Targeted imaging of hypoxia-induced integrin activation in myocardium early after infarction

Leszek Kalinowski,1,2,3 Lawrence W. Dobrucki,4,5 David F. Meoli,1 Donald P. Dione,1 Mehran M. Sadeghi,1 Joseph A. Madri,3 and Albert J. Sinusas1,4

1 Experimental Nuclear Cardiology Laboratory, Division of Cardiovascular Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut; 2 Department of Clinical Chemistry and Biochemistry, Medical University of Gdansk, Gdansk, Poland; and 3 Departments of Pathology and 4 Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut

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Kalinowski L, Dobrucki LW, Meoli DF, Dione DP, Sadeghi MM, Madri JA, Sinusas AJ. Targeted imaging of hypoxia-induced integrin activation in myocardium early after infarction. J Appl Physiol 104: 1504–1512, 2008. First published March 20, 2008; doi:10.1152/japplphysiol.00861.2007.—The αvβ3-integrin is expressed in angiogenic vessels in response to hypoxia and represents a potential novel target for imaging myocardial angiogenesis. This study evaluated the feasibility of noninvasively tracking hypoxia-induced αvβ3-integrin activation within the myocardium as a marker of angiogenesis early after myocardial infarction. Acute myocardial infarction was produced by coronary artery occlusion in rodent and canine studies. A novel 111In-labeled radiotargeter targeted at the αvβ3-integrin (111In-RP748) was used to localize regions of hypoxia-induced angiogenesis early after infarction. In rodent studies, the specificity of 111In-RP748 for αvβ3-integrin was confirmed with a negative control compound (111In-RP790), and regional uptake of these compounds correlated with 201Tl perfusion and a 99mTc-labeled nitroimidazole (BRU59-21), which was used as a quantitative marker of myocardial hypoxia. The ex vivo analysis demonstrated that only 111In-RP748 was selectively retained in infarcted regions with reduced 201Tl perfusion and correlated with uptake of BRU59-21. In canine studies, myocardial uptake of 111In-RP748 was assessed using ex vivo single-photon-emission computed tomography (SPECT), ex vivo planar imaging, and gamma well counting of myocardial tissue and correlated with 99mTc-labeled 2-methoxy-2-methyl-propylisocitrile (99mTc-sestambiti) perfusion. Dual-radiotargeter in vivo SPECT imaging of 111In-RP748 and 99mTc-sestambiti provided visualization of 111In-RP748 uptake within the infarct region, which was confirmed by ex vivo planar imaging of excised myocardial slices. Myocardial 111In-RP748 retention was associated with histological evidence of αvβ3-integrin expression/activation in the infarct region. 111In-RP748 imaging provides a novel noninvasive approach for evaluation of hypoxia-induced αvβ3-integrin activation in myocardium early after infarction and may prove useful for directing and evaluating angiogenic therapies in patients with ischemic heart disease.

angiogenesis; radiotargeter imaging; myocardial infarction

ANGIOGENESIS is the formation of new microvascular networks from preexisting capillaries and is part of the natural healing response to ischemic injury. Among the various triggers of angiogenesis, tissue hypoxia has been identified as being a particularly important stimulus for the activation of new vessel growth, especially at the capillary level (18). Ischemia-induced myocardial angiogenesis may thus be viewed as a feedback response acting to restore perfusion and tissue oxygenation supply through new vessel growth. It appears that the magnitude of the angiogenic response may influence infarct size, postinfarction remodeling, and the prognosis in patients with acute myocardial infarction (1, 8, 15, 20). Therefore, the noninvasive evaluation of angiogenesis in clinical practice may help predict postinfarction remodeling and permit risk stratification of patients following ischemic injury.

