CNS drugs, what are the main benefits that can be accrued from radiotracer studies of the drug itself? Are there any applications and potential benefits from pharmacodynamic assessment in patients being treated with these drugs using imaging procedures?

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What Is the Role of Molecular Imaging in the Management of Cardiac Disorders?

Recent progress in the knowledge of the molecular-genetic mechanisms in cardiovascular disease as well as technological development of new imaging strategies has led to the application of new biologically based approaches. Methods are actively being developed for controlled gene delivery to the cardiovascular system using novel gene constructs. Moreover, gene expression can be controlled and imaged using cell-specific, drug-controlled expression systems.

In contrast to direct, targeted imaging paradigms, indirect molecular imaging is more complex and involves multiple components. "Reporter imaging" is an example of an indirect imaging strategy. This paradigm includes a marker/reporter gene and a marker/reporter probe. Several groups have been exploring this approach in the evaluation of the cardiovascular system (*1–4*). The reporter gene product can be an enzyme that converts a reporter probe to a metabolite that is selectively trapped within transduced cells. The main advantage of this approach is the enzymatic amplification of the probe signal that facilitates imaging of the magnitude and location of reporter gene expression.

Another important novel imaging paradigm is the imaging of molecular markers and biological pathways that give insight into the pathogenesis and progress of diseases and assessment of therapeutic intervention. These include novel imaging strategies for heart failure, thrombosis, apoptosis, atherosclerosis, and angiogenesis. Several specific cardiovascular applications for molecular imaging are being investigated, including the imaging of the angiogenic process targeted at vascular endothelial growth factor (VEGF) (*5*) and αvβ3 integrins (*6*), matrix metalloproteases (MMP), apoptosis (*7*), tracking stem cell therapies (*8*), and imaging atherosclerotic plaques and vascular injury (*9*).

Imaging of Angiogenesis

Angiogenesis represents the formation of new capillaries by cellular outgrowth from existing microvessels and occurs as part of the natural healing process after ischemic injury (*10*). The angiogenic process is a complex multistep phenomenon that involves many stimuli, growth factors, and interactions between multiple cell types (*11*). Favorable conditions or molecular events associated with the initiation of the angiogenic process are potential imaging targets. This includes evaluation of the altered expression of αv integrins (αvβ3, αvβ5), VEGF receptors (in particular VEGF R2 and neuropilin-1), and fibroblast growth factor (FGF) receptors (FGF R1 and syndecan-4), among others.

VEGF receptors are reasonable targets for imaging mediators of ischemia-induced angiogenesis. ^{111}In-labeled $VEGF_{121}$ was evaluated in a model of hindlimb ischemia (*5*), an approach that takes advantage of the specificity of $VEGF_{121}$ for hypoxia-inducible endothelial cell (EC) VEGF receptors. However, this approach may be limited, in part, by the total $VEGF_{121}$ receptor density and the retention of ^{111}In-$VEGF_{121}$ in other critical organs. Additional studies in more clinically relevant models will be required to validate the concept of angiogenic receptor labeling as a clinically useful imaging approach.

The αvβ3 integrin is expressed in angiogenic vessels and is known to modulate angiogenesis and, therefore, represents another potential novel target for imaging angiogenesis. Haubner et al. (*12–14*) reported the synthesis and characterization of a series of radiolabeled αvβ3

antagonists, reporting kinetics in both in vitro and in vivo preparations. Harris et al. (*15*) recently reported the high affinity and selectivity of an ^{111}In-labeled quinolone (^{111}In-RP748) for the αvβ3 integrin using assays of integrin-mediated adhesion. Meoli et al. (*6*) were the first to report the potential of ^{111}In-RP748 for in vivo imaging of myocardial angiogenesis. ^{111}In-RP748 demonstrated favorable kinetics for imaging of ischemia-induced angiogenesis in the heart. Additional studies have demonstrated the value of a ^{99m}Tc-labeled peptide (NC100692) for targeted imaging of the αvβ3 integrin in rodent models of hindlimb ischemia using high-resolution pinhole planar imaging (*16*). These experimental studies suggest that the radiolabeled αvβ3 targeted agents may be valuable noninvasive markers of angiogenesis after ischemic injury. Additional experimental studies will be required to define the duration of αvβ3 integrin expression/activation after ischemic injury or after stimulated angiogenesis. The changes in expression/activation of αvβ3 integrin will also need to be related to changes in more functional parameters, such as myocardial perfusion, regional mechanical function, permeability, and regional hypoxia. The potential for targeted imaging of other integrins, such as αvβ5, must also be considered.

Imaging of Atherosclerosis and Vascular Injury

Integrins, particularly αvβ3, have also emerged as promising targets for imaging injury-induced vascular remodeling/proliferative processes. Sadeghi et al. (9) have demonstrated that ^{111}In-RP748 and homologues bind preferentially to activated αvβ3 on ECs in vitro and exhibit favorable binding characteristics for in vivo imaging. These investigators presented evidence that ^{111}In-RP748 uptake can track the proliferative process associated with carotid artery injury by targeting activated αvβ3 integrin expression in vivo in apolipoprotein E-negative (apoE$^{-/-}$) mice. These findings may potentially lead to the development of noninvasive imaging strategies for vascular cell proliferation–associated states, whether focal (e.g., postangioplasty restenosis) or diffuse (e.g., pulmonary hypertension).

