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IMAGING EVALUATION OF CARDIAC STEM CELLS TRANSPLANTATION

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SUMMARY

Cardiovascular Disease is the number one cause of death in the World. Although advances in treatment and intervention have reduced the mortality rate in patients with coronary artery disease, the number of patients with refractory myocardial ischemia and congestive heart failure is rapidly increasing. "Cellular therapy" represents an important new approach in the treatment of cardiovascular disease and the scientific community needs to establish not only the appropriate type and timing of cellular administration but also the diagnostic gold standard to better enable the study of subjects before and after cellular therapy.

Introduction

According to the World Health Organization, Cardiovascular Diseases (CVDs) are the number one cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke. By 2030, almost 23.6 million people will die from CVDs and they are also predicted to remain the single leading causes of death [1]. Thanks to advances in medical treatment and interventional procedures, the mortality rate in patients with coronary artery disease has reduced considerably; however, the number of patients with refractory myocardial ischemia and congestive heart failure is rapidly and constantly increasing. It is important for patient care that diagnostic and interventional radiologists are actively involved in the development of these therapies; both at the bench, and at the bedside through clinical studies. Specifically, the diagnostic radiologist has to become an expert in the imaging, tracking, and monitoring of stem cells and in the assessment of engraftment efficiency, whereas the interventional radiologist is already an expert in targeted stem cell delivery by means of different routes (percutaneous, selective intravenous, or intraarterial). The diagnostic radiologist should continue to develop and refine imaging techniques to study stem cell tracking and engraftment, cellular migrations, and their effect on organ functionality. In pursuit of these objectives, both the diagnostic radiologist and the interventional radiologist will be firmly integrated in this promising and still developing field of medicine and become valuable partners for basic science researchers and clinicians alike [2].

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The characteristics of an ideal imaging technology for stem cell tracking during clinical trials are as follows [3]:
1. is biocompatible, safe, and nontoxic
2. causes no genetic modification or perturbation to the stem cell: Several imaging techniques, such as enzymatic conversion of an injected substrate and receptor-based binding, require stable integration of transgenes. This strategy may be combined with genetic manipulation of stem cell populations to enhance the viability, differentiation, and coupling of these cells with the myocardium
3. allows quantification of cell number
4. detects single-cells in any anatomic location
5. allows for minimal or no transfer of contrast agent to non-stem cells
6. allows for minimal or no dilution with cell division
7. does not require an injectable contrast agent

As clinical trials undoubtedly will require long-term follow-up of tissue function or host survival, the ideal imaging technology would also permit the tracking of injected stem cells from months up to years after injection [3]. Imaging approaches could, not only improve the understanding of therapeutic mechanisms in preclinical studies, but may also have direct clinical applications. To summarize, the "ideal imaging technology" should give information regarding the correct amount and best kind of cells to use as well as the timing of administration. Presently the most commonly used imaging technologies are:
1.Two-dimensional and three dimensional echocardiography
2.Magnetic resonance imaging (MRI)
3.Direct labeling of cells using radionuclides.

Two-dimensional and three dimensional echocardiography

Two-dimensional echocardiography provides an inexpensive, clinically accepted method for evaluating cardiac function without ionizing radiation; however, this method has limited imaging windows and cannot provide three-dimensional imaging, which can be important in assessing left ventricular remodeling after infarction. Three-dimensional echocardiography can instead provide a more complete anatomy compared to traditional transthoracic echocardiography; however, this is more invasive and requires patient sedation. [4] Both two-dimensional and three dimensional echocardiography can only provide indirect information regarding cell homing and distribution (Figure 1a and 1b).

Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging can provide detailed morphological and functional information and, therefore, seems ideally suited to the integration of efficacy assessments with the capability of cell tracking [5]. Currently two kinds of contrast medium can be used; they are:
1.Superparamagnetic iron oxide (SPIO) contrast agents
2. Gadolinium-based contrast agents

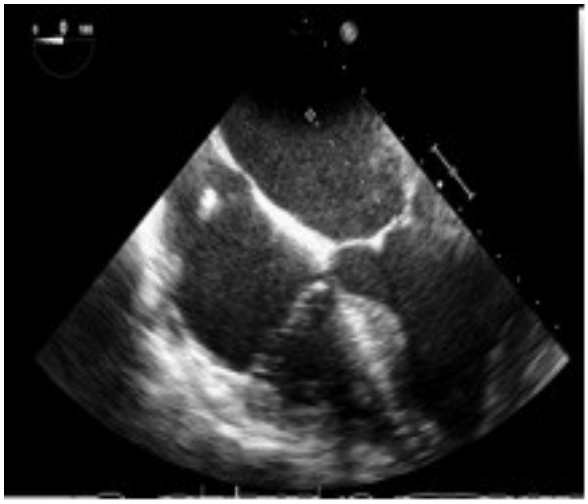


Figure 1a: Example of transesophageal echocardiographic 2D four chamber projection of an infarcted myocardium.