Traditionally, the angiogenic response has been examined by evaluation of the physiological changes associated with the process. Most investigators studying myocardial angiogenesis have focused on assessment of changes in myocardial perfusion, a late event in the process. Alternatively, the initial hypoxic stimulus could be evaluated. Radiotargeters containing nitroimidazole moieties have been shown to accumulate in ischemic myocardium in an amount related inversely to tissue oxygen content, supporting the idea that nitroimidazoles may potentially be used to identify the hypoxic myocardium (5, 22, 23). One of the most promising technetium-99m-labeled candidates for evaluation of tissue hypoxia is oxo[3,3,9,9-tetramethyl-5-oxa-6-(2-nitroimidazol-1-yl)-4,8-diazaundecane-2,10-dione dioximato(3-)]N,N′,N′′,N′′′-technetium (99mTc-BrU59-21) (11).

Molecular imaging is emerging as a new tool for the noninvasive detection of unique “biochemical signatures” that can differentiate and characterize tissues before manifestation of gross anatomical features. One of the most commonly used imaging modalities with potential for detection of early molecular signals is radionuclear imaging techniques (2). In particular, the proliferating neovascular endothelial cells present unique, transient cell surface markers, such as integrins, which may be used to differentiate angiogenic vessels from mature capillaries. The αvβ3-integrin (vitronecctin receptor) is a well-recognized biomarker of angiogenesis that is relatively selective for proliferating endothelial cells (21). We previously demonstrated that an 111In-labeled quinolone (111In-RP748, Bristol-Myers Squibb Medical Imaging, N. Billerica, MA) preferentially binds to activated αvβ3-integrin on cultured endothelial cells with high affinity and selectivity (16). 111In-RP748 also exhibits favorable biodistribution for in vivo imaging with a rapid blood clearance (12).

Recently, we documented the feasibility of noninvasive imaging of myocardial angiogenesis using 111In-RP748 in myocardial infarction.
IMAGING OF HYPOXIA-INDUCED αvβ3-INTEGRIN ACTIVATION

In the present work, we evaluated the efficacy of $^{99m}$Tc-BRU59-21 and $^{111}$In-RP748 for the assessment of ischemia-induced myocardial hypoxia and angiogenesis, respectively, in an acute model of myocardial infarction in rats and dogs. The quantitative changes in expression/activation of αvβ3-integrin were related to myocardial perfusion as well as regional hypoxia. We also examined the uptake of $^{111}$In-RP748 following ischemic injury in relation to immunohistochemical markers of the angiogenic process.

MATERIALS AND METHODS

All studies were performed with approval of the Institutional Animal Care and Use Committee, according to the guiding principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, 1996).

Rodent Experiments

Surgical preparation. We employed an established rat model of infarction (12, 19). Male Sprague-Dawley rats (200–250 g) were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg), intubated, and mechanically ventilated with 1% isoﬂurane-99% oxygen for the duration of the procedure. A left thoracotomy was performed in the fourth intercostal space, and after opening the pericardium the anterior coronary artery was ligated with 7.0 monofilament suture at a 6-0 proline suture at ~7 mm below the origin for 3 min followed 3 min later by a 45-min occlusion. The short preoclusion was used to reduce the incidence of fatal arrhythmias.

Protocol. Two hours after reperfusion, rats (n = 12) were injected into jugular vein with 4.0 ± 1.0 mCi $^{99m}$Tc-BRU59-21 (Bracco Research, Princeton, NJ), followed by an immediate injection through the same catheter with either $^{111}$In-RP748 (1.2 ± 0.1 mCi), the agent targeted at αvβ3-integrin, or $^{111}$In-RP790 (1.2 ± 0.1 mCi), a nonspecific control agent (Bristol-Myers Squibb Medical Imaging) (7, 12, 16). Sixty minutes after injection of one of the $^{111}$In-labeled agents, rats were also injected with thallium-201 ($^{201}$Tl; 0.78 ± 0.06 mCi) for evaluation of relative myocardial perfusion. A radioisotope was injected with 0.1 ml of saline vehicle.