Schäfers et al. (*17*) investigated the feasibility of scintigraphic imaging of MMPs in vivo using a radiolabeled broad-spectrum MMP inhibitor (CGS27023A) in an established animal model of arterial remodeling and lesion development where MMPs are induced and activated. The MMPs constitute a large family of proteolytic enzymes responsible for the degradation of myocardial extracellular matrix (ECM) that is associated with vascular remodeling. Others have employed radiotracers targeted at inflammatory cell markers such as MCP-1 (*18*).

Targeted radiotracer imaging of atherosclerosis or vascular remodeling presents a unique problem, in that the target lesion has a very low mass and may be located deep in the body. Existing PET and SPECT instrumentation may be insufficiently sensitive to detect these small, deep lesions. Accordingly, several groups of investigators are developing intravascular scintillation catheters that can be used to detect local uptake of radiotracers targeted to components of atherosclerotic or unstable vascular plaque (*19–21*). Most of these intravascular detectors employ plastic scintillators linked to fiber optics that may transmit down a flexible catheter system.

Imaging of Postinfarction Remodeling

MMPs are also responsible for degradation of the myocardial ECM that is associated with the post-myocardial infarction (MI) left ventricular remodeling that often leads to heart failure. The importance of detecting and quantifying MMP activity in vivo during the evolution of post-MI remodeling is the driving force to develop a noninvasive method that will help to translate basic observations into clinical applicability. ^{99m}Tc- or ^{111}In-labeled MMP-targeted SPECT radiotracers have been developed that display selective binding kinetics to the active MMP catalytic domain. Binding of these radiotracers to the exposed catalytic domain of active MMPs provides a means to detect and image MMP activation in vivo using SPECT. Su et al. (*22*) demonstrated the feasibility of noninvasive MMP imaging using an ^{111}In-labeled nonspecific MMP inhibitor (^{111}In-RP782) to evaluate temporal changes in MMP activation in a murine model of MI. ^{111}In-RP782 retention correlated well with MMP activity defined by in situ zymography.

Imaging of Apoptosis

Apoptosis, or programmed cell death, occurs in association with many cardiovascular diseases. This preprogrammed cell death often occurs in combination with cell death by necrosis. Cells undergoing apoptosis express on their cell membrane phosphatidyl serine (PS), a constitutive plasma membrane anionic phospholipid that is not expressed in normal cells; thus it presents a favorable target for imaging of apoptotic processes. Annexin-V is a medium-size physiologic human protein with a high Ca^{2+}-dependent affinity toward the PS on the outer leaflet of the cell membrane. Annexin-V could be readily labeled with either a fluorescent or radionuclide agent and used in apoptosis imaging. Investigators have used Annexin-V labeled with radionuclide agents to image apoptotic cell death in vivo (*23,24*). Recently, ^{99m}Tc-labeled annexin-V has been used to track heart transplant rejection. In fact, apoptosis has been noninvasively identified in an animal model of heart transplant rejection (*24*) and allograft rejection in rat liver transplantation. Others investigated the clinical role of imaging with annexin-V for the detection of apoptosis in cardiac allograft recipients (*7*). These studies have demonstrated the usefulness of radionuclide imaging with annexin-V in patients who had undergone heart transplantation. Noninvasive annexin-V imaging could help obviate the need for highly invasive endomyocardial biopsies. Apoptosis imaging would also be useful in various other cardiac diseases characterized by overexpressed or deficient apoptosis. Moreover, monitoring the efficacy of

interventions directed toward induction of apoptosis is possible.

Imaging Stem Cells

Preclinical studies suggest that stem cells may be able to home to sites of myocardial injury to assist in tissue regeneration. The success of these stem cell therapies in patients will require methods to determine the biodistribution and fate of stem cells without postmortem histology as well as noninvasive imaging techniques for assessment of cardiac function after stem cell therapy. Labeling of autologous stem cells with ^{111}In-oxine has been recently shown to permit study of the dynamic biodistribution and trafficking of these cells after administration (*25*). However, tracking stems cells using this approach is limited to the half-life of ^{111}In. Other investigators have proposed the use of reporter probe imaging as a more effective means of tracking stem cells over long periods of time (*8,26*).

Summary

With the advent of both novel instrumentation and new noninvasive imaging techniques, the range of possible applications to gather new information on biochemical processes and pathways within the cardiovascular system is ever expanding. The recent introduction of novel gene therapies for treatment of cardiac diseases created a remarkable need for noninvasive imaging approaches to track the progress of such therapies. The examples of molecular imaging in nuclear cardiology cited here provide insight into the future of noninvasive imaging. Progress may rest on the development of targeted biological markers of physiological processes. Moreover, we expect that in the near future, targeted imaging will be routinely used in nuclear cardiology laboratories for diagnosis and prognostication as well as evaluation of therapy strategies in conjunction with current imaging modalities.

The intense development of novel targeted tracers, more sensitive instrumentation, and better reconstruction software requires interdisciplinary teams of physicians, chemists, radiophysicists, bioengineers, and computer science specialists to tackle the sophisticated process of translation from discovery of a targeted molecule to clinical imaging in patients. Thus, there is a need for nuclear medicine societies to bring scientists from different basic science disciplines to the medical community. Increased investments in cost and labor appear to be worthwhile because targeted imaging promises to lead to the understanding of fundamental biological functions in living systems as well as to bring basic science into intimate contact with clinical medicine.

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