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The effect of nonuniform magnetic targeting of intracoronary-delivering mesenchymal stem cells on coronary embolisation

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ABSTRACT

Magnetic targeting has been recently introduced to enhance cell retention in animals with acute myocardial infarction. However, it is unclear whether the magnetic accumulation of intravascular cells increases the risk of coronary embolism. Upon finite element analysis, we found that the permanent magnetic field was nonuniform, manifestated as attenuation along the vertical axis and polarisation along the horizontal axis. In the *in vitro* experiments, iron-labelled mesenchymal stem cells (MSCs) were accumulated in layers predominantly at the edge of the magnet. In an ischaemic rat model subjected to intracavitary MSCs injection, magnetic targeting induced unfavourable vascular embolisation and an inhomogeneous distribution of the donor cells, which prevented the enhanced cell retention from translating into additional functional benefit. These potential complications of magnetic targeting should be thoroughly investigated and overcome before clinical application.

1. Introduction

Stem cell transplantation is a promising therapeutic strategy for acute myocardial infarction and heart failure. Intracoronary injection (IC) permits relatively homogeneous dissemination of donor cells to the target area in a more physiological manner with less myocardial injury than an intramyocardial injection [1]. IC has become the most commonly used approach in clinical studies, particularly when small cells, such as bone marrow mononuclear cells (MNCs), are used. However, a significant disadvantage of IC cell injection is the risk of myocardial damage resulting from coronary embolism [2,3], and this risk is most important if large cells are injected, particularly, into diseased, narrowed coronary arteries.

Magnetic targeting strategies, traditionally used in chemotherapy for tumours [4], have been introduced in recent years to localise magnetic nanoparticle-loaded cell delivery for targeting lesions *in vivo* [5–11]. The accumulation and retention of the magnetic responsive cells can be enhanced by focussing an external magnet on the area of interesting [12]. We previously demonstrated in an *in vitro* study that the cell capture efficiency reached 89.3% in a deep magnetic field with a magnetic flux density of 640 mT, a magnetic intensity gradient of 38.4 T/m, and a flow velocity of 0.8 mm/s [13]. The potency of magnetically enhanced cell accumulation was also recently confirmed in animal studies with intracoronary cell delivery, resulting in enhanced cell retention by 5–10 fold [14,15].

Considering the powerful attraction of the magnetic field, however, we wonder whether the magnetic accumulation of cells in the vascular lumen increases the risk of coronary embolism when intracoronary injections are given, especially when relatively

Abbreviations: IC, intracoronary injection; MagMSCs, magnetically-loaded mesenchymal stem cells; SPIO, superparamagnetic oxide nanoparticles; MSCs, mesenchymal stem cells; MNCs, mononuclear cells; NdFeB, neodymium–iron–boron; PLL, poly-L-lysine; B, magnetic field density; I/R, ischemia/reperfusion; MR, magnetic resonance; CE, capture efficiency; SRY, sex-determining region Y gene; $\partial B/\partial r$, magnetic field gradient in r direction; F_r, magnetic force on cells in r direction.

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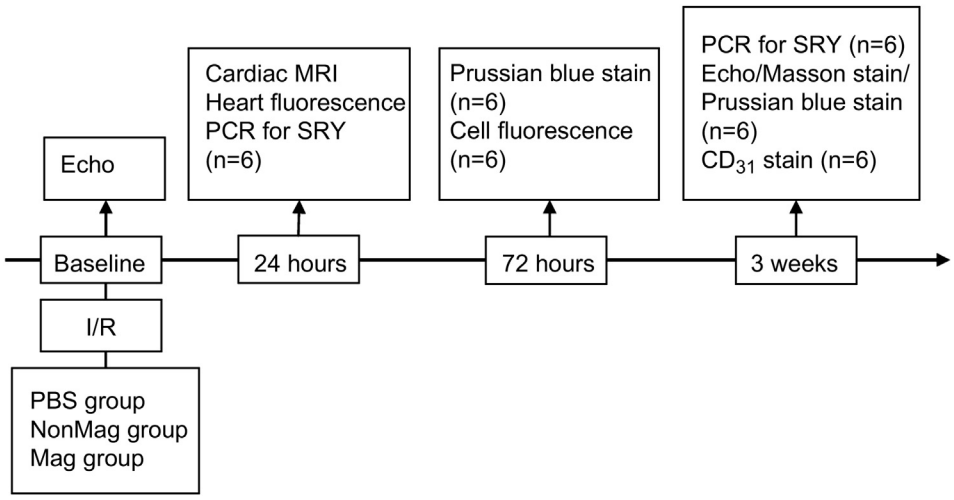

Fig. 1. Study protocol. Echo = echocardiography; I/R: Ischemia/Reperfusion; SRY: Sex-determining Region Y gene.

large cells, such as mesenchymal stem cells (MSCs; approximately 20–25 μm in diameter), were used. Based on an analysis of characteristics of a magnet cylinder and the accumulation of magnetically labelled MSCs in both static and flowing state, we explored the safety and effectiveness of magnetically targeting intracoronary-delivering bone marrow derived MSCs in a rat model of ischemia/reperfusion(I/R).

2. Material and methods

2.1. Magnetic material

2.1.1. Resovist

Resovist (SHU555A; Schering, Berlin, Germany) was a kind of superparamagnetic oxide nanoparticles (SPIO) (magnetite – Fe_3O_4/maghemite – γFe_2O_3) coated with carboxydextran and an overall hydrodynamic diameter of 62 nm as measured with photon-correlation spectroscopy. It has good stability and good magnetism [16,17]. The polycrystalline iron oxide core consists of multiple single crystals, each 4.2 nm in diameter as measured with electron microscopy. Resovist contains 0.5 mmol/L of iron per liter, including 40 mg/mL mannitol and 2 mg/mL of lactatic acid, adjusted to a pH of 6.5 at 37 °C. The solution has an osmolality of 0.319 osmol/kg H_2O and a viscosity of 1.031 MPas.