Postmortem analysis. Thirty minutes after $^{201}$Tl injection, rats were euthanized, and the hearts were rapidly excised and cast with dental impression material (alginate impression material, Quala Products, Milford, DE) to facilitate uniform cutting into 2-mm-thick slices. Heart slices were cut transmurally into eight radial slices. Heart slices were then divided into epicardial and endocardial sections for gamma-well counting using two energy windows (99mTc, 130–170 keV; $^{111}$In, 170–300 keV). Finally, tissue radioactivities were expressed as counts per minute per gram and then normalized to the nonischemic region of the heart.

Canine Experiments

Studies were also performed in dogs (n = 5) following acute myocardial infarction to better determine the spatial localization of αvβ3-integrin in relationship to the region of myocardial injury and perfusion, and to demonstrate the feasibility of in vivo imaging of angiogenesis.

Surgical preparation. Mongrel adult male dogs (30–35 kg) were anesthetized with thiopental sodium (10 mg/kg iv), intubated, and placed on a respirator for mechanical ventilation with nitrogen oxide and oxygen (3:1) and 0.5–1.5% halothane. A left lateral thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. Either the proximal left anterior descending (LAD) or left circumflex coronary artery was occluded for 2 h followed by reperfusion for 6 h.

Dual-radiotracer imaging. All dogs (n = 5) underwent dual-isotope single-photon-emission computed tomography (SPECT) imaging with $^{111}$In-RP748 and $^{99m}$Tc-labeled 2-methoxy-2-methyl-proply-isorositrile ($^{99m}$Tc-sestamibi). After the 6-h reperfusion period, $^{111}$In-RP748 (6.20 ± 0.42 mCi) was injected into the femoral vein, and six serial 15-min SPECT images were acquired starting at 15 min after injection. All SPECT images were acquired with a dual-head gamma camera (GE Millenium; General Electric, Waukesha, WI) coupled with medium-energy parallel-hole collimators. $^{111}$In-RP748 images were acquired using two energy windows, 180 keV ± 7.5% and 252 keV ± 10%, grouped into a single image. SPECT images were acquired in continuous advance mode, 15 s per 3° frame, with a zoom of 1.77 and 64 × 64 matrix. Following the $^{111}$In-RP748 SPECT imaging, $^{99m}$Tc-sestamibi (20.80 ± 4.36 mCi) was injected (each in 0.7–1.0 ml of saline vehicle). Fifteen minutes later, a 15 min SPECT $^{99m}$Tc-sestamibi image was acquired to provide a reference “hot spot” image facilitating reconstruction of the $^{111}$In-RP748 targeted “hot spot” image. Acquisition parameters were identical to those used for the $^{111}$In-RP748 SPECT acquisitions, except for an energy window centered at 140 keV ± 7.5%.

Postmortem analysis. Immediately after the final image was acquired, dogs were euthanized, and hearts were rapidly excised and cast with dental impression material and sliced into 5-mm-thick short-axis slices. Cast slices were placed directly on the collimator of the gamma camera to obtain registered sequential high-resolution (256 × 256) images using the same energy windows as the in vivo imaging. After ex vivo imaging, heart slices were stained with a buffered solution of 2,3,5-triphenyl-2H-tetrazolium chloride (TTCh) to identify myocardial infarction. Each slice was cut into eight radial pieces, and each pie was then divided into epicardial and endocardial sections for gamma-well counting using two energy windows ($^{99m}$Tc, 130–170 keV; $^{111}$In, 170–300 keV). Finally, tissue radioactivities were expressed as counts per minute per gram and then normalized to the nonischemic region of the heart.

