PET IMAGING AND ITS APPLICATION IN CARDIOVASCULAR DISEASES

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Abstract

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and represent a great challenge for modern research and medicine. Despite advances in preventing and treating CVD over the decades, there remains an urgent need to develop sensitive and safe methods for early detection and personalized treatment. With refinements of molecular imaging technologies such as positron emission tomography (PET), noninvasive imaging of CVDs is experiencing impressive progress in both preclinical and clinical settings. In this review, we summarize advances in cardiovascular PET imaging, highlight the latest development of CVD imaging probes, and illustrate the potential for individualized therapy based on metabolic phenotype.

Introduction

Cardiovascular disease (CVD), including stroke, is the leading cause of death in the United States. In 2011, approximately 787,000 deaths were attributable to CVD, accounting for more than 25% of all deaths in the United States.[1-4] Cardiovascular disease starts with impaired endothelial function followed by inflammation of the vessel wall, eventually leading to the formation of atherosclerotic lesions that cause myocardial infarction and stroke. The major risk factors of hypertension, obesity, and diabetes further increase the burden of CVD.[5] Currently, there are an estimated 62 million people with CVD in this country, but as the age of the population rises, so does the incidence of CVD and heart failure. Thus, developing sensitive and noninvasive methods for early detection and personalized treatment of CVDs and heart failure remains a top priority.

Molecular imaging is a biomedical discipline that enables the noninvasive visualization, characterization, and quantification of biological processes at the molecular and subcellular levels within living subjects.[6] It exploits specific imaging probes as well as intrinsic tissue characteristics as the source of image contrast. It also provides the potential for better understanding of integrative biology, earlier detection, and accurate diagnosis of disease for therapeutic monitoring.[7] The advantage of molecular imaging over more conventional readouts is that it reveals functional changes in tissue and organ with sufficient spatial and temporal resolution for studying biological processes in vivo. The imaging modalities in both preclinical and clinical studies include positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and optical imaging. Each modality has its inherent strengths and limitations as shown in Table 1.[8] The rapid evolution of multimodality imaging such as hybrid PET/CT and PET/MR imaging with advanced imaging probes offers a new perspective by allowing for a combined anatomic and functional evaluation of disease.

As a key component of molecular imaging, a highly sensitive imaging probe must specifically reach the target of interest in vivo and be detectable within a defined span of time. As shown in Figure 1, the imaging probe must incorporate two key elements: (1) an affinity ligand that recognizes the intended molecular or cellular target and (2) a signaling component. Development of a desirable molecular probe with the potential for clinical translation is frequently a challenging endeavor for medicinal and radiochemists. As we all know, molecular imaging has been routine in cancer research, clinical trials, and oncology-related medical practice.[9,10] Nowadays, with new developments in cardiovascular biology and remarkable advances in imaging technologies, molecular imaging is becoming a vital preclinical and clinical tool in the fields of atherosclerosis, thrombosis, vascular biology, and cardiovascular medicine.[11-14] Beyond molecular imaging, myocardial perfusion imaging with ^{82}Rb PET can also provide powerful and incremental risk stratification for patients with suspected or known coronary artery disease.[15] Additionally, 2-deoxy-2-(^{18}F)fluoro-D-glucose (^{18}F-FDG) has been used as the gold standard for assessing myocardial glucose metabolism.[16] Currently, SPECT myocardial perfusion imaging is considered a reliable and widely used tool in CVD, with the advantages of lower cost and compatibility with a wider variety of radiopharmaceuticals. However, it has technical weakness compared to PET, such as lower spatial resolution.[17] More importantly, PET/CT with 3-dimensional acquisitions demonstrated an important milestone in myocardial perfusion imaging that could signifi-

Imaging Modality	Tissue Penetration	Sensitivity	Spatial Resolution	Clinical Translation
Optical fluorescence/ bioluminescence	Limited (< 2 cm)	High	High	Limited[1]
MRI	Unlimited	Low	High	Yes
PET/SPECT	Unlimited	High	Low	Yes

[1] Fluorescence-guided surgery confers improved precision in tumor resection while preserving critical structures.[8] MRI: magnetic resonance imaging; PET: positron emission tomography; SPECT: single-photon emission computed tomography

Table 1. Imaging modality characteristics.[8]

