Improved protocol for in vivo stem cell monitoring in rat brain

U. Himmelreich[1], P. Ramos-Cabrer[1], R. Weber[1], K. Kandal[1], E. M. Shapiro[2], A. P. Koretsky[2], M. Hoehn[1]

[1]In-vivo NMR group, Max-Planck-Institute for Neurological Research, Cologne, NRW, Germany, [2]LFMI/NINDS, National Institutes of Health, Bethesda, MD, United States

Introduction:

Three-dimensional MRI can achieve microscopic resolution to monitor cellular processes. In order to discriminate implanted cells from host cells, they can be effectively labeled with ultrasmall paramagnetic iron oxide nanoparticles (USPIO), which generate a pronounced contrast in T2*-weighted images. This approach is well established and was used for monitoring stem cell migration in diseased animals *in vivo* [1, 2]. However, intrinsic T2 and $T2^*$ effects caused by paramagnetic deoxyhemoglobin in erythrocytes of blood vessels may complicate the data analysis. Changes in the deoxyhemoglobin level were the basis of MRI studies that have addressed the effect of blood oxygenation level-dependent (BOLD) contrast [3]. Appart from fMRI studies, BOLD MRI has been used for monitoring and characterisation of tumors and tumor treatment using carbogen (95% O_2/ 5% CO_2). Although, the effect of the inhalation gas mixture on the BOLD effect is known [3], little attention has been paid to its application and importance for cell tracking by MRI. We have utilised the BOLD effect for the suppression of intrinsic T2 and $T2^*$ effects by modifying the inhalation gas, allowing unequivocal detection of USPIO labeled stem cells in the rat brain.

Methods:

All experiments were performed in accordance with the NIH animal protection guidelines and approved by the governmental authorities. Wistar rats (n=10, weight 250-550g) were anesthetized with 1% halothane. Three animals were part of a study where USPIO labeled embryonic stem cells were implanted in a stroke model (for details see [2]). The gas mixture for anesthesia was varied between: (A) 30-35% oxygen (O_2) and 65-70% N_2O and (B) 95% O_2 and 5% CO_2 and (C) 100% O_2. The gas mixture was changed between (A), (B) and (C) after completion of one set of MRI experiments without repositioning of the animal with a 15 minute delay time. All MRI experiments were performed on an experimental animal scanner at 4.7 Tesla or 7 Tesla (Bruker BioSpec; Bruker, Ettlingen, Germany) equipped with actively shielded gradient sets of 100 and 200 mT m^{-1}, respectively. For rf irradiation and signal detection custom-built coils were used. A 12-cm-diameter Helmholtz coil arrangement served for rf excitation, whereas signal detection was achieved with a 2.3 cm (4.7T)/ 3.0 cm (7T) diameter surface coil. MR images were processed with the NIH software 'Image J'.

Results:

All rats tolerated the five hours procedure. Two animals were imaged multiple times (up to four times). The images with the gas mixture B (95% O_2, 5% CO_2) resulted in substantial reduction of intrinsic T2 and $T2^*$ effects by blood vessels compared to the commony used gas mixture A (30-35% O_2, 65-70% N_2O). Small blood vessels were not visible at all and major blood vessels were reduced in size by up to 75%. Gas mixture (C) had only a marginal effect on the blood vessels, confirming that the combined effect of O_2 (shift of equlibrium from deoxy- to oxyhemoglobin) and CO_2 (increase in blood volume and hereby further dilution of deoxyhemoglobin) is responsible for the suppression of intrinsic susceptibility effects. The hypointense signal of USPIO labelled embryonic stem cells was not affected by changed gas mixtures resulting in unequivocal detection of USPIO labelled stem cells with gas mixture B as shown in Figure 1.

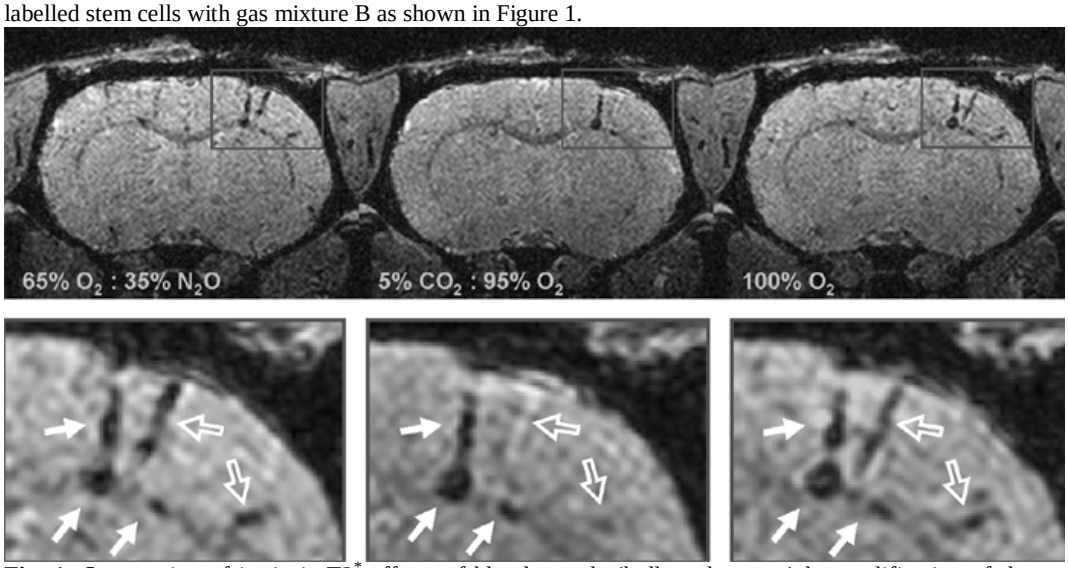

Fig. 1: Suppression of intrinsic $T2^*$ effects of blood vessels (hollowed arrows) by modification of the anesthesia gas. Hypointensity caused by USPIO labeled stem cells (solid arrows) was not affected. The resolution in the 3D MRI was 78 x 78 x 78 µm.

Conclusion:

The modified inhalation gas mixture (95% O_2/ 5% CO_2) enables unequivocal distinction between hypointensity effects caused by USPIO labeled cells and intrinsic hypointensity caused by paramagnetic deoxyhemoglobin in blood vessels. This will substantially improve the monitoring of cell migration *in vivo*.

References:

1. Bulte JWM, Douglas T, Witwer B et al.; Nat. Biotechnol. **19**, 1141 (2001).
2. Hoehn M, Küstermann E, Blunk J et al.; Proc. Natl. Acad. Sci. USA **99**, 16267 (2002).
3. Ogawa S, Lee TM, Kay AR and Tank DW; Proc. Natl. Acad. Sci. USA **87**, 9868 (1990).