Histological analysis. One transmural biopsy (~5 mm × 5 mm) was removed from the central infarct and remote noninfarcted territories after excising the heart and staining myocardial slices with TTC. This biopsy was split into two transmural pieces. One was immediately frozen for immunohistochemical staining and immunoblot analysis. The other was fixed in 10% formalin solution and later embedded in paraffin for histochemical and immunohistochemical stainings. Staining with Masson’s trichrome and hematoxylin and eosin (H-and-E) was performed to delineate the infarct region. Immunohistochemical analysis of angiogenesis was accomplished with the biotinylated endothelium-specific lectin, Bandeiraea simplicifolia Lectin I (Vector Laboratories, Burlingame, CA), an endothelial cell marker. The abundance of αvβ3-integrin was assessed with a specific monoclonal antibody for the αvβ3-integrin (LM609, Chemicon International, Temecula, CA). The positive control for anti-integrin αvβ3 antibody was MOPC21, a nonspecific mouse monoclonal IgG1 (BD Biosciences Pharmingen, San Diego, CA). All sections for immunohistochemical staining were counterstained with hematoxylin.

Integrin precipitation and immunoblot analysis. Frozen biopsy pieces were pulverized and homogenized in 5 vol (wt/vol) of a lysis buffer (pH = 7.4) containing 1% Triton X-100, 150 mM NaCl, 10 mM Tris·HCl, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium orthovanadate, 0.2 mM PMSF, 0.5% NP-40, and supplemented with protease inhibitor mixture (1 tablet/20 ml, Roche, Nutley, NJ). After centrifugation of the tissue suspensions at 3,000 × g for 15 min, the supernatants were recentrifuged at 15,000 × g for 15 min (both centrifugations at 4°C). Then, 500 μg of protein from the supernatants was incubated with 5 μl mouse monoclonal anti-integrin αvβ3 antibody (LM609, Chemicon International) or mouse IgG negative control and J Appl Physiol • VOL 104 • MAY 2008 • www.jap.org

Innovative Methodology
25 μl of protein A/G Plus-agarose beads (Santa Cruz Biotechnology) in 1 ml of total volume of the lysis buffer with agitation overnight at 4°C. The beads with bound immune complex were pelleted in the centrifuge (5804R Eppendorf) at 3,000 g for 5 min and washed three times with the lysis buffer. The beads were then boiled for 5 min in Laemmli sample buffer. After centrifugation at 3,000 g for 5 min, the supernatants were electrophoresed on 5% SDS-polyacrylamide gels. Proteins were then transferred to 0.45-μm pore nitrocellulose membranes with a polyblot apparatus (Bio-Rad Laboratories). Membranes were blocked with 5% nonfat dry milk in the washing buffer (pH = 7.5) containing 20 mM Tris·HCl, 500 mM NaCl, and 0.1% Tween-20 for 2 h at room temperature. Membranes were then incubated with rabbit polyclonal anti-integrin αv antibody (Chemicon International) diluted in blocking solution for 2 h at room temperature with gentle agitation. Subsequently, nitrocellulose strips were washed three times with the washing buffer and then reacted with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology, Santa Cruz, California) diluted 1:5,000 in the washing buffer for 1 h at room temperature. Strips were re-washed and incubated with the washing buffer, and bound secondary antibody was detected in incubation at room temperature with mixed peroxide solution and luminol solution of an enhanced chemiluminescence (ECL) detection system (ECL kit, Pierce Biotechnology). The density of ECL signals was quantified with an Epson Perfection 3490 Photo image scanner using the public domain software package National Institutes of Health ImageJ 1.38 (available at: http://rsb.info.nih.gov/ij/). Prestained markers (Bio-Rad Laboratories) were used for molecular mass determination. A control sample of Tenascin-C (Chemicon International) was run in parallel as a positive control. To compare αvβ3-integrin expression with the expression of another protein, we analyzed the expression of actin by immunoprecipitation following by Western blot using a rabbit polyclonal anti-actin antibody (H-196, Santa Cruz Biotechnology). Protein concentrations were measured by the method based on the Bradford dye-binding procedure (Bio-Rad Protein Assay) using bovine serum albumin as a standard.

Statistical Analysis

When applicable (comparison between 2 values), statistical analysis was done with Student’s t-test. For multiple comparisons, results were analyzed by ANOVA. Data are presented as means ± SD. Means were considered significantly different at \( P < 0.05 \).