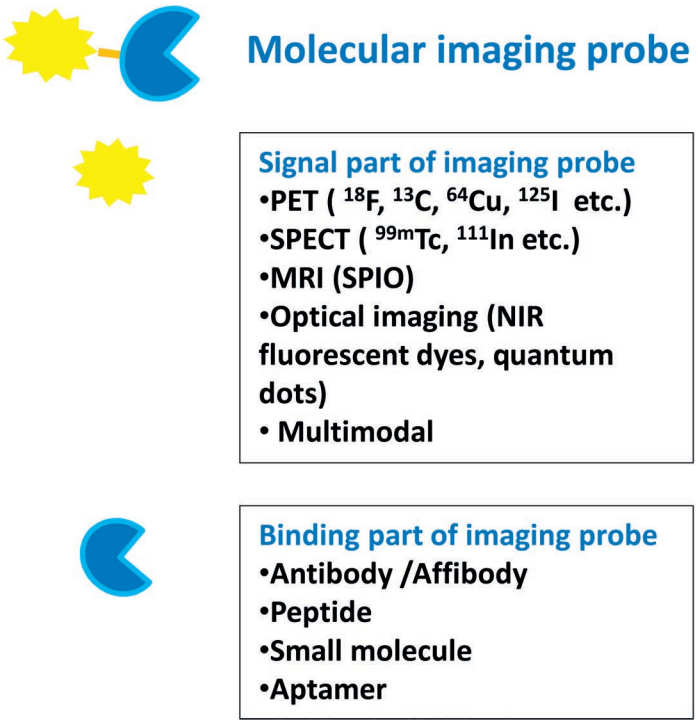

Figure 1. Illustration of molecular imaging probe. PET: positron emission tomography; SPECT: single-photon emission computed tomography; MRI: magnetic resonance imaging; SPIO: superparamagnetic iron oxide; NIR: near infrared

cantly shorten imaging protocols, reduce radiation exposure, and increase its application in CVD.

The Omnivorous Heart

The heart primarily relies on fatty acids as its source of fuel, with up to 70% of the adenosine triphosphate being derived from fatty acid oxidation; it also can utilize glucose, lactate, and ketone bodies.[18] The relative use of each substrate is tightly regulated, with each pathway having a considerable degree of plasticity and interdependence.[18] Under conditions of stress, pathology, and metabolic insults, the relative ratios of dependence on the various substrates has been shown to change. For instance, reduced glucose uptake in type 2 diabetes can further increase the heart's dependence on fatty acids,[19] whereas heart failure with reduced ejected fraction (HFrEF), left ventricular (LV) hypertrophy, and ischemic heart failure are associated with a shift from fatty acid to glucose utilization.[18,20,21] It is thought that this metabolic shift occurs because glucose consumes less oxygen per molecule than fatty acids, making it a more efficient substrate, particularly in oxygen-deprived or stressed conditions.[18,22] Understanding this relative substrate use may be beneficial in assessing pathology and the underlying mechanisms and ultimately determining the best course of treatment.

Identifying Metabolic Changes in the Heart

Assessment of Substrate Changes

To assess changes in substrate use in a diseased heart, PET imaging can be used with the help of isotope-labeled substrate compounds. For example, to assess glucose uptake, the myocardial rate of ^{18}F-FDG uptake can be measured as K_i (mL/min/g) using PET imaging.[23] However, since FDG is 2-deoxy glucose, it does not undergo glycolysis and is not oxidized the same as glucose. It therefore quantifies glucose uptake and not oxidation. But a dynamic scan of FDG over time can reveal important information about the rate and dynamics of glucose utilization in the heart (Figure 2). Use of 1-^{11}C-glucose can give more information on the complete oxidation of glucose. Furthermore, fatty acid uptake and oxidation can be assessed by measuring uptake using ^{11}C-labeled palmitate followed by dynamic scanning for compartmentalization studies.[24] After accounting for myocardial blood flow rates and oxygen consumption (mVO_2) with ^{15}O-water and 1-^{11}C-acetate, one can determine metabolic compartmentalization and oxidation of substrates.[25] These data must be combined with substrate blood levels and mathematical compartmental modeling to get accurate information on availability, uptake, and oxidation of the substrate.

Investigators at the Center for Bioenergetics and the Small Animal Imaging Core at the Houston Methodist Research Institute are working on developing these capabilities to enable assessment of metabolic changes in preclinical mouse models of heart disease followed by human studies. These studies and capabilities will allow us to design personalized therapies in line with the Houston Methodist Institute for Academic Medicine's (IAM) Precision Medicine concept, in which treatment is personalized for a particular patient's molecular signature and anatomical/temporal information. For instance, knowing the metabolic substrate use signature of an individual with LV hypertrophy may help in designing treatment that would help reverse the substrate switch to facilitate adaptive rather than maladaptive changes in the myocardium.

