Perfusion and Specific Radioprobes for Cardiac Imaging

Filipa Mendes, Lurdes Gano, Célia Fernandes, António Paulo and Isabel Santos
Radiopharmaceutical Sciences Group, IST/CTN, Instituto Superior Técnico,
Universidade Técnica de Lisboa, Estrada Nacional 10, km 139,7, 2695-066 Bobadela LRS, Portugal

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Abstract: Major advancements have been made in treating cardiovascular disease. However, improving diagnosis is crucial, because the detection of the early stages of disease would allow preventative approaches therapy. Myocardial perfusion imaging is in clinical use for decades and is an effective tool for diagnosis, and long-term follow-up of patients with suspected or known coronary artery disease. The technetium-based agents, 99mTc-sestamibi and 99mTc-tetrofosmin, are widely used myocardial blood flow tracers. However, since both present drawbacks in their biodistribution properties, there is now resurgence in the study of both neutral and cationic technetium agents to further improve the characteristics of perfusion radiopharmaceuticals. Despite all the success of perfusion imaging, a unique strength of nuclear imaging is its ability to provide tools for imaging processes at molecular and cellular levels in intact organisms under a wide variety of physiologic conditions. Advances in new specific imaging agents that identify myocardium injury and cellular dysfunction may contribute to the improvement of diagnosis and eventually better therapeutic approaches. In this communication, we will review perfusion agents and their biological mechanism of uptake. We will also discuss examples of target-specific radiopharmaceuticals for cardiac imaging, including advances in pre-clinical imaging approaches.

1 MYOCARDIAL PERFUSION IMAGING

In the past few decades, major improvements have been made in treating some types of cardiovascular disease. However, new treatment options are urgently needed for all types of cardiovascular disease. Moreover, improving diagnosis is crucial, because by detecting the early stages of disease, the focus of therapy could be shifted from treatment to prevention.

Myocardial perfusion imaging has been in clinical use for over 30 years, serving as an effective, reliable, and relatively simple tool for diagnosis, risk stratification, and long-term follow-up of patients with suspected or known coronary artery disease (Notghi and Low, 2011). Thallium-201 chloride was the first pharmaceutical to be widely used clinically for imaging myocardial perfusion. Because of its relatively long half-life and low energy X-ray emission, it is not the ideal agent for imaging, giving a relatively large radiation dose with lower image quality than technetium agents. It enters the cells via the Na/K-ATPase, and is redistributed fairly rapidly.

Technetium-based agents, ^{99m}Tc-sestamibi (Cardiolite) and ^{99m}Tc-tetrofosmin (Myoview), are now widely used myocardial blood flow tracers (Figure 1). These perfusion agents have with minimal redistribution, better imaging characteristics and less radiation to the patient. ^{99m}Tc-sestamibi enters the cell via a passive pathway due to its lipophilicity and accumulates in the mitochondria in response to the physiologically negative mitochondrial and plasma membrane potentials. Due to their elevated number of mitochondria, the heart, muscles, liver and kidneys present a high uptake of this radiopharmaceutical. Because the in vivo behavior of ^{99m}Tc-tetrofosmin demonstrates similarities with ^{99m}Tc-sestamibi it was initially suggested that the mechanism determining cellular distribution was also similar. The first studies indicated that the uptake is through a metabolism-dependent process, most likely by potential-driven transport of the lipophilic cation. However, subsequently it was shown that inhibition of the Na+/K+ ATPase, partly inhibited the uptake of

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^{99m}Tc-tetrofosmin indicating that lipophilicity is not the only factor involved in the cellular uptake. Moreover, this agent appears to be more associated with the cytosol than with mitochondria. Nevertheless, it is consensual that ^{99m}Tc-tetrofosmin uptake depends on both cell membrane and mitochondrial potentials.

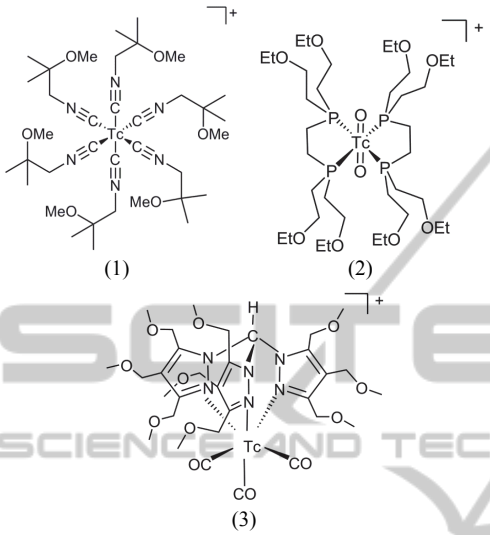

Figure 1: Structures of ^{99m}Tc-sestamibi (1), ^{99m}Tc-tetrofosmin (2) and ^{99m}Tc-TMEOP (3).

