In vivo and In Vitro MR Imaging of Magnetically Labelled Human Embryonic Stem Cells

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Introduction

Human embryonic stem cells have emerged as a potentially new therapeutic approach for treatment of heart and other diseases applying the concept of regenerative medicine. A method for *in vivo* visualisation and tracking of transplanted hES would increase our understanding of *in vivo* hES behaviour in both experimental and clinical settings. The aim of this study was to evaluate the feasibility of magnetic labelling and visualisation of hES with magnetic resonance imaging both in vitro and in vivo.

Subjects and methods

Human ES were established and expanded according to standard procedures. After expansion, the cells were further cultured in feeder-free culture conditions and magnetically labelled by addition of SPIO agent dextran-coated Ferrum-oxide particles (Endorem®, Gothia Medical) to the culture medium. Accumulation of SPIO in hES was assessed by Prussian Blue staining and electron microscopy. Viability, proliferation and differentiation potential of magnetically labeled cells was assessed by trypan-blue and embrionic body formation. For *in vitro* imaging, the labelled and unlabelled hES were placed in a standard 1 cm probe embedded in the agarose gel to reduce the susceptibility effects. The hES (~ 300 000 in 10 μl) were then injected into excised mouse hearts with injection placed within anterior left ventricular (LV) wall. MRI experiments were performed on a Bruker Avance DMX 500 with a standard vertical magnet (52 mm diameter) operating at 11.75 T. A saddled RF coil tuned to 500 MHz for proton was used for transmission and detection. A multi-slice multi spin-echo imaging pulse sequence consisting of 32 echoes and an echo spacing of 6 ms was used to obtain T_2-weighted images. In vivo imaging was performed on male Sprague-Dawely rats. Magnetically labeled hES (~ 300 000 in 50 μl) were injected within anterior LV wall. Cardiac gated gradient echo pulse was used with the following parameters, FOV = 8 cm, matrix = 256 x 192, TR 150 ms, TE 8 ms. In vivo MRI was perforemed 24 h and 5 dayes after the transplantation.

Results

hES appeared to be unaffected by magnetic labelling and maintained their ability to proliferate and differentiatet and in the culture. No agent for membrane permeabilisation was needed for facilitation of intracellular SPIO accumulation. Prussian Blue and electron microscopy have revealed numerous iron particles in the cytoplasm of hES. On T_2- weighted images, the labelled cells have shown well-defined hyopintense areas at the site of injection in anterior LV wall both in vitro in the mouse heart (Figure 1) and in vivo in the rat heart (Figure 2.).

Conclusions

It is feasible to magnetically label and visualise hES. MR visualisation of magnetically labelled hES may be a valuable tool for *in vitro* and *in vivo* tracking of hES.

Figure 1.

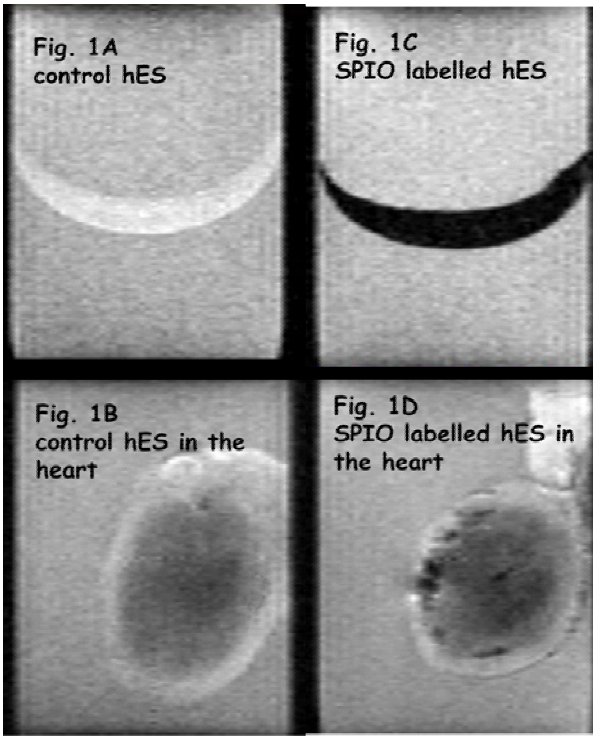

Figure 2.