Detection of Transplanted Stem Cells by Molecular Magnetic Resonance Imaging

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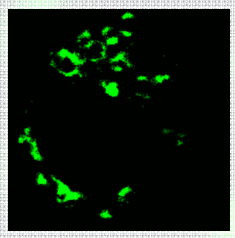
Introduction

Stem cell transplantation is a promising approach for the therapy of various neurodegenerative diseases including Parkinson´s disease (PD). However the mechanisms of differentiation, migration and long term survival of the transplanted stem cells are still not clear. In this study we are magnetically labelling murine embryonic stem cells with iron-oxide particles (VSOP) *in vitro* to make them detectable by molecular Magnetic Resonance Imaging (MRI).

Magnetic Labelling

We used for cellular labeling Very-Small-Superparamagnetic-Iron-Oxide-Particles (VSOP) C200 (Ferropharm). The VSOP consist of an iron-oxide core, coated by monomer citrate giving a total diameter of 9 nm. The particles are internalized by the stem cells via endocytosis (Fig. 1A, B). As shown in Fig. 2 the incubation with iron-oxide-particles (VSOP) led to a significant uptake of iron by the stem cells measured by Atomic Absorption Spectroscopy (AAS). The intracellular iron concentration is increased by the factor of 49 (1.5 mM VSOP). Therefore, the incorporated VSOP-particles are responsible for 98% (1.5 mM VSOP) of the total iron in the cell after incubation.



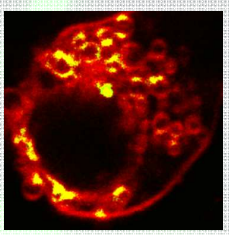


Figure 1
Confocal-Laser-Scanning-Microscopy images of a cluster of stem cells. Cells were incubated with Rhodamine-Green labeled iron-oxide-particles (green) and the membrane marker ANNEPS (red). A green channel, B overlay green and red channel.

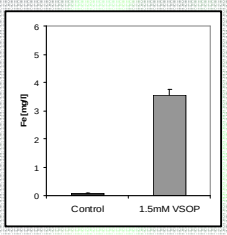


Figure 2
Iron-oxide-particle uptake by embryonic stem cells measured by AAS

Lipofection

To further enhance the uptake of iron-oxide-particles we performed lipfection. We used as transfection reagent FuGENE6 (Roche), which is composed of lipids and other components. The iron-oxide-particles are incorporated into the liposomes formed by FuGENE6 and the complexes are incubated with the cells. Dependend on the incubation time we found a highly significant increase of iron-oxide-particle uptake by embryonic stem cells (Fig. 3 and Fig. 4).

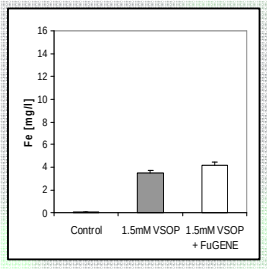


Figure 3
Iron-oxide-particle uptake by embryonic stem cells measured by AAS after 1.5 h incubation

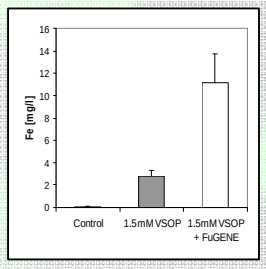


Figure 4
Iron-oxide-particle uptake by embryonic stem cells measured by AAS after 24 h incubation

Iron-Oxide-Particles Cause Transient Oxidative Stress

Up to now the effects of this intracellular labelling on the biology of the cells were not thoroughly investigated. Therefore we investigated whether the magnetic labelling of macrophages with iron-oxide-particles *in vitro* results in an increase of oxidative stress. We showed that the incubation of macrophages with iron-oxide-particles results in a highly significant increase of oxidative stress. The decrease of oxidative stress to control levels one day after incubation indicates that the augmentation of oxidative stress is transient and closely linked to the incubation of the cells with iron-oxide particles (Fig. 5). The magnetic labelling is stable over an extended period of time (Fig. 6). The oxidative stress can be reduced by the application of iron-chelators (Desferal).

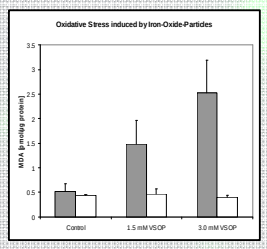


Figure 5
Grey bars show MDA concentration immediately after incubation, whereas white bars show MDA concentration one day after incubation.

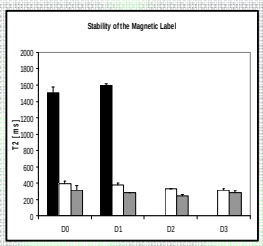


Figure 6
Time course of T2 Relaxation measured by NMR over three days after incubation of macrophages with VSOP. Black bars represent the control cells, white bars cells incubated with 1.5 mM VSOP, grey bars cells incubated with 3 mM VSOP.

Detection of Minimum Cell Numbers by High-Resoltution Magnetic Resonance Imaging (17 T)

The embryonic stem cells were labelled with 1,5 mM VSOP-particles *in vitro*. In gel-phantoms 1000 (Fig. 7) and 100 (Fig. 8) magnetically labelled embryonic stem cells can be detected by high-resolution MRI at 17 T. Transplantation of magnetically labelled embryonic stem cells in 2µl PBS in the striatum of Wistar-rats led to a significant contrast at T2* weighted images. The hypointensity is dependent on the cell number. Figure 9 shows *in vivo* MRI at 17 T of 1000 cells, Figure 10 of 100 cells. After transplantation of 1000 unlabeled cells (Fig. 11) only weak areas of hypointensity can be detected. In Histology clusters of embryonic stem cells can be shown , which seemed to be already differentiated (Fig. 12).

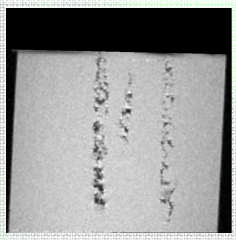


Figure 7

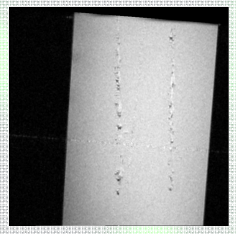


Figure 8

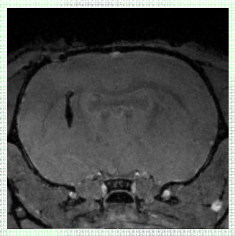


Figure 9

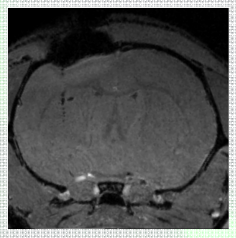


Figure 10

Figure 11

Figure 12

Conclusions

This results show that a non-invasive tracking of transplanted embryonic stem cells by molecular MRI is possible. The cells are detectable at very low cell numbers (<100). The next steps are the long term monitoring of the transplanted cells and the establishment of the magnetic labelling as vitality marker also *in vivo*.

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