Molecular Imaging in Nuclear Cardiology

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State-of-the-art techniques have been used to measure key aspects of cardiovascular pathophysiology from the birth of radionuclide cardiovascular imaging. However, during the last 30 years, there have been few innovative imaging advances to further our understanding of the complex physiologic processes. Molecular imaging now offers an array of tools to develop advanced diagnostic approaches and therapies for patients with coronary artery disease and heart failure. For example, the enhanced understanding of the pathophysiology of atheroma makes it possible to identify vulnerable plaque based on its metabolic signature or the presence of excessive apoptosis. Because the metabolic and apoptotic signals are large, it is likely that even small lesions will be visible. Of the many approaches that are being developed, 2 tracers appear most likely to be tested in the near future: (1) [^{18}F]-fluorodeoxyglucose, to determine macrophage metabolism; and (2) radiolabeled annexin, to measure apoptosis of the inflammatory cells. Using existing techniques such as perfusion imaging, appropriate patients can be selected for treatment with novel therapies, such as stem cell transplantation or vascular gene therapy. Using positron tomography in place of single photon imaging adds the capability for the measurement of absolute perfusion and perfusion reserve to the information on regional perfusion. Flow reserve detects global decreases in perfusion and refines the determination of lesion severity available from perfusion imaging.

> Above all, we forget that we ourselves are a part of history, that we are the product of growth and are condemned to perish if we lose the capacity for further growth and change.
>
> Hermann Hesse

SINCE ITS inception more than 85 years ago, cardiovascular nuclear medicine has used cutting edge technology. One of the earliest applications of radionuclides to study the circulation was Blumgart's determination of the circulation time in man.[1] The measurement used intravenously injected radon gas (a daughter product of the decay of radium) as the radioindicator and a Wilson Cloud chamber as the radiation detector. This innovative research took advantage of the discovery and purification of radium by Marie Curie, which led to Ms. Curie receiving the Nobel prize in 1903 and 1911 for her participation in the discovery of radioactivity, and her identification and purification of radium.[2] Similarly, the development of the Cloud Chamber resulted in Charles Wilson receiving the Nobel prize in 1927. The concept of using innovative technology continued with the description of the radiocardiogram by Myron Prinzmetal in 1947.[3] Prinzmetal placed a single Geiger tube over the precordium to record the passage of bolus of radioiodinated albumin through the heart. An estimate of cardiac function and pulmonary blood volume could be derived based on the shape and heights of the curves. In 1948, described by Hofstader (another Nobel laureate), the Geiger tube was replaced by the sodium iodide scintillation detector to improve the quality of this test.

Benedict Cassen developed the rectilinear scanner in 1950, a device that created images by transforming the radioactivity over a particular region into dots on a piece of paper.[4] By 1958, Rejali and coworkers had applied rectilinear scanner technology to image the cardiac blood pool.[5] These images were used to detect pericardial effusion and ventricular hypertrophy. Cohen and colleagues also in 1958 designed a system to measure total myocardial blood flow with the positron emitting isotope of rubidium.[6] The measurement system used 2 pairs of detectors, one set placed anteriorly and posteriorly over the right lung to measure the background, the other placed over the heart to measure myocardium plus background. It was only 4 years later, in 1962, that the first myocardial perfusion scan was recorded by Carr and coworkers.[7,8] The next decade saw the description of gated blood pool imaging for the determination of ventricular volumes, ejection fraction,[9] regional wall motion,[10] the change in regional function from rest to stress,[11] myocardial perfusion imaging for the detection of ischemia,[12] hot spot imaging for myocardial infarction, and metabolic imaging with fatty acids. Although we have improved the technology and now include positron emission tomography (PET) in our repertoire, we have not enhanced the spectrum of information we can offer to expand clinical decision making for

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nearly 30 years. Molecular imaging offers the promise of ending this drought.

