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EDITORIAL COMMENT

Targeted Imaging Offers Advantages Over Physiological Imaging for Evaluation of Angiogenic Therapy*

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A large population of patients suffering from advanced coronary artery disease and chronic myocardial ischemia cannot be adequately managed by a combination of antianginal medication and angioplasty or coronary artery bypass surgery. Therefore, therapeutic stimulation of vascular growth in the management of chronic myocardial ischemia seems to be a useful strategy in treating such patients. A number of recent clinical trials have examined the role of therapeutic administration of molecular regulators of blood vessel growth to promote vascular development to treat ischemic heart disease (1). The patients eligible for these therapeutic angiogenic trials often have underlying ischemic but viable "hibernating" myocardium, characterized by

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persistent myocardial dysfunction in the presence of resting hypoperfusion or chronic myocardial stunning due to repetitive episodes of stress-induced ischemia in myocardial regions with severely impaired coronary flow reserve. Fallavollita et al. (2) have established a porcine model of surgical ameroid implantation in which the physiologic and structural features of chronically ischemic myocardium remain constant for months. A recent review suggests that such porcine models of chronic myocardial ischemia may be ideally suited for evaluation of therapeutic angiogenesis or other interventions targeted to improve flow and function of chronically dysfunctional myocardium (3).

In their paper in this issue of *iJACC (JACC: Cardiovascular Imaging)*, Johnson et al. (4) use a porcine ameroid constrictor model in the evaluation of a radiolabeled peptide targeted at $\alpha v\beta 3$ integrin for tracking the therapeutic efficacy of endomyocardial injection of a naked plasmid-deoxyribonucleic acid encoding the 165-amino-acid isoform of human vascular endothelial growth factor ($phVEGF_{165}$), demonstrating that targeted single-photon emission computed tomographic (SPECT) imaging of angiogenesis may be a useful approach for monitoring angiogenic therapy in the heart. This catheter-based endovascular delivery approach was previously shown to be feasible for gene transfer in porcine studies by Vale et al. (5) using Noga electromechanical mapping; they were also the first to establish Noga-directed myocardial gene therapy in humans in 2001, injecting a plasmid encoding vascular endothelial growth factor-2 (phVEGF-2) (6). A follow-up randomized, double-blind, placebo-controlled trial of vascular endothelial growth factor-2 therapy demonstrated a statistically significant improvement in angina class and a trend toward improvement in exercise duration and quality of life in phVEGF-2–treated patients compared to placebo-treated patients (7). Recently, the Euroinject One clinical trial was reported for patients with chronic angina and no-option for conventional revascularization randomized to receive direct endomyocardial injection of $phVEGF_{165}$ or placebo (8). In the $phVEGF_{165}$ treatment group, there was a significant improvement in stress-induced perfusion defects at 3 months compared with baseline, although this change was not different than the control group. However, there were significant improvements in wall motion in the $phVEGF_{165}$ treatment group compared with the control group.

Despite the promising results seen in experimental animal studies and preliminary phase 1 clinical trials with angiogenic therapy, randomized, placebo-controlled, double-blind clinical trials of VEGF and fibroblast growth factor-2 have in gen-

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eral yielded mixed or somewhat disappointing results (9). This might be related to insensitivity of our existing imaging approaches for evaluation of angiogenesis.

In theory, myocardial angiogenesis should result in improved perfusion and tissue oxygenation, resulting in reduced hypoxia, diminished myocardial ischemia, and improved regional left ventricular function. Therefore, angiogenesis may be indirectly evaluated noninvasively by analysis of standard physiological parameters, such as regional myocardial perfusion and function. However, it remains uncertain whether the effects of angiogenesis on perfusion would be better evaluated by analysis of changes in intravascular blood volume, or vascular and/or perfusion reserve. Unfortunately, angiogenesis may also alter vascular permeability and the effective vascular surface area for substrate exchange, which can confound imaging approaches that use diffusible tracers. Nevertheless, demonstration of physiological benefit remains a critical step in the development and evaluation of therapeutic angiogenesis. Positron emission tomographic (PET) perfusion tracers such as ^{13}N-ammonia, ^{15}O-water, or ^{82}Rb would theoretically be preferred due to the favorable characteristics of PET imaging in general and the ability to define absolute regional myocardial blood flow.