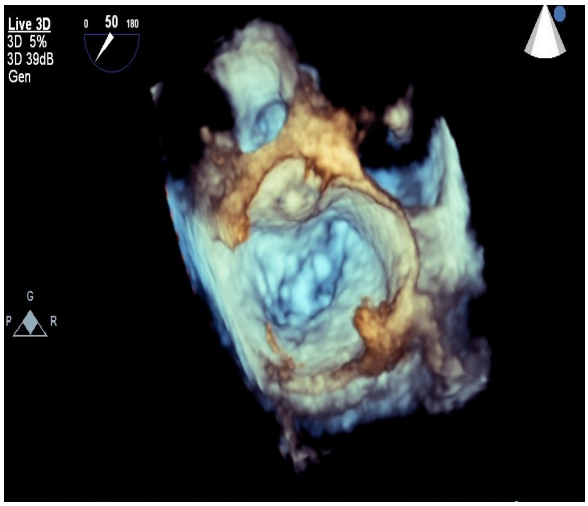


Figure 1b: Transesophageal echocardiographic 3D image.

Although there are several MRI cellular labeling methods, direct cellular labeling with superparamagnetic iron oxide (SPIO) contrast agents has been mostly widely used for many reasons [6]. Cells can be labeled directly with super-paramagnetic iron oxide agents or radionuclides before their application, for subsequent in vivo visualization of their distribution. The use of non-invasive imaging modalities in preclinical cell therapy studies reveals key aspects of cell biology that are not observed through other approaches except for histological analysis. In particular, the possibilty of following cell trafficking and survival dynamically over longer periods of time has contributed to the understanding of the potential mechanism of benefit.

MRI can offer information regarding cell homing and distribution, molecular effects, structural tissue and organ remodeling, left ventricular regional and global function, perfusion and metabolism. Furthermore, MRI offers two important advantages: high spatial resolution and no radiation. However, on the other hand, the important disadvantages are low sensitivity and the fact that the signal may not reflect viable cells (Figure 2). The potential for assessing engraftment of therapeutic cells was quickly understood, and investigations are now focusing on refining contrast agents to ensure maximum signal for minimum labeling.

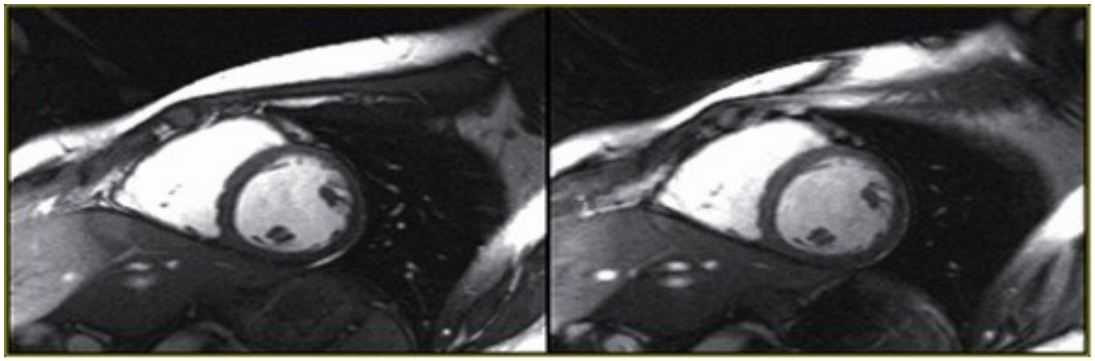
There are potential theoretical disadvantages to the use of magnetic labeling. Most importantly, the imaging signal is not directly linked to cell viability. There is a risk of the release of iron oxide after cell death and its accumulation in bystander cells, confounding any quantitative assessment of cell trafficking. In addition, cell division can dilute the magnetic label within only a few cell divisions [5].

Several clinically approved formulations of SPIO-based contrast agents are available and have been used for cell labeling in a variety of diseases. Toxicity of these agents is low, since the SPIO nanoparticles that are released from dying cells can be degraded in the normal iron recycling pathways [7]. Compared to gadolinium-based contrast agents, SPIOs become more effective upon cell internalization due to particle clustering and, thereby, create large “blooming” hypointensities on standard clinical MRI scanners. There are two common methods used for cell internalization, they are: Magnetofection and Magnetoelectroporation [7]. The MR contrast agent gadolinium has proved extremely safe in tens of millions of patients. Recently, however, nephrogenic sclerosing fibrosis has been reported as a possible adverse effect of the use of high doses in patients with severe renal impairment with glomerular filtration rate < 30 mL/min. The risk appears extremely low, but many centers now use gadolinium tightly bound to a cyclic chelate, for which the incidence of nephrogenic sclerosing fibrosis is near zero. MR with cyclic gadolinium is still used in renal failure because of the significant dangers of iodinated contrast agents [8].