RESULTS

Rodent Acute Myocardial Infarction Experiments

Gamma-well counting permitted quantification of relative myocardial \(^{111}\text{In-RP748}\) retention in relationship to myocardial hypoxia and perfusion. Decreased myocardial \(^{201}\text{Tl}\) retention was consistently observed in the anterior wall, as shown in representative myocardial count profiles (Fig. 1A). The retention of \(^{111}\text{In-RP748}\) was increased in areas of decreased \(^{201}\text{Tl}\) retention. In contrast, myocardial uptake of the control compound \(^{111}\text{In-RP790}\) was homogenous across the heart regions. Tissue gamma-well counting results are summarized in Fig. 1B. The retention of \(^{111}\text{In-RP748}\) was found to be inversely related to the degree of myocardial hypoperfusion. On average the relative myocardial retention of \(^{111}\text{In-RP748}\) in the low-flow infarcted regions was about three to four times that in normal regions at 3.5 h after reperfusion. In contrast, there was no selective retention of the nonspecific control \(^{111}\text{In-RP790}\) compound.

\(^{111}\text{In-RP748}\) was selectively retained in the hypoxic region of myocardium, as defined by \(^{99m}\text{Tc-BRU59-21}\) uptake. The retention of \(^{111}\text{In-RP790},\) the negative control compound, was not related to the myocardial retention of \(^{99m}\text{Tc-BRU59-21}\) (Fig. 2A). The comparison of the gamma-well counting results for \(^{111}\text{In-RP748}\) or \(^{111}\text{In-RP790}\) and \(^{99m}\text{Tc-BRU59-21}\) are summarized in Fig. 2B. Myocardial segments with the highest relative \(^{99m}\text{Tc-BRU59-21}\) uptake (\(>75\%\)), suggesting hypoxia, showed significantly higher uptake of relative \(^{111}\text{In-RP748}\) than those segments with low \(^{99m}\text{Tc-BRU59-21}\) uptake.

![Fig. 1. Myocardial radiotracer activity in relationship to relative \(^{201}\text{Tl}\) perfusion in acute rat infarct model as assessed by gamma-well counting. Representative circumferential \(^{201}\text{Tl},\) \(^{111}\text{In-RP748},\) and \(^{111}\text{In-RP790}\) count profiles are shown for a short-axis slice through the infarct region (A). Myocardial segments from all rats were segregated into 4 categories based on relative \(^{201}\text{Tl}\) perfusion (%nonischemic). Regional myocardial \(^{111}\text{In-RP748}\) activity (%nonischemic) was significantly increased in the ischemic regions. Myocardial \(^{111}\text{In-RP790}\) activity (%nonischemic) was not significantly increased in the ischemic regions (B). \(\ast P < 0.05\) vs. \(^{111}\text{In-RP790},\) \(\dagger P < 0.05\) vs. >80%.]
The control compound, $^{111}$In-RP790, was not significantly increased in the hypoxic areas of the myocardium. These findings suggest that the retention of $^{111}$In-RP748 following ischemia-reperfusion may be caused by hypoxia-induced activation of the $\alpha\beta_3$-integrin associated with the initiation of the angiogenic process.

**Canine Acute Myocardial Infarction Experiments**

**Postmortem analysis.** In the canine studies, gamma-well counting showed that myocardial segments with decreased perfusion, as determined by $^{99m}$Tc-sestamibi retention, demonstrated increased retention of $^{111}$In-RP748. Figure 3A demonstrates the regional differences in myocardial retention of $^{111}$In-RP748 in relationship to $^{99m}$Tc-sestamibi activity for both endocardial and epicardial segments. The gamma-well counting data for all of the canine studies are summarized in Fig. 3B. The uptake of $^{111}$In-RP748 was found to be directly proportional to the degree of myocardial ischemia as defined by relative $^{99m}$Tc-sestamibi retention. A 2.5-fold increase in the relative retention of $^{111}$In-RP748 was observed in the most ischemic regions at \( \sim 10 \text{ h} \) after onset of the ischemic insult.

