Discrepancies between the fate of human myoblasts transplanted into immunocompetent mice after loading with Gd-chelates or with SPIO and the time-course of label detection by in vivo NMR imaging

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INTRODUCTION: High spatial resolution and non-invasiveness are essential characteristics of NMR imaging, which make the technique highly suitable for longitudinal assessment of therapies. In cell therapy protocols, there is unfortunately no intrinsic contrast of transferred cells with respect to host tissues and, to be detected, therapeutic cells must be pre-loaded with an appropriate NMR contrast agent (CA). To date, most NMR studies have used iron oxide particles (SPIO) as CA to monitor grafted cells *in vivo* [1], mainly because of the high detection sensitivity they provide. However, SPIO have some drawbacks: quantitative analysis of labeling is impossible and recapture by macrophages may result in overestimating therapeutic cell survival. Recently, lanthanide complexes have been proposed as an alternative to visualize stem cells in vivo [2]. The aim of this comparative study was to assess the ability of these two classes of NMR CA to accurately monitor the fate of labeled myoblasts transplanted into skeletal muscle.

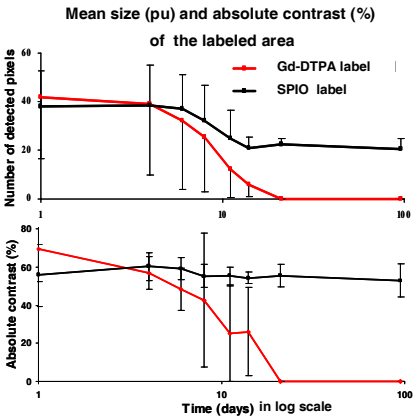
MATERIALS AND METHODS: *Cell labeling and grafting:* Primary myoblasts isolated from human biopsies were grown until confluence and then loaded by direct incubation with 25 mM of Gd-DTPA (Magnevist, Schering, Germany) or 100µL/mL of SPIO (Endorem, Guerbet, France) during 24 hours at 37°C and washed after treatment. Transplant experiments were then performed on eight C57/Bl6 mice. For each subject, 2.10^6 labeled myoblasts were injected along the length of the *tibialis anterior* (TA) in both legs, left with Gd-DTPA and right with SPIO.

In vivo NMR experiments: All acquisitions were performed in a 4T Bruker Biospec NMR spectrometer equipped with a 20 cm diameter 200 mT/m gradient insert. Interleaved axial T1-weighted spin echo images (TR/TE/NEX: 150/5.7/20, in-plane resolution:120x120µm²) of the lower limbs were acquired immediately after cell injection (D0) and were repeated on days D1, D4, D6, D8, D11, D14, D21 and until 3 months after cell transplantation. T1 and T2 measurements were also systematically performed.

Image analysis: NMR data were analyzed using the Image Sequences Analysis Tool of ParaVision. T1 and T2 maps were calculated. In order to identify labeled cell pixels from muscle pixels, we defined the detection threshold as ($\overline{S}_{muscle}$+1.96.SD) for Gd-DTPA label and as ($\overline{S}_{muscle}$-1.96.SD) for iron oxide label, where $\overline{S}_{muscle}$ is the mean intensity of skeletal muscle surrounding the injection site and SD is the associated standard deviation. Number of pixels and signal intensity of the detected area were measured. Label contrast was calculated as follows, with N the number of pixels detected as label: $\left|\sum_{pixels=1}^{N}(S_{pixels}-\overline{S}_{muscle})/(N.\overline{S}_{muscle})\right|$.

Immunohistochemistry: Skeletal muscle specimens were taken on either D8, D11, D16 or after 3 months. Serial transverse sections were performed and processed for immunohistochemistry staining with polyclonal anti human COX-2 (mitochondrial localization) or anti-human lamine (nuclear localization) antibodies. In addition, Prussian blue staining was performed to detect ferric iron in tissue.

Statistics: Data are presented as mean values ± SD. Non parametric statistics were performed with Friedman Q-test and Mann-Whitney U-test.



RESULTS: As expected, T1-weighted images showed an hyper-intensity signal at the injection site on D0 for Gd-DTPA label and an hypo-intensity signal for SPIO label. T2 maps of TA revealed a diffuse increase in T2 at D0 and until D8, attributed to an inflammatory lesion. With the SPIO label, the number of enhanced pixels did not significantly decrease between D1 and D11 and reached a plateau of 20 pixels (0.3mm²), whereas the Gd-DTPA label significantly decreased and totally disappeared at D21, as shown in the figure. The negative contrast of SPIO remained stable over time and differed from the Gd-DTPA induced contrast, which was found to decrease ($p<0.004$) between D1 and D11. Maps confirmed that T1 was reduced in labeled areas. Immunostainings performed on D8 and D11 were negative, showing there were no human cells left in muscles, confirming their immunorejection. On the other hand, Prussian blue staining revealed the presence of iron at D8 and D11, which was in good agreement with the persistence of the negative NMR contrast over time.

CONCLUSION: In this study, time-courses of in vivo label detection were very different after intramuscular injection of myoblasts loaded with Gd-DTPA or with SPIO. The Gd-DTPA signal enhancement disappeared within two weeks after injection while the SPIO label persisted over three months. In addition, and most importantly, the in vivo remanence of both CA in muscles exceeded the known survival of xenografts in immunocompetent tissues, which is of approximately 6 days in our experimental model, and was confirmed here by the absence of anti-human COX2 staining at D8. These results indicate that transplanted cell survival cannot be estimated directly from the NMR signal changes induced by CA loading of grafted cells. Label re-uptake by other cell types was already known for SPIO [3]. It has also been shown here for Gd-DTPA labeling, despite rapid renal clearance which was expected to prevent the phenomenon.

References: **1**. Bulte, J.W. &D.L. Kraitchman, *Monitoring cell therapy using iron oxide MR contrast agents.* Curr Pharm Biotechnol, 2004, 5:567-84. **2**. Crich, S.G., et al., *Improved route for the visualization of stem cells labeled with a Gd-/Eu-chelate as dual (MRI and fluorescence) agent.* Magn Reson Med, 2004, 51:938-44. **3**.Cahill, K.S., et al., *Noninvasive monitoring and tracking of muscle stem cell transplants.* Transplantation, 2004, 78:1626-33.