As reviewed in the preceding text, in the absence of a direct biological marker of angiogenesis, the evaluation of therapeutic angiogenesis has focused on a number of clinical end points or imaging of the physiological consequences of the therapeutic intervention (10). However, there is a tremendous need for development of noninvasive approaches for direct evaluation of the molecular events associated with angiogenesis to more effectively track therapeutic angiogenesis.

Angiogenesis is a complex process, involving: 1) degradation of the basal membrane surrounding the parental vasculature; 2) migration of endothelial cells or progenitor cells; 3) proliferation of quiescent endothelial cells to form new vessels; and 4) alignment and organization of endothelial cells to form tubes (11). The process of angiogenesis involves the interplay of many cells including monocytes/macrophages, mast cells, lymphocytes, connective tissue cells, pericytes, endothelial cells, and pluripotent progenitor cells, all of which influence the process by secreting soluble angiogenic and antiangiogenic molecules and proteolytic enzymes. Several approaches appear to be feasible for imaging of the molecular events. These include the use of labeled oligonucleotides targeted to specific messenger ribonucleic acid (mRNA) sequences, short peptides targeted to specific intracellular and cell surface receptors, and labeled ligand-avid imaging. Potential biological targets for imaging angiogenesis fall into 3 principal categories; 1) endothelial cell markers of angiogenesis; 2) nonendothelial cells involved with angiogenesis; and 3) markers of the extracellular matrix (ECM).

A potential target for early detection of angiogenesis would relate to the imaging of the favorable conditions or molecular events associated with the initiation of the angiogenic process. The potential for targeted imaging of $VEGF_{121}$ receptors and $\alpha v\beta 3$ integrin as markers of angiogenesis has been demonstrated by several groups of investigators (12–17). Expression of VEGF is markedly increased by hypoxia and mediates many cellular functions, including release of other growth factors, cell proliferation, migration, survival, and angiogenesis (18). At least 5 isoforms of human VEGF-A mRNA encoding for monomer VEGF proteins of 121, 145, 165, 189, and 206 amino acids are produced by alternative splicing from a single gene. In the study by Johnson et al. (4), they administered $phVEGF_{165}$ directly into the myocardium to induce local angiogenesis.

The angiogenic response is modulated by the composition of the ECM and intercellular adhesions, including integrins. Integrins are a family of $\alpha\beta$ heterodimeric cell surface receptors that mediate cell–cell and cell–matrix adhesion through tightly regulated interactions with ligands. Integrin-ligand binding is dependent on conformational changes in the integrin structure (19). From the imaging standpoint, targeting of $\alpha v\beta 3$ integrin has promise, because expression and activation in quiescent endothelial cells is low, whereas "angiogenic" endothelial cells demonstrate marked up-regulation of $\alpha v\beta 3$ integrin expression and activation. Investigators first proposed the potential noninvasive detection of tumor angiogenesis in vivo using magnetic resonance imaging and a paramagnetic contrast agent targeted to endothelial $\alpha v\beta 3$ integrin via the LM609 monoclonal antibody (20). However, targeted in vivo imaging using similar monoclonal antibodies has been limited in the past by slow clearance of the tracer from the blood. Haubner et al. subsequently reported the synthesis and characterization of a series of radiolabeled $\alpha v\beta 3$ integrin antagonists, reporting kinetics in both in vitro and in vivo preparations (21). Their work has focused on the use of cyclic Arg-Gly-Asp (RGD) peptides, which are known to bind to $\alpha v\beta 3$ integrin.

Meoli et al. (12) were the first to report the potential of a radiolabeled $\alpha v\beta 3$ integrin-targeted peptidomimetic, ^{111}In-RP748, for in vivo imaging of myocardial angiogenesis. Subsequently, several other studies have demonstrated the potential of targeted imaging of $\alpha v\beta 3$ integrin for imaging of angiogenesis in skeletal muscle or the myocardium in response to ischemic injury (13–17). The study by Johnson et al. (4) confirms the value of the $\alpha v\beta 3$ integrin-targeted imaging approach for assessment of myocardial angiogenesis using an ^{123}I-labeled arginine-glycine-aspartic acid (RGD) peptide in pigs with chronic ischemia treated with direct intramyocardial injection of phVEGF$_{165}$. In their study, changes in expression/activation of $\alpha v\beta 3$ integrin were related to changes in more functional parameters, such as myocardial perfusion and regional mechanical function, although they did not evaluate potential changes in permeability, regional hypoxia, or metabolism.