For the foreseeable future, myocardial perfusion imaging will continue to be used for assessment of ischaemia. However, both Cardiolite and Myoview present biodistribution properties that suffer from well-known drawbacks, the most important being the high liver uptake, which can interfere in the analysis of cardiac imaging, particularly of the inferior left ventricular wall (Germano, et al., 1994; Kailasnath and Sinusas, 2001; Kapur, et al., 2002; Llaurado, 2001; Parker, 2001). As a consequence, there is now a resurgence and development of both neutral and cationic technetium agents to further improve the characteristics of perfusion radiopharmaceuticals (Hatada et al., 2004); (Kim et al., 2008); (Liu, 2007); (Liu et al., 2010).

Recently we were able to identify a new class of organometallic complexes based on tris(pyrazolyl)methane as lead structure (Garcia et al., 2009); (Santos and Correia, 2005); (Maria et al., 2007). This type of complexes has a cationic character and they are stable both in vitro and in vivo. In particular, we discovered that the tricarbonyl complex ^{99m}Tc-TMEOP (Figure 1) exhibited high heart uptake and biodistribution properties suitable for myocardial imaging (Maria et al., 2009); (Goethals et al., 2010) (Figure 2).

Data collected so far suggest that the pharmacokinetic profile of ^{99m}Tc-TMEOP may allow high quality imaging early after tracer injection. Biodistribution and cardiac pinhole-gated SPECT imaging studies in rats showed that ^{99m}Tc-TMEOP has a cardiac uptake comparable to 99mTc-sestamibi and ^{99m}Tc-tetrofosmin, but has a significantly faster liver clearance (Goethals, et al., 2010). At 40 min post injection, the heart/liver ratio of ^{99m}Tc-TMEOP is twice that of ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin (6.98±1.66, 2.48±0.30 and 2.66±0.40, respectively). Altogther, the data collected so far suggest that the pharmacokinetic profile of ^{99m}Tc-TMEOP may allow high quality imaging early after tracer injection.

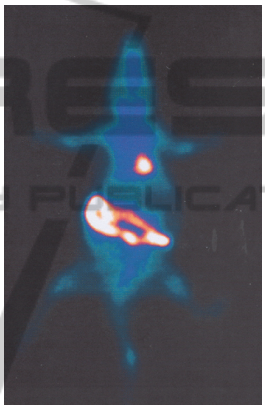

Figure 2: Planar image of a rat administered with ^{99m}Tc-TMEOP, at 60 min pi. Image was performed in supine position and acquired using a 128×128 matrix in a GE gamma camera. Adapted from (Mendes et al., 2012).

Therefore, to get a better insight on the in vivo behaviour of ^{99m}Tc-TMEOP, its mechanisms of myocardial uptake and excretion have been investigated. Our results indicate that the heart uptake of ^{99m}Tc-TMEOP is related to its accumulation in the mitochondria due to the negative plasma and mitochondrial transmembrane potentials (Mendes et al., 2012).

It is well know that cancer cells and tumours also maintain a more negative potential owing to increased metabolic requirements, and as a result, there is an increased accumulation of ^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin and ^{99m}Tc-TMEOP in malignant tumours. This feature permits the use of these radiotracers for imaging cancers of the breast, lung, brain and parathyroid adenomas (reviewed in Mendes et al., 2011).

Despite all the success of perfusion imaging, a unique strength of nuclear imaging is its ability to provide tools for imaging biochemical and metabolic

processes and receptor and transporter functions at molecular and cellular levels in intact organisms under a wide variety of physiologic conditions.

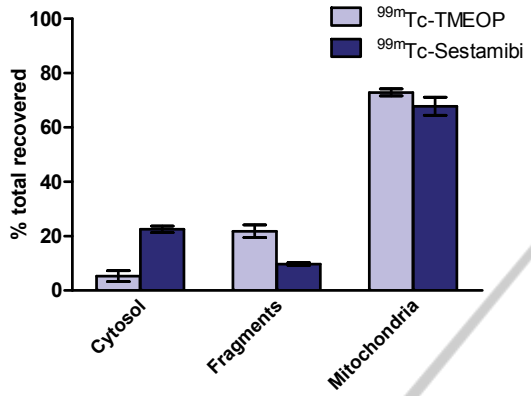

Figure 3: Subcellular distribution of ^{99m}Tc-TMEOP and ^{99m}Tc-Sestamibi in isolated rat's heart tissue. Adapted from (Mendes et al., 2012).

2 CARDIAC MOLECULAR IMAGING

Molecular imaging studies are shedding important light on the cellular and molecular biology underlying important cardiovascular diseases (Osborn and Jaffer, 2012). Therefore within the field of cardiovascular medicine, potential applications of molecular imaging include the analysis of vulnerable plaques, heart failure, neurohormonal dysfunction, myocardial metabolism, stem cell engraftment, protein–protein interactions, and angiogenesis. (Table 1).

Myocardial Pathology. Metabolic adaptation probably represents one of the earliest responses to myocardial ischemia. The application of a metabolic radiotracer, as opposed to a perfusion tracer, potentially extends the time window for noninvasive imaging of an ischemic event beyond the resolution of symptoms. Targeting intracellular metabolic processes could expand our ability to diagnose and treat subclinical or progressive cardiovascular disorders that often remain elusive with traditional imaging approaches. These therapeutic strategies in turn create a demand for accurate, sensitive, and physiological evaluation of therapeutic effects.