POTENTIAL OF MOLECULAR IMAGING

The purpose of molecular imaging is to identify and track a sequence of cellular events as they occur, to provide a more complete understanding of how a process works over the life of a cell or organism. This approach can be far more useful in the laboratory than just looking at multiple snapshots of the process over time. Molecular imaging uses a large number of technologies, including ultrasound, magnetic resonance, optical, and radionuclide approaches, to trace these processes. For example, the distribution of radiolabeled cells can be tracked until the tracer decays. To follow the process for more than a few days, the cell needs a unique characteristic that can be relabeled repeatedly (eg, a novel receptor or metabolic process). Incorporating a marker gene in the deoxyribonucleic acid (DNA) causes each daughter cell to synthesize the receptor or metabolic marker. Subsequent generations of these cells can be identified by administering the appropriately labeled ligand.

A snapshot of the process is useful for most clinical purposes. The snapshot of receptor expression, perfusion, or metabolism, when integrated with clinical symptoms, laboratory and anatomic findings, provides information critical for patient treatment. Using this definition, it is clear that "Nuclear Medicine imaging has always been molecular imaging."[13] The remainder of this review will focus on major, current clinical problems in cardiology and the advances made in each of these areas with molecular imaging.

THE CLINICAL PROBLEM

The major problems in heart disease have not changed significantly during the last few decades: mortality remains high, and the number of hospital discharges has increased. Cardiovascular disease kills approximately 46,575 people each day worldwide[14] (~2,600 in the United States[15]). In the United States, there are 12.9 million patients with a diagnosis of coronary heart disease. An additional, growing problem is congestive heart failure. There are 4.9 million patients in the United States with a diagnosis of congestive failure, and approximately 550,000 new cases are diagnosed each year. Overall, diseases of the heart and blood vessels are responsible for ~40% of all deaths in the United States. Coronary artery disease, stroke, and congestive heart failure are responsible for 77% of all cardiovascular deaths.

The major procedure in nuclear cardiology to address the issues of coronary disease and heart failure is gated myocardial perfusion imaging. Unfortunately, perfusion imaging often identifies the disease process after it is well established and often has caused significant damage. Frequently, patients are not considered candidates for perfusion imaging unless they have symptoms of either angina or an angina equivalent. Because approximately half (6.3 million) of the patients with known coronary disease do not have angina, most undergoing the test have advanced disease at the initial diagnosis.[16] Therapy at this phase is usually invasive and expensive. A promise of molecular imaging is the ability to define the disease process early, when less expensive, less complex approaches to therapy may be efficacious.

Coronary Artery Disease

Coronary artery disease is an immune inflammatory process, which, over decades, results in arterial narrowing. Few diseases have longer "incubation" times.[17] Early atheroma may begin in infancy. The American Heart Association has classified atheroma into 6 classes. Classes I, II, and III are early lesions of progressively increasing complexity.[18] All lesions have lipoprotein cholesterol insudated beneath the endothelial cells in the artery. Although the initiating event for the process is still debated, injury to the endothelium is involved. During the process of repair, lipoprotein cholesterol is trapped between the endothelium and the subintimal layer of the vessel. The subendothelial lipid is a source of inflammation, causing the endothelial cells to signal monocytic cells to enter the area. The monocytic cells are transformed into macrophages, which are seen in the intima. In the region of the lipid, the macrophages are usually filled with phagocytized lipoprotein cholesterol and oxidized cholesterol, giving them a characteristic foam cell appearance. Early in the disease, the vessel responds to this inflammatory lesion with abluminal (compensatory) enlargement, as described by Glagov and coworkers.[19] Over time, usually decades, additional lipid is deposited, the lesion grows in size, abluminal compensation is exhausted, and the additional inflammation and/or lipid deposition compromises the vascular lumen.