**Immunohistochemistry.** Representative immunohistochemical stains are shown in Fig. 4. After coronary ligation, myocardial necrosis was evident in the injury zone based on Masson’s trichrome and H-and-E stains. Immunohistochemical staining with an endothelium-specific lectin did not demonstrate an alteration in capillary density in the central ischemic region early postinfarction. However, immunostaining with LM609, a specific antibody for the $\alpha\beta_3$-integrin, demonstrated early activation in the $\alpha\beta_3$-integrin in this same region of the left ventricular myocardium. Interestingly, increased capillary staining was strongly localized in the peri-infarct region, while arteriolar smooth muscle displayed mostly positive staining with LM609 within the central infarct lesion. However, there was no discernable staining of $\alpha\beta_3$-integrin in remote noninfarcted regions.

![Fig. 2. Relative myocardial radiotracer activity in relationship to $^{99m}$Tc-labeled BRU-5921 in acute rat infarct model. Representative circumferential $^{99m}$Tc-BRU-5921, $^{111}$In-RP748 and $^{111}$In-RP790 count profiles are demonstrated (A). Myocardial segments from all rats were segregated into 3 categories based on relative $^{99m}$Tc-BRU-5921 activity (% nonischemic). $^{99m}$Tc-BRU-5921 and $^{111}$In-RP748 distributions were very similar and corresponded well with the area of infarct. In contrast, $^{111}$In-RP790 distribution did not change significantly throughout myocardium (B). *P < 0.05 vs. $^{111}$In-RP790.](image1)

($\leq 100\%$). The control compound, $^{111}$In-RP790, was not significantly increased in the hypoxic areas of the myocardium. These findings suggest that the retention of $^{111}$In-RP748 following ischemia-reperfusion may be caused by hypoxia-induced activation of the $\alpha\beta_3$-integrin associated with the initiation of the angiogenic process.
Quantitative analysis of αβ3-integrin expression. Relative quantification of protein bands, normalized to actin, revealed ~2.3-fold increased levels of αβ3-integrin in the infarct region compared with the remote (noninfarct) region of myocardium (Fig. 5). This finding suggests that the retention of 111In-RP748 within the hypoxic region of myocardium is associated with a rapid upregulation of αβ3-integrin expression.

Dual-tracer in vivo and ex vivo imaging. Reconstruction of the in vivo 111In-RP748 SPECT images required registration and batch reconstruction of 111In-RP748 image data with 99mTc-sestamibi perfusion images. Figure 6A shows an example of registered 111In-RP748 and 99mTc-sestamibi SPECT reconstructions in standard short- and long-axis planes from a dog following LAD occlusion (2 h) and reperfusion (6 h). The
increase in myocardial $^{111}$In-RP748 activity correlated with a $^{99m}$Tc-sestamibi perfusion defect. Thus, as early as 8 h after the onset of ischemia, in vivo targeted $^{111}$In-RP748 retention was seen within the infarct region and demonstrated the activation of the angiogenic process. In each dog, focal $^{111}$In-RP748 activity was generally greater than that of the surrounding tissues with a favorable heart-to-background activity ratio.

The high resolution of dual isotope $^{99m}$Tc-sestamibi and $^{111}$In-RP748 imaging of ex vivo slices demonstrated clear focal retention of $^{111}$In-RP748 within the hypoperfused infarcted area of myocardium (Fig. 6B). Additionally, these images allowed direct comparison of myocardial $^{111}$In-RP748 retention with topography of necrosis and the presence of $\alpha\beta3$-integrin in immunohistochemical stains (see Fig. 4) and its abundance in immunoblot quantitative analysis (see Fig. 5).

