in the anterolateral wall of the myocardium after 3 days reperfusion (0.28 ± 0.03 %ID/g, $p < 0.05$). ^{18}F -AIF-NOTA-PRGD2 localization in the MI/R was maximal between 1 and 3 weeks (0.59 ± 0.16 and 0.55 ± 0.13 %ID/g respectively, $p < 0.01$). Uptake of the radiotracer decreased by 4 months but localization of the radiotracer was still greater than that in the sham group (0.31 ± 0.01 %ID/g, $p < 0.05$).

In order to further confirm integrin receptor binding specificity of ^{18}F -AIF-NOTA-PRGD2, we used non-radiolabeled integrin specific ligand, E[c(RGDyK)]₂, as a blocking agent. Both imaging and quantification demonstrated that tracer uptake in the infarcted myocardium could be blocked by excess amount of unlabeled RGD peptide. The tracer uptake was significantly lower than that in the unblocked rats at 1 week after MI/R (0.22 ± 0.08 vs. 0.59 ± 0.16 %ID/g, $p < 0.05$). We also labeled a RAD peptide with a similar procedure and found that the localized tracer uptake was 0.21 ± 0.01 %ID/g at 1 week after MI/R, which is significant lower than that of ^{18}F -AIF-NOTA-PRGD2 ($p < 0.01$). These results further substantiated the specificity of integrin targeting imaging.

Ex vivo autoradiography

In parallel with *in vivo* PET imaging, we also performed *ex vivo* autoradiography to validate the quantitative results based on PET images. Typical autoradiographic images and averaged uptake ratios at different time points are shown in Figure 5. At 3 days, 1 week and 3 weeks after MI/R, increased signal intensity was clearly shown in the infarcted and ischemic area, evidenced by TTC stains and HE staining. The results confirmed the localized high level of ^{18}F -AIF-NOTA-PRGD2 uptake as presented by non-invasive PET imaging. Quantification data demonstrated that the trend of uptake ratio of ^{18}F -AIF-NOTA-PRGD2 reached maximum at 1 week and 3 weeks after MI/R, which is consistent with PET imaging results. Once blocked with excess amount of unlabeled RGD peptide, the decreased uptake of ^{18}F -AIF-NOTA-PRGD2 was also reflected on the autoradiographic images. Much lower signal intensity was also observed with the control tracer ^{18}F -AIF-NOTA-RAD ($p < 0.05$).

CD31 and integrin immunostaining

To further investigate the mechanism of increased ^{18}F -AIF-NOTA-PRGD2 uptake after MI/R, we performed CD31 and CD61 immunostaining to follow the changes of microvasculature and integrin β_3 expression at different time points after the surgery. As shown in Figure 6 in the normal heart from the sham group, positive CD31 staining was distributed homogeneously among myocardium, representing an orderly architecture of the microvasculature. At the same time, very low level of CD61 signal was observed. At 3 days after MI/R, the pattern of CD31 signal was different from that in the normal myocardium. Increased CD61 signal was observed along with CD31 positive region but without apparent co-localization. At 1 week after MI/R, stronger and more diffusive CD31 and CD61 signals were recognized with dominant co-localization. The similar pattern was also observed in the infarcted/ischemic myocardium at 3 weeks after MI/R. After 4 months, the vascular density was dramatically lower than that in the normal myocardium, indicated by sparse CD31 positive region. CD61 signal still remained, but with a considerably lower level as compared with that at 1 or 3 weeks after MI/R.

Discussion

In a mouse MI model, it has been found that the maximum quantitative uptake in the infarct area was highest at 2 weeks after MI with SPECT imaging [28]. With ^{18}F -Galacto- RGD as

the PET tracer, Higuchi *et al.* also demonstrated that focal accumulation in the infarct area started at day 3, peaked between 1 and 3 weeks after reperfusion [14]. In this study we applied for first time a one-step labeled PET tracer ^{18}F -AIF-NOTA-PRGD2 to visualize and quantify the temporal changes of integrin $\alpha_v\beta_3$ expression during ischemia-induced angiogenesis in a rat myocardial ischemia/reperfusion model. Increased focal tracer retention was observed as early as 3 days after onset of ischemia and the tracer accumulation peaked at 1 and 3 weeks in the infarcted and peri-infarct zones. At 4 months after MI/R, ^{18}F -AIF-NOTA-PRGD2 uptake within the myocardium decreased from the peak level but was still higher than that in the remote myocardium. Thus, the imaging results with ^{18}F -AIF-NOTA-PRGD2 PET revealed a similar pattern of tracer uptake in the infarct area.