Table 1. Atheroma Imaging Agents

Agent	Comments
^{125}I Autologous human LDL	Human carotid arteries[27] imaged in vivo. At endarterectomy, uptake corresponded to sites of lipid.
^{99m}Tc-LDL	Rabbit injured aorta.[28,29]
^{99m}Tc-LDL	Human carotid arteries,[30] xanthomas located on the arms.[31]
^{111}In-IgG, Fc, and Fab fragments	Rabbit injured aorta.[32]
^{125}I Oxidation specific monoclonal antibody	Rabbit injured aorta.[33]
^{99m}Tc AP4A	Rabbit aorta.[34]
^{125}I HDL	Apo e−/−mice.[35]
^{125}I Monocyte chemoattractant	Rabbit injured aorta and iliac artery.[36]
Peptide:	
F-18 FDG	Injured rabbit aorta,[37] human carotid,[38] aorta,[39,40] human coronary artery.[40]
^{111}In Z2D3 Antibody recognizing proliferating vascular smooth muscle	Human carotid arteries.[41]
^{99m}Tc Endothelin derivative	Rabbit aorta.[42]
^{99m}Tc Annexin V	Apo e−/−mice.[43]

Abbreviation: FDG, [^{18}F]-fluorodeoxyglucose.

Increased levels of low-density lipoprotein (LDL) cholesterol, homocysteine, lipoprotein (a), and decreased high-density lipoprotein (HDL) cholesterol accelerate the process, particularly at sites of major shear stress in the vessels. Occasionally, there is a rupture of nonocclusive plaque, leading to thrombosis at the lesion site and the acute onset of symptoms.

Late stage atheroma also contains remarkable amounts of cellular debris.[20] Many foam cells die in the lesion because intracellular digestion of the phagocytized material produces toxic material (particularly free cholesterol), which induces apoptosis.[21] However, if the macrophage filled with toxic material dies rapidly, apoptosis may be incomplete, cell membrane integrity may be lost, releasing the intracellular contents into the local environment. One consequence of macrophage cell death with the loss of cell membrane integrity is increased concentrations of matrix metalloproteinase in the lesion. High levels of matrix metalloproteinase reduce the integrity of the cap separating the lesion from blood flowing in the vessel, making the lesion prone to rupture.

In its late phases, the atheroma looks like an abscess in the wall of the vessel. There are large numbers of inflammatory cells, lakes of lipid, and infiltration of granulocytes and lymphocytes at the lesions. In addition to changing the vessel shape, the inflammatory response also interferes with vessel function. The inflammatory lesions interfere with production of nitric oxide by the endothelium, causing a miscommunication between the endothelium and underlying smooth muscle cell. The loss of this paracrine function causes abnormal vasoreactivity, manifest as inappropriate vasoconstriction. Abnormal vasoreactivity can cause decreased perfusion distal to the site(s) of abnormal constriction, especially when the cold pressor test is used as the stressor.[22] In the catheterization laboratory, abnormal vasoreactivity can be detected by comparing coronary arteriograms recorded before and after direct coronary artery infusion of acetylcholine.[23] Vessels with abnormal vasoreactivity dilate normally when nitroglycerin is infused. Therapy known to reduce serum lipids, such as dietary restriction of fats, aggressive therapy with statins,[24] ingestion of arginine,[25] or controlling blood sugar levels in diabetics, reduces the inflammatory component of the lesion, restoring production of nitric oxide and normal vasoactivity.

The evolution of atheroma from a fatty streak to ruptured atheroma with associated thrombus has been divided into 6 lesion types by an expert panel of the American Heart Association.[26] Type V and VI lesions are associated with coronary events. One goal of molecular imaging has been the direct identification of advanced atheroma, lesions of class IV and V, to direct therapy before an acute event occurs, reducing the risk of sudden death.

MOLECULAR IMAGING OF ATHEROMA

The biology of atheroma provides a number of targets for imaging. Table 1 lists a number of nuclear medicine approaches that have been tested in experimental animals and man.