Direct labeling of cells using radionuclides

Direct labeling of cells with radionuclides provides the advantage of a lower background signal when compared with MRI, but at the same time it is important to mention that higher sensitivity is achieved at the cost of lower spatial resolution. Various clinically applicable radionuclides have been used, based on previously es-

Figure 2: Magnetic Resonance images: Examples of images obtained in short axis fiesta. Observe the good contrast between epicardial and endocardial contours.



tablished protocols for leukocyte or thrombocyte scintigraphy.

Direct labeling with radionuclides provides information about addressing homing and biodistribution after cell injection and also about perfusion and tissue metabolism. Radiolabeling of cells has already been used for cell tracking in many experimental and several clinical applications. Indium oxine and 99mTc HMPAO have been used in conjunction with Single Photon Emission Tomography (SPECT) imaging, and 18FDG with Positron Emission Tomography (PET) for tracking bone marrow derived or endothelial progenitor cells delivered to the myocardium intravenously or via the coronary arteries [9]. Both SPECT and PET can be used for tracking radiolabeled stem cells. SPECT is widely available, simple and requires tracers that are applied in everyday clinical practice. However, there are problems with acquiring accurate quantitative data, mainly due to errors derived from photon scattering (Figure 3a and 3b). Research using SPECT is conducted at the University of San Raffaele in Milan. They are looking at the effects of direct intramyocardial injection of autologous bone marrow cells (CD34+ selected cells versus all mononuclear cells) in patients with chronic myocardial ischemia with a mapping system named NOGA. Preliminary results have showen that an improvement in symptoms, in the first 6 months, appears to be achieved in approximately 50% of patients. Additionally, these first results have demonstrated an improvement of quantitative scintigraphic stress test imaging; however, this study lacks information about the homing mechanism of injected stem cells and about their microenvironment [9]. PET results are more sensitive, have higher spatial resolution and quantification is more straightforward and clinically applicable. Unfortunately, PET scanners are more expensive, not widely available and usually require an on-site or cyclotron nearby (for production of the necessary tracers) [10].

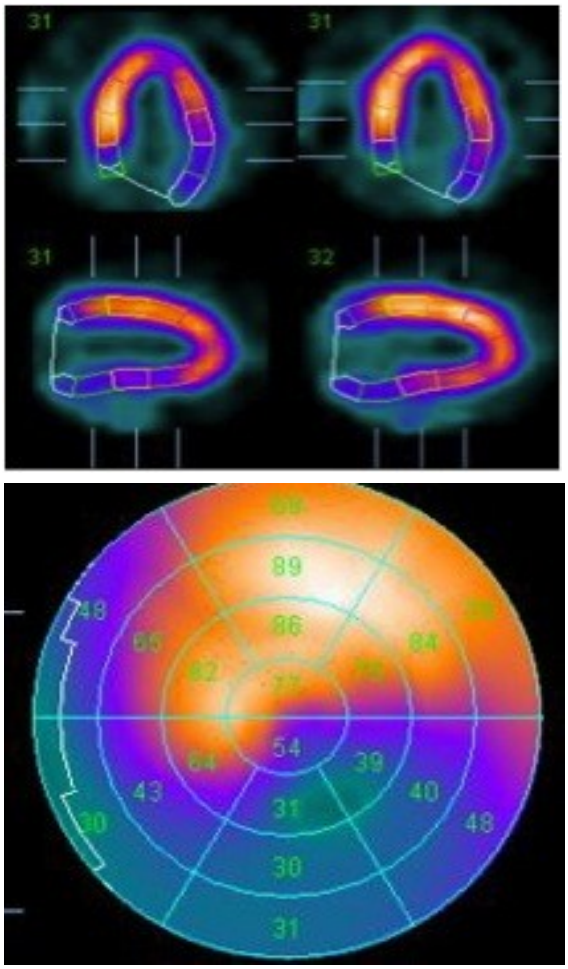


Figure 3a (upper) and 3b (lower): SPECT images: Note the presence of an extended myocardial ischemia.

Conclusions

MRI evaluation of stem cell transplantation results is biocompatible, non-invasive, safe and nontoxic and it does not require genetic manipulation. All of these characteristics are common to three dimensional echocardiography but the first of them can provide a more detailed morphological analysis and can offer information about perfusion and metabolism. Direct labeling of cells with radionuclides provides the advantage of a lower background signal if compared with MRI but at the same time higher sensitivity is achieved at the cost of lower spatial resolution. We can conclude that this field of research offers new hope for subjects with chronic heart disease and that collaboration with radiologists is essential to its advancement.

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