Extracellular matrix (ECM) proteins such as vitronectin, fibrinogen, and fibronectin interact with integrin via the RGD sequence. Due to its high affinity and selectivity for the receptor, the pentapeptide cyclo(RGDfK) became the most prominent structure for the development of molecular imaging compounds to evaluate integrin $\alpha_v\beta_3$ expression [29]. Compared with RGD monomer including Galacto-RGD, the dimeric RGD peptide tracers showed higher tumor integrin-specific accumulation and more favorable *in vivo* kinetics [30]. The use of multimeric cyclic RGD peptides to improve the integrin $\alpha_v\beta_3$ binding affinity was based on the fact that the interaction between integrin $\alpha_v\beta_3$ and RGD-containing ECM proteins involves multivalent binding sites with clustering of integrins [31]. With the dimeric RGD peptide tracer ^{18}F -AIF-NOTA-PRGD2, significant focal myocardial uptake was observed on PET images (Fig. 4). Quantitative data showed that the tracer uptake reached a high level plateau on 1 and 3 weeks after MI/R with 0.59 ± 0.16 and 0.55 ± 0.13 %ID/g, respectively, which is significantly higher than those reported using the monomeric RGD tracer ^{18}F -Galacto-RGD (less than 0.3 %ID/g) [14].

The dimeric RGD peptide tracer ^{18}F -FPPRGD2 we developed previously [30] has been approved by the Food and Drug Administration (FDA) through an exploratory investigative new drug (eIND) mechanism (IND 104150). The initial pilot studies in human demonstrated that the administration of ^{18}F -FPPRGD2 was well tolerated, with no marked effects on vital signs, ECG readings, or laboratory values [32]. Synthesis of ^{18}F -FPPRGD2 usually requires two HPLC purifications, which is time-consuming and consequently results in low labeling yield. Alternatively, one-step route for labeling has been investigated by several groups including us [17, 23, 24]. By taking advantage of the reaction of ^{18}F -fluoride-aluminum complex to pre-attached chelator on RGD peptides, a novel dimeric RGD peptide tracer, ^{18}F -AIF-NOTA-PRGD2, has been prepared without the need of HPLC purification and still maintaining reasonable specific activity level [24]. Also it has been demonstrated that modification of RGD peptides with NOTA did not significantly change the biological binding affinities [23]. In this study, the labeling efficiency for ^{18}F -AIF-NOTA-PRGD2 was consistently between 20–25%. The total synthesis time was about 25 min without HPLC purification. The radiochemical purity was over 97%. It is reasonable to surmise that the simplicity of the labeling process of ^{18}F -AIF-NOTA-PRGD2 would be very attractive for clinical translation, especially when applied to patients with cardiovascular diseases.

Increased ^{18}F -AIF-NOTA-PRGD2 uptake in the peri-infarct and infarcted zones was associated with increased integrin $\alpha_v\beta_3$ expression induced by angiogenesis and tissue remodeling after MI/R. As confirmed by anti-CD61 staining, normal myocardium showed minimal integrin β_3 expression. To the contrast, increased integrin β_3 expression was observed as early as 3 days after MI/R and kept increasing to a peak level between 1 week and 3 weeks after MI/R (Fig. 6). In a canine model of MI/R, Frangogiannis *et al.* [33] found that capillary density peaked at week 3. We also identified the changes of morphology and density of microvasculature after MI/R based on anti-CD31 staining. It is worth noting that the signals from CD31 and CD61 were not always co-localized. One possible explanation is

that inflammation occurred after MI/R since infiltrated macrophages do express high levels of $\alpha_v\beta_3$ integrin [34]. Indeed, HE staining at 1 week after infarction (Fig. 5a) suggested inflammatory cell infiltration among affected myocardium. We also cannot exclude the possibility of proliferating myofibroblasts taking up RGD peptide tracer in the infarcted and peri-infarct area, as reported previously [28, 35]. As shown in Figure 5a, significant wall thinning was observed at 4 months after MI/R, indicating the formation of myocardial scar. Consequently, how to delineate angiogenesis and tissue remodeling after MI/R by ^{18}F -AIF-NOTA-PRGD2 PET imaging will still need further investigation.

