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Imaging and 1-day kinetics of intracoronary stem cell transplantation in patients with idiopathic dilated cardiomyopathy

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ABSTRACT

Background: Stem cell transplantation is an emerging method of treatment for patients with cardiovascular disease. There are few studies completed or ongoing on stem cell therapy in patients with idiopathic dilated cardiomyopathy (IDCM). Information on stem cell homing and distribution in the myocardium after transplantation might provide important insight into effectiveness of transplantation procedure.
Aim: To assess early engraftment, retention and migration of intracoronarily transplanted stem cells in the myocardium of patients with advanced dilated cardiomyopathy of non-ischaemic origin using stem cell labeling with ^{99m}Tc-exametazime (HMPAO).
Materials, methods: Thirty-five patients with IDCM and advanced heart failure were included in the study. Autologous hematopoietic (CD34+) stem cells were harvested by peripheral blood apheresis after bone marrow stimulation, labeled with ^{99m}Tc-HMPAO, tested for viability and injected into coronary vessel supplying areas of myocardium selected by myocardial perfusion scintigraphy as dysfunctional yet viable. Imaging was performed 1 h and 18 h after transplantation.
Results: Myocardial stem cell retention ranged from 0 to 1.44% on early and 0–0.97% on delayed imaging. Significant efflux of stem cells occurred from site of delivery in this time period ($p < 0.001$). Stem cell viability was not affected by labeling.
Conclusion: Stem cell labeling with ^{99m}Tc-HMPAO is a feasible method for stem cell tracking after transplantation in patients with IDCM.

1. Introduction

Stem cell transplantation is an emerging method of treatment for patients with cardiovascular disease, resulting in improvement of cardiac haemodynamics and functional capacity [1]. While clinical research is predominantly concentrated on ischaemic heart disease in its acute and chronic presentation, there are few studies completed or ongoing in advanced heart failure of non-ischaemic etiology [2–5].

Stem cell engraftment, retention, distribution and differentiation are the likely important events that determine the success of treatment [6]. Assessment of long-term fate of transplanted stem cells requires use of imaging techniques involving genetic manipulation which are unlikely to be used in human studies due to ethical restraints [7]. However, it is possible to evaluate the important early events of stem cell engraftment, retention and distribution in the heart after transplantation using established methods of cell labeling [7,8]. ^{99m}Tc-hexamethyl-propyleneamine-oxyme (HMPAO) is a well established cell label, used primarily for localization of infectious/inflammatory processes. Half-life of the radionuclide allows for tracking of labeled cells within 24 h of labeling. To date, there are no published studies using labeled stem cell imaging for assessment of cardiac stem cell transplantation in patients with non-ischaemic cardiomyopathy.

The aim of this study was to assess early engraftment, retention and migration of intracoronarily transplanted stem cells in the myocardium of patients with advanced dilated cardiomyopathy of non-ischaemic origin.

2. Material, Methods

2.1. Patients

We included 35 patients with (Table 1) depressed left ventricular ejection fraction on echocardiography (<30%; standard Simpson technique)

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and who had been in NYHA class III or IV for at least 6 months on optimal medical therapy. Dilated cardiomyopathy was defined based on the absence of any stenotic lesions on coronary angiography, no primary valve disease on echocardiography, and no history of hypertension or alcohol abuse, according to the European Society of Cardiology position statement.

2.2. Myocardial perfusion scintigraphy

Myocardial perfusion scintigraphy in resting state was performed using viability protocol. Prior to radiopharmaceutical injection, 2 puffs (0.4 μg) of nitroglycerin were applied sublingually and blood pressure and heart rate was monitored. After 10 min, 600 MBq of ^{99m}Tc-sestaMIBI was injected intravenously.

Dual-detector single-photon emission computed tomography (SPECT) was performed 1 h pi., using ECG-gating, 64 × 64 matrix, 36 projections, 25 s per frame (GE Millenium dual-head gamma camera, GE Corporation, USA). Myocardial perfusion was quantified using 20-segment model, normalized to maximum uptake in the myocardium.

2.3. Stem cell mobilization and collection

Patients received bone marrow stimulation using G-CSF after testing for appropriate bone marrow response (single test dose of G-CSF, transient increase in absolute neutrophil count > = 50%). Peripheral blood stem cells were mobilized by daily subcutaneous injections of G-CSF (5 μg/kg bid). On the fifth day a full blood count and peripheral blood CD34 + cell count were performed. Peripheral blood stem cells were then collected on the same day with the Amicus cell separator (Baxter Healthcare, IL, USA). The magnetic cell separator Isolex 300i (Nexell Therapeutics Inc., Irvine, CA, USA) was used for the immunomagnetic positive selection of CD34 + cells. In the closed system the collected cells were washed to remove the platelets, sensitized with mouse monoclonal anti-CD34 antibodies and then incubated with immunomagnetic beads coated with polyclonal sheep anti-mouse antibodies (Dynabeads-Dynal AS, Oslo, Norway). The bead/CD34 + cell rosettes were separated in the magnetic field from other cells and CD34 + cells were released from the Dynabeads using an octapeptide with an affinity for anti-CD34 antibodies. The remaining CD34 + cells were free of surface contaminants and the CD34 cell surface antigen was left intact. Final infusate was concentrated to a volume of 100 ml.