The study by Johnson et al. (4) appropriately uses an established pig model of hibernating myocardium to test ^{123}I-Gluco-RGD for targeted imaging of the $\alpha v\beta 3$ integrin as a marker of angiogenesis in evaluation of phVEGF$_{165}$-stimulated angiogenesis. They observed focal retention of ^{123}I-Gluco-RGD in the SPECT ^{201}Tl perfusion defect only in the phVEGF$_{165}$-treated pigs and not in the control pigs, although they observed no differences in ^{201}Tl perfusion between the treatment groups. It is not clear why the investigators did not demonstrate increased uptake of ^{123}I-Gluco-RGD in the control animals, because angiogenesis is part of the natural response to chronic ischemia. It is important to note that $\alpha v\beta 3$ integrin is expressed on endothelial cells as well as inflammatory cells and smooth muscle cells. The investigators did not define what specific cell type ^{123}I-Gluco-RGD is binding to within the myocardium, and they did not correlate tracer uptake directly with $\alpha v\beta 3$ expression on histological staining. The cause for the observed relationship of ^{123}I-Gluco-RGD retention with the endothelial cell marker lectin is not clear, because other studies have suggested that similar $\alpha v\beta 3$ integrin-targeted radiolabeled RGD probes bind primarily on actively proliferating endothelial cells, not on all endothelial cells. There were substantial circulating blood levels of the ^{123}I-Gluco-RGD at the time of imaging, suggesting that focal myocardial retention in the treated animals might simply reflect differences in intramyocardial blood volume or activity within the interstitial space associated with changes in vascular permeability, not necessarily active angiogenesis. These issues raise some concern about potential nonspecific binding or retention of ^{123}I-Gluco-RGD.

The presence of an early VEGF-dependent hyperpermeable phase associated with angiogenesis makes magnetic resonance (MR)-based vascular permeability imaging with contrast agents ideal for early identification of the angiogenic process. Perfusion-sensitive first-pass imaging of ischemic myocardium represents an alternative to MR permeability imaging that also is well suited to assessment of the effects of new vessel growth in the heart (22). A number of preclinical and clinical studies have demonstrated the value of nontargeted MR imaging for assessment of angiogenesis. These MR approaches have been used to evaluate angiogenic therapy with VEGF (23,24). High-resolution contrast ultrasound also has been used to evaluate the changes in perfusion and intramuscular blood volume associated with therapeutic angiogenesis (25).

Beyond physiological imaging and the targeted imaging of VEGF or $\alpha v\beta 3$ integrin, there are many other potential targets for in vivo radiotracer-based imaging of angiogenesis. Targeted imaging of syndecan-4 and aminopeptidase-N (CD-13) represent potential alternative endothelial cell markers for imaging of angiogenesis. Another essential component of angiogenesis is signaling by molecules in the ECM. The developmental endothelial locus-1 (Del-1) matrix protein and perlecan, a basal lamina proteoglycan, interact with other ECM components during the process of angiogenesis and represent other potential targets for imaging of angiogenesis. Imaging aimed at detecting the ongoing neovascularization could also monitor the influx of blood-derived macrophages and circulating endothelial precursor cells, as well as expression of new markers specific for developing arteries in veins (ephrins, ephrin receptors, semaphorins, and so on).

Although radiotracer-based targeted imaging offers the highest sensitivity for in vivo clinical imaging, there may also be a role for targeted MR or ultrasound imaging of angiogenesis. A number of MR agents have been evaluated for imaging of angiogenesis; including antibodies, peptides, and peptidomimetics targeted at $\alpha v\beta 3$ integrin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin, and CD-13. Because of the lower sensitivity of MR imaging, a strategy to improve the signal-to-background noise ratio is needed. The use of targeted nanoparticles or macromolecules to deliver a higher "payload" of a

contrast agent to the molecular address may solve this issue.

The dual-isotope SPECT imaging approach outlined in the Johnson et al. (4) study offers an advantage over clinical indices or imaging of only the physiological consequences of these therapies, allowing for the simultaneous evaluation of critical molecular signals coincident with standard physiological indices such as perfusion. This type of targeted imaging will benefit from the availability of hybrid SPECT-CT imaging systems which will permit registration of molecular and physiological information with 3-dimensional anatomical structure. This will facilitate correction for attenuation and partial volume errors and provide absolute quantification of radiotracer retention within the specific regions of the heart treated. Quantitative targeted imaging of biological markers will be critical for understanding the angiogenic process and tracking novel molecular or genetic therapies directed at stimulating angiogenesis.

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