2.1.2. Permanent magnet cylinder

A Neodymium-iron-boron (NdFeB) permanent magnet cylinder with a diameter of 8 mm (Shanghai yahao Interument Equipment Co., China) was used in this study. The magnetic flux density (*B*) of the magnet surface is up to 600 mT measured using a model 51,662 Leybold T m. The distribution of the magnetic flux density was calculated by a finite element analysis.

2.2. Preparation of magnetically labelled cells

2.2.1. Isolation and cultivation of rat MSCs

The animal experiments were approved by the Animal Care and Use Committee of Fudan University and were in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Academy Press (NIH Publication No. 85-23, revised 1996).

Bone marrow MSCs were isolated from 4-week-old male Sprague–Dawley (SD) rats weighing 100–120 g as described before [13,18]. All cells that were used were harvested with 0.25% trypsin when they reached 80–90% confluence at passage 4 for the subsequent experiments.

2.2.2. Preparation of fluorescently and magnetically labelled cells (Mag-Dir-MSCs)

MSCs were cultured in media containing SPIO and poly-L-lysine (PLL, 0.15 mg/mL) for 24 h, with the concentration of iron 50 mg/mL and PLL 0.15 mg/mL [13]. The magnetic SPIO-labelled MSCs (MagMSCs) were then incubated with 1 μmol/L ethyl indotricarbocyanine iodide (Dir; ABD Bioquest, Inc.) for 20 min at 37 °C according to the manufacturer's protocol.

Prussian blue staining was used to indicate the presence of iron within the Mag-Dir-MSCs, and transmission electron microscopy (Pilips CM120) was used to evaluate the presence and localisation of intracellular iron particles, as well as the structural changes that resulted from the labelling procedures. The iron content in the cells at different time after labelling was quantified by using atomic absorption spectrometry (Thermo E.IRIS Duo ICP). Inverted microscopy was used to examine the staining efficacy of the Dir dye. The diameter of cells was measured in suspensions using a Scepter 2.0 Handheld Cell Counter, with 50 pk of 60 μM Sensors (Millipore; Billerica, MA, USA).

2.3. Magnetically guided distribution of MagMSCs in vitro

2.3.1. In vitro magnetic attraction of static MagMSCs

The MagMSCs suspension, at a concentration of 5×10^4 cells/mL, was replaced in a culture plate that was 34.8 mm in diameter and had a bottom thickness of 1.3 mm. The cell cultures, with or without an NdFeB magnet cylinder beneath the bottom glass, were kept for 24 h with 5% CO_2 at 37 °C. The MagMSC distributions were observed by inverted microscopy and cellular magnetic resonance (MR) imaging. Cellular MR imaging was performed use a 1.5-T clinical MR scanner (CV/i, GE Medical Systems) applying a T_2WI-flash sequence. The imaging parameters were as follows: repetition time (TR) = 800 ms; echo delay time (TE) = 26 ms, flip angle = 25°; 256 × 160 matrix and slice thickness = 2 mm, with no gap and a 90-cm^2 field of view (FOV).

2.3.2. In vitro magnetic capture of flowing MagMSCs

A total of 20 mL MagMSCs suspension, at a concentration of 5×10^4 cells/mL, was placed in a 50 mL syringe and flowed through a quartz tube (ID 2.3 mm, OD 4.3 mm, length 20 mm). This tube was positioned vertically, and the aforementioned magnet cylinder was placed tightly at the mid-segment of the tube. The flow velocity was set at 1 mm/s, 10 mm/s, 30 mm/s and 50 mm/s, controlled by a syringe pump. The capture efficiency (CE) was calculated as previously described [13]. All experiments were performed in triplicate for each condition.

To roughly analyse the distribution of MagMSCs in the tube, the middle part of the tube that was influenced by the magnetic force was imaged digitally and analysed using NIH ImageJ software 1.37v (NIH, Bethesda, Md). Briefly, the image was inverted and transformed into a grey picture, and then the signal intensity distribution along the axis of the tube was presented as a plot profile. Signal intensity was calculated as the actual signal intensity in position of interest minus the noise signal intensity in remote position 2 cm distant from the magnet.

2.4. Magnetic targeting of MagMSCs in rats with I/R

2.4.1. Animal model, cell infusion and magnetic targeting

Fig. 1 summarised the diagnostic and surgical steps of animal experiments. A I/R model was developed in female SD rats (150–200 g). Rats underwent left thoracotomy in the 4th intercostal space under general anaesthesia. The heart was exposed and a myocardial infarction was produced by ligation of the left anterior descending coronary artery (LAD) for 90 min, using a 6-0 silk suture. After that, the suture was released to allow coronary reperfusion.

A total of 108 survival rats were randomly divided into three treatment groups. The Mag group received 1×10^6 Mag-Dir-MSCs with magnetic guidance ($n = 36$). The NonMag group received 1×10^6 Mag-Dir-MSCs without magnetic guidance ($n = 36$), and the PBS group received PBS alone ($n = 36$). In the Mag group, 20 min after the establishment of I/R model, 1×10^6 Mag-Dir-MSCs resuspended in 1 mL PBS were infused into the left ventricle cavity with 5 s of temporary aorta and pulmonary artery occlusion, as previously described [19,20]. Intracoronary delivery was