**DISCUSSION**

One of the mechanisms by which the heart adapts to myocardial hypoxia and/or ischemia is stimulation of angiogenesis, the growth of new microvessels from the existing microvasculature within the ischemic area. This response may limit regions of impairment and ultimately preserve tissue function. The present study reports the potential of using $^{111}$In-RP748, a radiotracer targeted at activated $\alpha\beta3$-integrin, to noninvasively evaluate the angiogenic process in acute models of myocardial infarction. We have demonstrated preferential uptake and retention of $^{111}$In-RP748 in the reperfused infarcted regions of the heart within about 3.5–10 h of reperfusion, in both rodent and canine models of ischemic injury.

In an ischemia-reperfusion rat model, $^{111}$In-RP748 was selectively retained in the infarcted regions with reduced $^{201}$TI perfusion; there was almost a fourfold increase in relative myocardial $^{111}$In-RP748 retention in the most ischemic regions at 3 h postreperfusion. Moreover, the extent of regional $^{111}$In-RP748 retention correlated well with the uptake of $^{99m}$Tc-BRU59-21, a radiolabeled nitroimidazole, that is trapped in hypoxic myocardium.

The most hypoxic low-flow regions, identified by increased $^{99m}$Tc-BRU59-21 retention and reduced $^{201}$TI uptake, correlated with the areas of maximum $^{111}$In-RP748 retention. These data are consistent with the role of regional hypoxia as an important stimulus of myocardial angiogenesis and may provide a potential marker of the initiation of the angiogenic process. The concern regarding possible nonspecific uptake of $^{111}$In-RP748 early after ischemia-reperfusion led to our direct comparison of myocardial retention of $^{111}$In-RP748 with the retention of a negative control compound ($^{111}$In-RP790), having similar chemical structure, although no in vitro specificity for $\alpha\beta3$-integrin (12). There was no increase in myocardial $^{111}$In-RP790 retention in the ischemic region of a comparable group of rats postinfarction, supporting the specificity of $^{111}$In-RP748. The uptake and retention of the control compound tracked flow as would be expected for any diffusible radiotracer. Also, in contrast to $^{111}$In-RP748, the uptake of $^{111}$In-RP790 did not correlate with the uptake of $^{99m}$Tc-BRU59-21, which was shown to be selectively retained in hypoxic myocardium. These rat studies support the use of $^{111}$In-RP748 as a specific marker for the noninvasive imaging of the early events of myocardial angiogenesis following an ischemic episode.

An acute canine model of infarction was employed that permitted noninvasive serial evaluation of changes in myocardial $^{111}$In-RP748 uptake in relationship to changes of myocardial perfusion, to further explore the potential value of $^{111}$In-RP748. In these in vivo canine imaging studies, we used $^{99m}$Tc-sestamibi as the perfusion radiotracer since it provides higher count images relative to $^{201}$TI and therefore improves image quality, facilitating quantitative assessment of myocardial perfusion (13). In vivo imaging with $^{111}$In-RP748 demonstrated a favorable myocardium-to-liver ratio and rapid clearance from blood, facilitating noninvasive imaging of myocardial angiogenesis within 60 min of radiotracer injection (16).

