In vivo detection of small numbers of magnetically labeled embryonic stem cells

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Introduction:

The potential of stem or progenitor cells to regenerate damaged tissue in neuronal diseases has attracted the focus of neurological research over the recent years. By using cells which are labeled with small iron oxide particles as a contrast agent, MRI gives new possibilities for detection [1] or tracking the migration [2] of stem cells in vivo. To study the migration of the cells in more detail the critical factor is the number of cells that can be detected. In this study we used a 17.6 Tesla magnet, the highest magnetic field available for imaging of rodents, in combination with an optimized labeling procedure to explore the detection limits of low numbers of grafted cells in the rat brain in vivo.

Subjects & Methods:

Mouse embryonic stem cells (CRL-1934, ATCC, Manassas, USA) were labeled with very small superparamagnetic iron-oxide particles (VSOP) C200 (Ferropharm, Teltow, Germany). After performing experiments with different amounts injected in gel phantoms (1000 unlabeled; 1000 and 100 labeled cells), the cells were transplanted into the brain of anesthetized (isoflurane) female Wistar rats (130 - 150g). Each animal received an injection of 2 µl cell suspension into the midbrain of the left hemisphere via a Hamilton micro syringe (500 µm outer diameter) containing a defined number of labeled (1000 three animals, 100 three animals and 20 four animals) or unlabeled (1000 six animals) cells. The animals were imaged immediately after, and up to six weeks after, the transplantation. MRI experiments were performed on a Bruker 17.6 T widebore Avance System with a maximum gradient strength of 200 mT/m and an 8 leg linear birdcage resonator (inner diameter 38mm). A standard 3D – FLASH sequence with an echo time of 4.7 ms was used, optimizing the effect of the signal loss due to T_2^* relaxation of the iron labeled cells, the signal to noise, and the contrast in the image. With a field of view of 25 x 25 x 12.5 mm^3 and a matrix size of 256 x 256 x 128 the resolution of the experiment was 98 µm isotropic and the scan time was 32 minutes.

Results:

In the gel experiments the injection canal could be identified and in the case of the labeled cells dark spots along the canal are visible (more and larger spots in the 1000 labeled cell case). After transplantation of 1000 labeled cells in the rat brain a clearly visible area of signal extinction extending to the end of the transplantation canal was observed (Figure 1 middle). In contrast, none of the six control animals showed comparable signal loss at the transplantation site (Figure 1 left). In the case of transplantation of 100 cells the area of signal loss became smaller but was still easily visible in several slices of the 3D data set (Figure 1 right). The small signal attenuation detected in the brains of rats that had received only 20 labeled cells could not be assigned unambiguously to the grafted cells.

Discussion & Conclusion:

With high field strength and the appropriate cell labeling method 100 labeled cells are clearly observable in gels and in vivo. The dominant problem in vivo at cell numbers of 20 or below is the distinction between cells, anatomical structures and blood. In monitoring stem cells in different animal models the problem of blood will be of less importance, since cells are not grafted at the lesion site. Time course studies of animals will also facilitate the distinction between anatomical structures and cells migrating towards the lesion sites.

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References:

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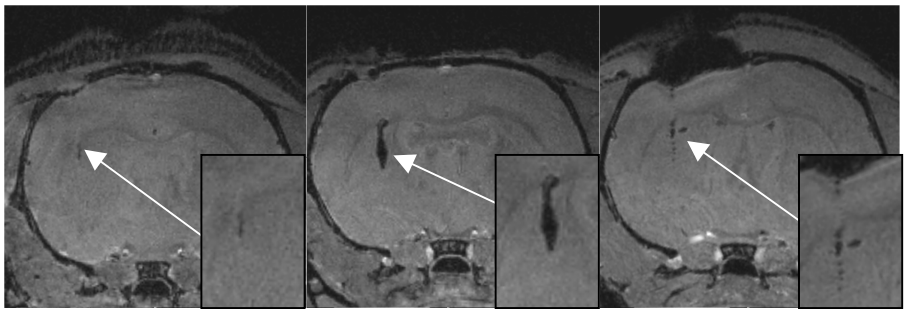

Figure 2: Three rat brains with different number of cells. (left) 1000 unlabeled stem cells (control). (middle) 1000 and (right) 100 labeled stem cells.