The autonomic nervous system plays an important role in many cardiac functions, including cardiac rhythm, conduction, and repolarization.

Several specific neurotransmitters interact with receptors on pre- and post-synaptic binding sites regulating the complex system of the heart. Abnormalities in this interaction result in a variety of cardioneuropathies.

Receptor imaging can be helpful for prognosticating patients with heart failure, diabetes, ischemic heart disease, heart transplantation, drug-induced cardiotoxicity, and dysautonomias.

Table 1: Examples of targets for molecular imaging of different cardiac pathologic events.

Pathological/Biological Process	Cellular/Molecular Targets
Ischemia / myocardial damage	Renin-angiotensin system Chemokine receptor VEGFR receptor
Metabolic imaging	Fatty acids metabolism Glucose metabolism
Cardiac neuronal imaging	Pre-/Post-synaptic – sympathic and parasympathic innervation Receptors / Channels
Acute myocardial infarct - Acute Necrosis	Disrupted myocytes Calcium rich-areas Myosin
Atherosclerosis - vulnerable plaques	Apoptosis Inflammation Adhesion Lipoproteins Angiogenesis

Vascular Pathology. The diagnosis of vulnerable plaques remains an elusive goal in clinical medicine. The most widely accepted features of vulnerable plaques, such as large lipid core, increased inflammatory milieu and thin fibrous caps, have been well characterized through pathological studies. The ability to image a vulnerable plaque in susceptible patients should theoretically result in useful prognostic information that can be used to either monitor or treat patients at risk more aggressively.

The relatively poor correlation between risk of plaque rupture and the degree of luminal obstruction exposes the crucial need for in vivo detection of the processes underlying progressive plaque destabilization.

In addition to the morphologic characteristics, apoptosis and inflammation are two other important indicators of plaque instability. Apoptotic macrophage death results in enlargement of the plaque necrotic core and positive vascular remodeling, whereas apoptosis of the smooth muscle cells leads to attenuation of the fibrous cap.

Finally, angiogenesis is defined as the formation of new capillaries by cellular outgrowth from existing microvessels. It plays a crucial role in the response to ischemia that is associated with

peripheral arterial disease and myocardial infarction. Imaging angiogenesis would therefore be valuable in assessing risk stratification of patients with arterial occlusive disease.

Selected Examples of Molecular Imaging Probes. The biodistribution of molecular imaging probes is determined by specific interactions between the radioactive molecule and its target, which can be for example antigen, enzymatic or receptor-binding. Therefore the probe should present a high affinity to its target, and also a high specificity, resulting in its selective uptake and distribution at the target tissues.

Different types of biomolecules and radionuclides, both metallic and non-metallic, have been explored in nuclear imaging.

Within the field of cardiac molecular imaging fluorine-18 fluorodeoxyglucose (^{18}F-FDG) is the most widely used agent (Figure 4).

^{18}F-FDG is a radiolabeled glucose analogue transported into metabolically active cells, and therefore it is an ideal agent for the assessment of viable myocardium.

Moreover, ^{18}F-FDG presence is also correlated with plaque macrophage content, and, therefore, could be used as a surrogate reporter of this critical cell involved in atherogenesis and plaque rupture (Osborn, Jaffer, 2012).

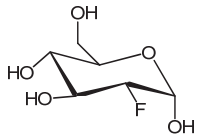

Figure 4: Structure of ^{18}F-FDG.

The renin-angiotensin system (RAS) plays an important role in regulating blood volume, arterial pressure, cardiac and vascular function, and may contribute to the pathogenesis of atherosclerosis. The renin-angiotensin system is frequently activated early in heart failure and is linked to left ventricular remodeling and myocardial fibrosis.

A comprehensive in vivo approach to the study of the RAS and its many components has been made difficult by the complexity of the system. However, this system has, at the same time, provided a number of targets for nuclear imaging via radiolabeled ligands, with special emphasis on the angiotensin-converting enzyme (ACE).

The initial attempts at developing specific ACE-binding radiotracers were made by use of ^{18}F–labeled captopril, the first clinically available ACE inhibitor. In normal rats, in vivo biodistribution at 30 minutes after injection revealed high uptake values in the lungs, kidneys, and aorta, organs with known high concentrations of ACE. This agent, however, had a number of shortcomings that reduced its potential as a suitable tracer for examining ACE distribution, as it is believed to have a higher affinity for vascular ACE than for tissue ACE and, thus, to be less suited for examination of tissue-bound ACE activity.

Another ^{18}F-labeled ACE inhibitor, lisinopril, showed higher affinity for tissue ACE and allowed higher resolution during in vitro autoradiography when compared with ^{18}F-labeled captopril. ^{99m}Tc-labeled lisinopril derivatives have been also developed (Femia, et al., 2008) and recently it has been shown that ^{99m} Tc-lisinopril localizes in the heart of transgenic rats that over-express human ACE-1 (Dilsizian, et al., 2012). The combination of these studies has shown the feasibility of in vivo imaging of ACE both by PET(^{18}F) and SPECT (^{99m}Tc).

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