Metabolic Alterations in Diastolic Dysfunction

Heart failure with preserved ejection fraction (HFpEF) is characterized by normal systolic function but impaired diastolic relaxation, resulting in reduced cardiac output. HFpEF is common in postmenopausal women and the elderly, and its prevalence is similar to that of HFrEF. However, the underlying mechanisms of HFpEF are poorly known. Although the metabolic changes in HFpEF are not well defined, some studies in humans indicated that, contrary to HFrEF, there may be decreased myocardial glucose uptake in diastolic dysfunction (DD) and diabetic cardiomyopathy.[23,26] HFpEF is closely associated with insulin resistance, therefore it remains to be determined whether the decrease in glucose uptake was due to the associated insulin resistance. It is thought that greater dependence on fatty acids and reduced glucose uptake due to insulin resistance may lead to overburdening the mitochondria, accumulation of acylcarnitines, mitochondrial uncoupling, and production of reactive oxygen species primarily at the levels of complexes I and III of the respiratory chain.[27,28] Oxidative stress can further trigger fibrotic processes that result in stiffer ventricles and diminished ventricular relaxation. The fact that HFpEF is associated with many systemic metabolic abnormalities—such as diabetes, insulin resistance, loss of estrogen, and aging—makes it even more crucial to study and monitor metabolic changes in the heart before the onset of overt heart failure.[29]

Determining both fatty acid and glucose uptake using PET imaging and sophisticated mathematical modeling can lead to development of personalized treatments for patients with HFpEF. The noninvasive nature of these studies along with the plethora of information they can yield make them indispensable to the IAM's focus on precision medicine. For example, for a woman who has recently entered menopause and has other comorbidities such as insulin resistance, it may be beneficial to assess the cardiac metabolic substrate use profile with PET imaging and then design medication that may increase or decrease the use of one substrate over