Instrument and Radiopharmaceutical Requirements to Image Atheroma

Imaging atheroma is a challenge, particularly in the coronary arteries. Atheromas are contained within the wall of the vessel, making the lesions very thin. Lesions typically occupy a fraction of the vessel circumference and often extend from 1 to 2 cm. Total lesion volume is often <0.1 mL.[44] Because single photon nuclear medicine imaging devices have spatial resolutions of approximately 4 mm at the surface of the collimator and 8 to 10 mm at the center of the object with planar imaging, and 10 to 15 mm resolution with single photon emission computed tomography, the relationship of small lesion size to image spatial resolution makes the disease very difficult to image. PET offers significant advantages, with spatial resolution at the center of the field from 4 to 6 mm (at the sweet spot) but decreases to 7 to 8 mm at the edge of the field of view.[45] Although the imaging devices do not have the spatial resolution to provide detailed lesion characterization, the lesions can be detected if there is an exceptionally high target and/or background ratio. Unfortunately, the majority of agents listed previously have ratios that are too low to permit reliable lesion detection in vivo. Residual activity in the blood pool and tracer in nonvascular tissue adjacent to the target vessels make these lesions more difficult to detect. As lesions enlarge, approaching a size that can be resolved by the imaging device, the required target and/or background ratio needed for detection decreases. If a lesion larger than the resolution of the instrument can be identified with a target to background ratio in the image of 1.5:1 (usually approximately 3:1 in the tissue), smaller lesions will require a progressively higher target to background ratios for detection (Fig 1).

LDL imaging can achieve lesion and/or background ratios that approach 6:1, which are good enough for the carotids where the lesions are large but not good enough for lesions in the aorta, where the lesions are distant from the detector, decreasing spatial resolution, or coronaries, where the lesions are small. Inflammatory markers, such as Fc fragments or IgG (as a marker of inflammation), also may achieve 6:1.[32] Because the resolution of radionuclide imaging instrumentation is not likely to improve markedly in the immediate future, it is more fruitful to seek other approaches to image atheroma.[46]

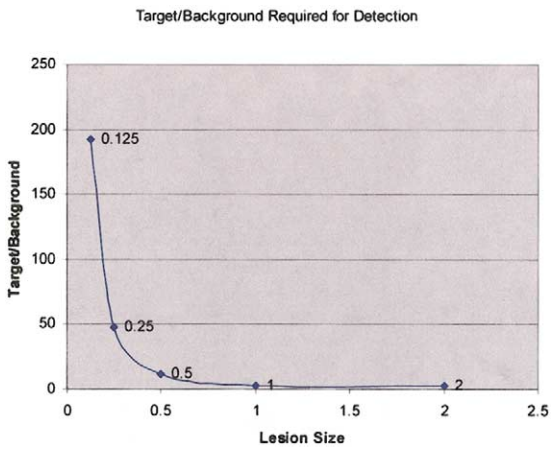

Fig 1. This graph assumes that the instrument has a resolution of 1 cm, and the target-to-background ratio in the tissue is 3:1 (giving a 1.5:1 ratio in the image). The graph depicts the increase in lesion-to-background ratio necessary for detection as the lesion decreases in size.

Severe inflammation is a hallmark of unstable lesions. These lesions contain a large number of inflammatory cells, each stimulated to phagocytize LDL, oxidized LDL cholesterol, and cellular debris. The number of macrophages in these lesions is sufficient to permit imaging with the receptor agonist monocyte chemoattractant peptide-1 (MCP-1).[47] Radiolabeled MCP-1 was detected in the injured rabbit aorta model because of the increase in receptor expression in each cell from approximately 5,000 per cell at rest to approximately 50,000 when stimulated, multiplied by the marked local increase in cell concentration. However, even with this agent, the maximum in vivo target/background ratio was ~6:1.

[^{18}F]-fluorodeoxyglucose (FDG)

An alternative approach is needed to image the lesions that will cause acute coronary syndromes. Metabolic activity in these lesions is so high that it increases temperature in the tissue in patients with acute coronary syndromes.[48,49] To generate this much heat, cells participating in the lesion have a marked increase in metabolism. For a short time, macrophages can use endogenous sources of energy, but their prolonged needs require exogenous substrate. Major external substrates are glucose, glutamate, and fatty acids.[50] Selection of the preferred substrate depends on the local environment. Fatty acid metabolism requires oxygen to generate adenosine triphosphate, but the conversion of glu-

cose to lactate is associated with the production of 2-adenosine triphosphate, without the requirement for oxygen. Atheroma, even in the presence of increased vasa vasorum, has a limited supply of oxygen, making the local environment acidotic and hypoxic. As a result, glucose serves as a major substrate for macrophages in atheroma.