2.4. Stem cell labeling, viability and labeling stability assessment

2.4.1. Stem cell labeling

Predefined volume (20%) of collected CD34 + cells was radiolabeled with 600 MBq of ^{99m}Tc-exametazime (HMPAO) and incubated for 20 min at room temperature. Unbound radioactivity was removed by washing the cells with cell free media. Labeled stem cells were resuspended with the remaining infusate.

2.4.2. Stem cell viability, labeling efficiency and stability assessment

Viability of labeled stem cells was assessed by Trypan Blue exclusion assay 1 h after labeling at the time of intracoronary injection. A small proportion of unlabeled stem cells was used for viability assessment at identical time-points for comparison.

2.5. Stem cell transplantation/injection

Using the standard right femoral approach a microcatheter (Progreat Microcatheter System, Terumo, Leuven, Belgium) was positioned in a mid portion of the target coronary artery, and repeated injections of stem cell solution were performed. Before the procedure patient was fully heparinized. To avoid trauma of the target vessel we performed no balloon inflations at any time during the procedure. Target vessel was determined by myocardial perfusion scintigraphy as one supplying areas of viable yet dysfunctional myocardium (segments of tracer accumulation of > = 50% of maximal activity in the myocardium and regional dysfunction). In patients with diffusely inhomogenous tracer distribution and regional dysfunction LAD was selected for stem cell injection as a vessel supplying the largest proportion of myocardium.

2.6. Stem cell transplantation imaging and quantification

1 h after transplantation, stem cell imaging was undertaken to assess myocardial engraftment and distribution. Planar anterior/posterior and LAO/RPO projections of the thorax and upper abdomen (128 × 128 matrix, 15 min per projection) and tomographic imaging of cardiac region (64 × 64 matrix, 36 projections, 25 s per frame) were performed on a dual – head gamma camera (GE Millenium, GE Corporation, USA). After 18 h, planar imaging was repeated to detect potential stem cell migration.

Quantification of stem cell retention was performed from anterior and posterior planar images (at 1 h and 18 h post injection) using mirrored regions of interest (ROI) placed over areas of stem cell accumulation after background correction (small periventricular ROI). Whole – organ ROIs were used for quantification of activity in the liver and spleen. Round ROI, normalized at 100 pixels was used for quantification of activity in the lung parencyhma and rectangular ROI, normalized at 200 pixels and placed at the mediastinum (i.e., representing activity in the sternum and vertebral column) was used for quantification of

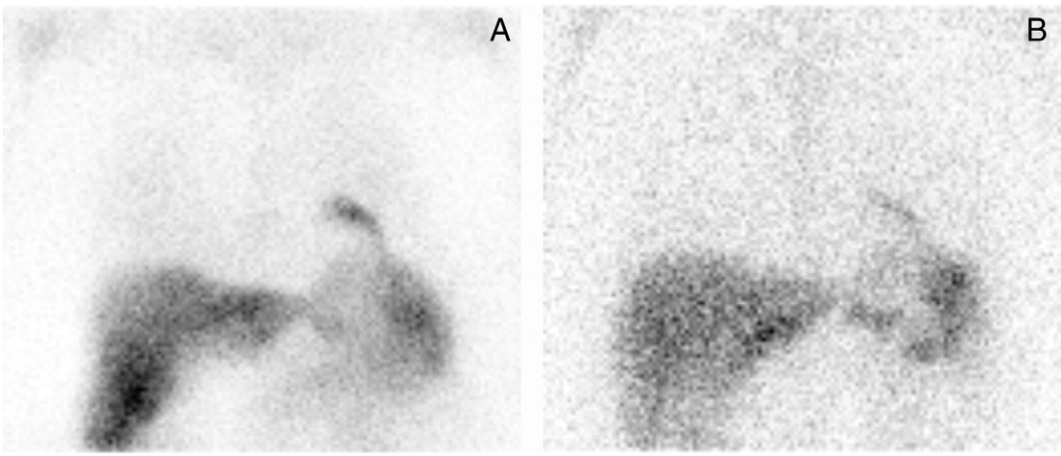

Fig. 1. Anterior planar image of intracoronary stem cell transplantation at 1 h (panel A) and 18 h (panel B) post injection. Target vessel for stem cell delivery was left anterior descending artery. Transplanted stem cells are distributed in the anterior myocardial wall. At delayed imaging, stem cell distribution does not appear to change.