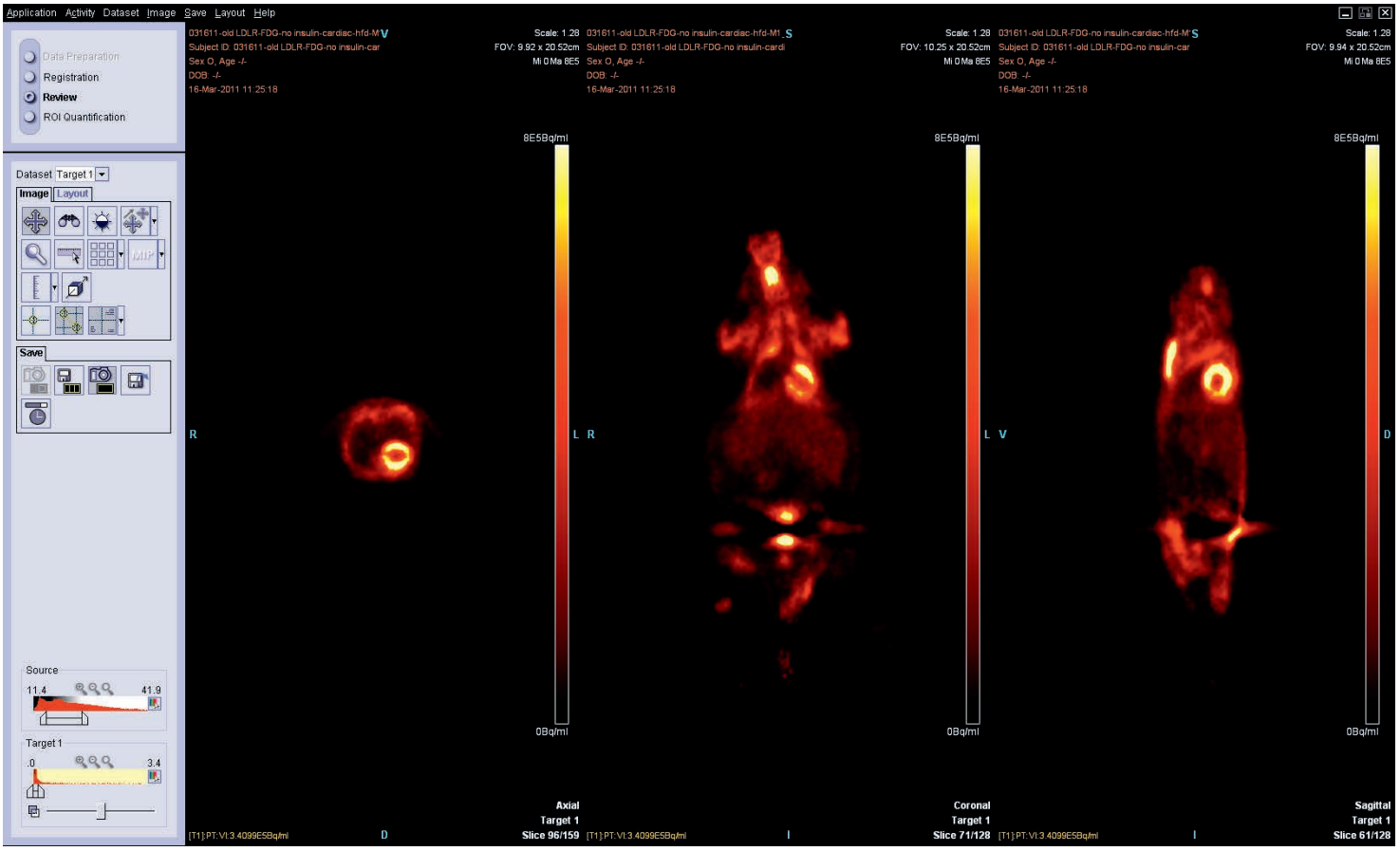

Figure 2. Positron emission tomography images showing FDG uptake in the mouse heart. FDG: [^{18}F]-2-fluoro-2-deoxy-D-glucose

the other. These studies may prevent the progression of diastolic dysfunction to HFpEF and potentially even prevent the need for a transplant in cases of heart failure.

PET Imaging Targeting Mitochondria

The mitochondrion is a double membrane-bound organelle considered to be the powerhouse of the cell, converting oxygen to CO_2 while oxidizing nutrients to provide free energy for adenosine triphosphate synthesis. Mitochondrial dysfunction is related to many disorders, including heart disease, neuronal degeneration, and cancer. Cardiac mitochondria are dynamic organelles that occupy more than 30% of cell volume,[30] and they continuously undergo fusion and fission within the cell. The changes in mitochondrial morphology may affect not only respiratory chain function but also many other pathway regulations such as apoptosis, cardiac ischemia/reperfusion, or heart failure. There are several reports on PET probes targeting mitochondrial complex I (MC-I) for imaging of myocardial perfusion and neuronal degeneration. MC-I is the first and largest multisubunit complex of the electron transport chain. Huisman et al. reported a pyridazinone analog, flurpiridaz F-18 (^{18}F-BMS-747158-02), as a promising new PET probe for the quantitative imaging of myocardial perfusion, which is currently being evaluated in phase 3 clinical trials.[31] Flurpiridaz F-18 binds to MC-I with high affinity in myocardial tissue. Its uptake into the heart is selective due to the high density of mitochondria in myocardial tissue relative to the liver. Flurpiridaz F-18 can detect milder perfusion defects with better accuracy, reflecting the true extent of perfusion defects compared to SPECT probes such as the ^{99m}Tc-labelled tracers.[32] The results in phase 2 studies also proved that flurpiridaz F-18 PET imaging has higher sensitivity than SPECT.[33] The phase 3 study to further confirm these results is currently ongoing.

Based on the structure of flurpiridaz F-18, another MC-I targeting probe, ^{18}F-BCPP-EF, was developed to detect neuronal damage in a rat model of ischemic brain damage. The study demonstrated that ^{18}F-BCPP-EF could detect neuronal degeneration associated with impaired MC-I activity.[34] On the other hand, alteration in mitochondrial potential is an important characteristic of cancer and is caused by mitochondrial dysfunction such as DNA mutation and oxidative stress. Studies show that the mitochondrial potential in carcinoma cells is significantly higher than in normal epithelial cells. MitoTracker rhodamine (Thermo Fisher Scientific Inc., Canoga Park, CA) is a mitochondrial membrane potential-dependent fluorescent dye that stains mitochondria. A ^{64}Cu radiolabeled rhodamine derivative was reported as a dual modality imaging probe (PET and optical) for both in vitro cellular staining assays and tumor imaging by PET.[35]

PET Imaging Targeting Angiogenesis

Angiogenesis is the growth of new blood vessels from existing microvessels. It modulates post-myocardial infarction healing and subsequent ventricular remodeling. Myocardial ischemia and infarctions usually cause hypoxia that stimulates angiogenesis. Proangiogenic therapy is currently undergoing clinical testing in patients suffering from ischemic heart disease, peripheral vascular disease, chronic wounds, and stroke.[36] Many factors have been found to contribute to the process of angiogenesis; among them, vascular endothelial growth factor (VEGF) and integrin $\alpha_V\beta_3$ have