Increased FDG concentration has been identified in patients with carotid disease, diffuse vasculitis, and coronary artery disease.[38,40,51] A study comparing FDG uptake to the number of macrophages in atheroma of hyperlipidemic rabbits showed a linear correlation of $r = 0.81$.[52] It is likely that FDG uptake was seen at these sites because of both the increased number of cells and the increased use of glucose as a result of the severity of inflammation.

Apoptosis in Atheroma

Apoptosis, involving smooth muscle, macrophages, and endothelium, is a prominent feature of atheroma.[17,21] Blankenberg and coworkers showed the use of radiolabeled annexin V as an agent to image apoptosis in vivo.[53] Mari and colleagues reported the feasibility of using radiolabeled annexin to detect atheroma in experimental animals.[43,54,55] Although some apoptosis is seen histologically in most atheromas, the number of apoptotic cells increases with the stage of the disease (class IV to VI lesions). Experimental studies with radiolabeled annexin show striking focal concentration in some lesions. Markedly increased circulating lipid levels develop in Apo e-/-mice, which are deficient in hepatic receptors for LDL cholesterol. As these mice age, characteristic atheroma develops in the aorta. The lesions bear a striking histologic resemblance to human atheroma, with the exception that these lesions rarely rupture. To compare molecular markers for the characterization of atheroma, Mari and coworkers compared ^{18}FDG, ^{125}I MCP-1, and ^{99m}Tc annexin V in apo e-/-mice.[55] The investigators observed some lesions concentrating tracers similarly, but other lesions had either no tracer or only a concentrated one. Histologically, lesions concentrating the tracers had more inflammatory cells. The maximum uptake ratio observed with this agent in selected lesions was approximately 10:1, suggesting that it is a candidate for identifying advanced atheroma.

Improving Cardiac Function After Myocardial Infarction

A promising technique for the restoration of regional ventricular function after myocardial infarction is the implantation of autologous bone marrow stem cells directly into the regions of acute necrosis.[56] This approach is based on the observation that true progenitor cells exist in mature adults. Techniques to identify these cells, grow them in vitro, and re-administer them have been developed. However, the technique requires careful timing to optimize the chance for the transplanted cells to survive and differentiate. During the course of infarction, cardiac myocytes are lost due to an acute, severe limitation of blood flow. The healing infarct causes inflammation, which brings some perfusion to the area of necrosis. This level of perfusion is sufficient to support the minimal metabolic requirements of fibrous tissue, which replaces the lost muscle cells. If the patient is reperfused, either through spontaneous or pharmacologic thrombolysis, there is a significant source of perfusion for the new tissue. Administering cultured autologous stem cells in the coronary artery, perfusing the area of acute infarction approximately 7 to 10 days after the event, appears to provide fertile soil for the implantation and differentiation of these cells. The follow-up of these patients showed improved wall motion in the treated areas.

Autologous cells, then, have several advantages:

1. They eliminate the need for immunosuppression of the host.
2. The cells are complete units.
   a. They have a complete complement of the genetic material required for the task.
   b. Rather than requiring specific genes, the cells require an appropriate environment to differentiate.
   c. The cells can differentiate into myocytes, as well as other cells required to produce “working” myocardium (eg, the cells can differentiate into cardiac myocytes and vascular cells).
3. Differentiation resulting in participation in contractile function can occur relatively quickly (ie, weeks) rather than requiring extended incubation to result in enhanced production of a few individual proteins.
4. Autologous marrow is unlikely to result in local inflammation, which may hinder incor-

poration of the "new" cells into the local environment.