$^{111}$In-RP748 was shown to identify initiation of the angiogenic process following nontransmural myocardial infarction and inversely correlated with perfusion assessed with $^{99m}$Tc-sestamibi. In vivo dual-isotope $^{111}$In-RP748/$^{99m}$Tc-sestamibi SPECT images demonstrated focal uptake of $^{111}$In-RP748 within the $^{99m}$Tc-sestamibi defect shortly after the ischemic injury. It is noteworthy that reconstruction and interpretation of the $^{111}$In-RP748 “hot spot” images of the activated $\alpha\beta3$-integrin required careful coregistration of the targeted images with the perfusion images. The focal retention of $^{111}$In-RP748 observed in vivo SPECT images within the perfusion defect was confirmed by ex vivo imaging of myocardial slices. Additional postmortem immunohistochemistry (Masson’s trichrome and H-and-E staining, and staining with monoclonal antibody LM609) and immunoblotting analysis demonstrated ischemic injury and early upregulation of $\alpha\beta3$-integrin expression/activation. Taken together, the data suggest the angiogenic process is initiated early (within a few hours) after myocardial infarction in response to hypoxia and that this process can be identified noninvasively with multi-isotope SPECT imaging.
The angiogenic process is tightly regulated and induces a complex sequence of events involving pericellular proteolysis, migration and proliferation of cells, final tube formation, and recruitment of periendothelial cells (6, 21). Each step of this process offers potential diagnostic and therapeutic targets. We employed a radiotracer that binds to the activated conformation of the αvβ3-integrin that is activated early following ischemia (4, 26) and mediates the adhesive interactions of angiogenic endothelial cells with components of the extracellular matrix (9, 14, 21). This approach is based on findings that endothelial cells express specific adhesion receptors, including the vitronectin-type integrin-αvβ3 (3). Cultured endothelial cells are dependent on αvβ3 for survival (24). In view of the important role for integrin-αvβ3 in angiogenesis, it is logical to expect that hypoxia, a fundamental stimulus for angiogenesis, would modulate expression and/or activation levels of this integrin. Indeed, previous studies demonstrated that hypoxia gradually increased levels of αv and β3 mRNAs, and in parallel active
αβ3 protein levels, with peak response at 24 h after exposure of cultured endothelial cells to hypoxic conditions (26). Similarly, the peak expression of αβ3 protein occurred 12–24 h after initiation of angiogenesis in vessels with basic fibroblast growth factor (bFGF) (4). The final effects of the αβ3-integrin on the cellular processes may be modulated by changes in αβ3 protein expression, but also by its changes between (at least two) distinct functional states: an “inactive” (i.e., non-adhesion promoting) and an “active” (adhesion promoting) state (25). Integrin activation is very rapid (<1 s) and is associated with changes in affinity (conformational changes) and in avidity (lateral mobility and clustering) of the molecule (27). Hence, it is conceivable that the high affinity of RP748 for activated αβ3-integrin supports the value of RP748 for in vivo imaging of the processes associated with angiogenesis (16). The mechanisms by which hypoxia influences αβ3-integrin expression/activation are not clear. The ability of vascular endothelial growth factor (VEGF) to modulate αβ3 protein expression on dermal endothelial cells suggests that hypoxia’s effect in modulating αβ3 levels may be mediated by increasing VEGF levels (17). In this regard, it is known that hypoxia-inducible factor 1 (HIF-1) is a transcriptional activator of VEGF and is critical for initiating early cellular responses to myocardial ischemia or infarction (<24 h after onset) (10).

A sensitive and specific imaging technique that can detect the early angiogenic response to hypoxia/ischemia may have prognostic implication for predicting late myocardial remodeling. In addition, a noninvasive method for serial evaluation of angiogenesis would be beneficial in monitoring novel therapies directed at stimulating angiogenesis. On the basis of our previous studies (12, 16) and the present report, it appears that dual-isotope SPECT imaging of myocardial angiogenesis with 111In-RP748 and a perfusion tracer may be valuable for this purpose. We have also shown in the experimental studies of angiogenesis that targeted SPECT imaging of the αβ3-integrin offers the possibility of assessing the angiogenic process in combination with evaluation of more conventional physiological measures of myocardial perfusion or hypoxia.

In conclusion, in the present study we have demonstrated that regional retention of 111In-RP748 in myocardium early after infarction correlated with upregulation of αβ3-integrin expression/activation, supporting the role of 111In-RP748 as an early targeted marker of angiogenesis. The early uptake of 111In-RP748 postinfarction was localized to the hypoxic infarct region. The targeted imaging of hypoxia-induced angiogenesis provides a novel approach for the assessment of angiogenesis in combination with imaging of physiological consequences of angiogenesis. We suggest that the evaluation of the angiogenic process using 111In-RP748 SPECT imaging may allow for a better risk stratification of patients with ischemic heart disease and provide a new surrogate imaging endpoint in clinical trials evaluating genetic or stem cell therapies directed at stimulating angiogenesis in patients with ischemic heart disease.

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