been identified as favorable biomarkers for PET imaging of cardiac and tumor angiogenesis. For example, a PET probe ^{64}Cu-VEGF121 was reported to visualize cardiac angiogenesis in a rat myocardial infarction model, suggesting that this probe was useful for assessment of angiogenic therapy response.[37] Studies also reported that RGD (Arg-Gly-Asp)-based integrin PET probes, such as ^{18}F-AlF-NOTA-PRGD2, can noninvasively image ischemia/reperfusion-induced myocardial angiogenesis with favorable pharmacokinetics.[38] Another clinical trial study with this PET probe demonstrated that ^{18}F-AlF-NOTA-PRGD2 PET/CT enabled the noninvasive visualization of glioblastoma multiforme lesions and the prediction of sensitivity to concurrent chemoradiotherapy as early as 3 weeks after treatment initiation.[39] Our group at the Houston Methodist Research Institute developed novel small molecular-based PET probes targeting VEGF receptor (VEGFR) and integrin $\alpha_V\beta_3$ with two patents filed.[40,41] We reported on the successful optimization and initial preclinical performance of a VEGFR-targeting PET probe that was based on the FDA-approved oncologic drug in current clinical use, vandetanib. This designed probe demonstrated approximately two orders of magnitude improvement in VEGFR binding affinity in vitro compared to the vandetanib, and it significantly enhanced tumor angiogenesis imaging with favorable biodistribution and pharmacokinetic properties for clinical translation and potential commercialization.[40] These studies will enable us to initiate the myocardial angiogenesis imaging project in the near future.

Metabolic Validation: Procuring and Analyzing Failing Heart Samples

Metabolic analysis of actual tissue samples provides the validation for imaging interpretations. The progression to heart failure that may result from any of several etiologies—e.g., hypertension, diabetes, or ischemia—is characterized by utilization of different metabolic substrates. These include combinations of glucose, fatty acid, lactate, and amino acids. As noted earlier with HFpEF, the use of imaging to identify a predominate metabolic phenotype could personalize and guide management decisions. Traditionally, metabolic analysis has been conducted on preclinical small animal models with acute ischemia or pressure overload as a source of myocardial stress. It is strategically difficult to obtain timely, adequately sized samples from the human heart for oxygen consumption studies with relative substrates since the tissue must be procured and processed to be studied within 60 to 90 minutes. Our close collaboration with the Houston Methodist transplant team of cardiologists, cardiothoracic surgeons, and cardiovascular researchers has facilitated prompt isolation and study of failing human heart tissue. These ventricular wall samples are procured from failing hearts with and without support from LV assist devices,[42] and the close proximity of our laboratory to the operating suites allows transfer for studies within minutes after hand-off.

Cardiac fiber and isolated mitochondria from human heart tissue have been interrogated for mitochondrial metabolic function studies using high-resolution Seahorse XF (Agilent Technologies, Santa Clara, CA) and Oroboros respirometers (Oroboros Instruments Corp, Innsbruck, Austria). The Seahorse XF measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) simultaneously.[43] OCR represents mitochondrial respiration while ECAR indicates glycolysis. The Seahorse XF can easily monitor the shifting between mitochondrial respiration and glycolysis. The Oroboros high-resolution respirometer can measure OCR only, but it can perform multiple injections sequentially for substrates and chemicals. This makes the Oroboros more flexible for comparison purposes. Both of these complement each other and are used to study the mitochondrial oxidative phosphorylation pathway regulation by applying different substrates, uncouplers, and inhibitors.

We can interrogate various metabolic pathways by using substrates labeled with the isotopes of carbon or nitrogen (^{13}C, ^{14}C, ^{15}N) and quantifying by mass spectrometry analysis. This technique also can be used for drug metabolism and pharmacokinetics studies. The results will help reveal how the citric acid cycle, beta-oxidation, and glycolysis pathways are altered in various diseases. This collaborative team approach and physical proximity facilities the ability to validate metabolic imaging for substrate presence and mitochondrial function in disease states.

Conclusion

With advances in molecular biology, biotechnology, chemistry/radiochemistry, and imaging techniques, cardiovascular molecular imaging is experiencing impressive progress. For cardiovascular PET imaging, the ultimate goal is to provide molecular and cellular information to better understand biological processes and to detect diseases early before manifestation of gross anatomical features or physiological consequences. This information would then guide clinical decision making. Cardiac PET will also be a useful tool to assess new therapeutic strategies and ultimately help patients suffering from cardiovascular disease.

Key Points:

- Unique metabolic patterns are associated with the underlying pathophysiology of heart disease.
- Metabolic imaging such as PET can be used to identify these unique patterns.
- Knowledge of the specific patterns can guide individualized clinical decision making

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Keywords: heart failure, metabolic imaging, PET imaging, personalized therapy

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