FDG PET imaging was used to provide an anatomical reference for ^{18}F -AIF-NOTA-PRGD2 PET images, because the latter approach lacks detailed anatomical information. Normal myocardium showed very high FDG uptake due to the high glucose metabolism. At 1 week after MI/R, the ischemic myocardium showed decreased FDG uptake, which may probably be due to lower viability caused by MI/R. However, at 2 weeks after MI/R, FDG PET imaging showed increased uptake in the infarct area. The exact reason needs further investigation, but we speculated that it might be related to remodeling and inflammation induced by MI/R.

In summary, longitudinal PET imaging using ^{18}F -AIF-NOTA-PRGD2 provided temporal information of integrin $\alpha_v\beta_3$ changes after MI/R in a non-invasive and quantitative mode. Moreover, the localization and quantification of PET imaging was further validated by *ex vivo* autoradiography, *in vivo* FDG PET imaging, and corresponding HE and TTC staining. However, one inherent limitation of PET imaging is that the spatial resolution was not high enough to define exactly the subarea of myocardial infarction and its border zone.

Conclusions

PET imaging using a one-step labeled tracer, ^{18}F -AIF-NOTA-PRGD2, allows non-invasive visualization of ischemia-reperfusion induced myocardial angiogenesis longitudinally. The favorable *in vivo* performance and easy production method of this integrin targeted PET tracer facilitates its future clinical translation for lesion evaluation and therapy response monitoring in patients with occlusive cardiovascular diseases and pro-angiogenic drug screening in preclinical setting.

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Fig. 1.
a, Chemical structure of ^{18}F -AlF-NOTA-PRGD2. **b**, Radiochemical purity by HPLC.

Fig. 2. Characterization of the myocardial infarction/reperfusion (MI/R) model. **a**, ECG change during and after MI/R. **b**, ^{18}F -FDG PET imaging of the sham-operated rats and the animal with MI/R at 3 days after reperfusion. Decreased ^{18}F -FDG accumulation was observed in anterolateral wall (white arrow) in the heart with MI/R. **c**, M-mode ultrasound at midventricle level. At 7 days after MI/R, there was akinesis of anterolateral wall and significant decrease in fractional shortening compared with that of sham-operated animals (red arrow).

Fig. 3.

In vivo PET images of ^{18}F -FDG, ^{18}F -AIF-NOTA-PRGD2, and their fusion in transaxial view. Coronary artery ligation resulted in decrease of ^{18}F -FDG uptake (red arrow) and increased uptake of ^{18}F -AIF-NOTA-PRGD2 (white arrow). Fusion of both scans results in perfect co-localization of decreased ^{18}F -FDG uptake and increased ^{18}F -AIF-NOTA-PRGD2 signals. The increased ^{18}F -AIF-NOTA-PRGD2 uptake could be blocked with excess amount of unlabeled RGD peptide E[c(RGDyK)]₂.

Fig. 4.

a, Representative transaxial PET images using ^{18}F -AIF-NOTA-PRGD2 or ^{18}F -AIF-NOTA-RAD at different times after MI/R. The high ^{18}F -AIF-NOTA-PRGD2 uptake region from the infarcted/ischemic myocardium is indicated by white arrows, and the triangle indicates ^{18}F -AIF-NOTA-PRGD2 signal from the surgical wound, which is present in both sham-operated and MI/R animals. **b**, Quantification of ^{18}F -AIF-NOTA-PRGD2 uptake after MI/R over time, expressed in %ID/g of tissue. ^{18}F -AIF-NOTA-PRGD2 uptake was highest at 1 week 1 postoperatively compared with sham group. * $p < 0.05$; ** $p < 0.01$.

Fig. 5.

a, Representative autoradiographs of ^{18}F -FAI-NOTA-PRGD2 and ^{18}F -AIF-NOTA-RAD uptake in the myocardium (upper row) and corresponding HE staining (middle row) and TTC staining (bottom row) on adjacent slices from the same animal at different time points after MI/R. **b**, Time course of myocardial ^{18}F -FAI-NOTA-PRGD2 uptake ratios (vs. remote myocardium) after MI/R determined by autoradiography. * $p < 0.05$ as compared with sham group, ** $p < 0.01$.

Fig. 6. Immunofluorescence staining of CD31 and CD61 (integrin $\beta 3$) in sham-operated and MI/R animals over time.