In light of these advantages, investigators have initiated human trials of autologous mesenchymal cells.

PET

Recording serial PET images following injection of N-13 ammonia or O-15 water allows quantitation of myocardial perfusion at rest and stress.[57,58] The ratio of stress-to-rest perfusion defines coronary flow reserve (CFR), which provides a functional assessment of the severity of coronary stenosis.[59,60] Yoshinaga and colleagues showed that myocardial areas with abnormal perfusion at stress had more markedly reduced CFR than zones supplied by a stenotic vessel but with normal perfusion at stress, where the decrease of CFR was intermediate between the areas with a perfusion abnormality and normally perfused segments.[61] Reduced CFR is seen in patients with hyperlipidemia and in asymptomatic patients with diabetes mellitus, usually caused by abnormal vasoreactivity.[62,63] Lowering cholesterol improves the CFR, suggesting that PET flow measurements can define changes in vascular reactivity with risk factor modification.

The uptake and oxidation of fatty acids are increased with increased cardiac work and decreased with ischemia.[64] Heart failure also is associated with decreased efficiency in the catabolism of fatty acids. The efficiency of aerobic myocardial metabolism can be determined by evaluating the uptake and clearance of C-11 acetate, a tracer catabolized by the Krebs cycles, and comparing the metabolic rate to cardiac work.[65,66]

Evaluating The Effectiveness of Cardiac Transgene Therapy

Administration of genes associated with angiogenesis is a novel method being developed as a therapeutic modality to treat vascular disorders. This procedure may be used in the correction of peripheral or coronary insufficiencies. The delivery of genetic material in high concentrations with minimal side effects is performed with catheter based, endovascular delivery systems. Imaging plays an extremely important role in the evaluation of targeting, binding, and gene expression, and the determination of the therapeutic results.

A major application of gene therapy in cardiology is augmenting the production of vessels in areas of ischemia. There have been several studies using genes coding for the production of vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF) to accomplish this goal. The gene can be administered as naked DNA directly into the myocardium or through a coronary artery catheter.[67,68] To promote angiogenesis in a territory perfused by a severely stenosed artery, direct intracoronary administration of the genes and angioplasty balloons coated with the gene encoding vascular endothelial growth factor, VEGF, have been tried.[69,70] Direct myocardial administration increased the number of vessels seen angiographically, while the catheter based approach resulted in increased myocardial perfusion at rest, especially in patients receiving a higher dose of VEGF. On the other hand, administering the growth factor itself appears less efficacious. In a dose increase study of 337 patients treated with intracoronary FGF, there was no significant change in perfusion at either rest or stress.[53]

The limited improvement in patient performance at stress has tempered the initial enthusiasm for angiogenic therapy with VEGF and FGF in patients with severe ischemic heart disease.[71] A novel application of gene therapy is the pretreatment of the autologous vein grafts used in coronary artery bypass surgery. Vein grafts have a failure rate of approximately 20% during the first year following engraftment. Pretreating these vessels with the E2F decoy gene, which targets smooth muscle and reduces proliferation of smooth muscle cells in the graft, has successfully minimized graft stenosis in preliminary human trials.[72,73]

Molecular imaging has provided an array of tools and major leads on new therapies to enhance the effectiveness of the treatment of patients with coronary artery disease and heart failure. A major concern with new treatments is the selection of appropriate patients to treat and the development of an objective "yardstick" to determine the efficacy of treatment. Established radionuclide imaging techniques, such as perfusion and function imaging with single photon tracers, can provide the information needed in many patients. PET provides additional quantitative and qualitative information of perfusion and metabolism that can be integrated with other parameters to increase the understanding of pathophysiologic mechanisms and the success of

various therapies. For example, flow reserve detects global decreases in perfusion and refines the determination of lesion severity available from perfusion imaging. It is likely that in the near future, even smaller lesions, such as vulnerable plaque, may be imaged directly. The most promising agents for evaluation are FDG, to determine macrophage metabolism, and radiolabeled annexin, to measure apoptosis of the inflammatory cells.

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