

LONG TERM EVALUATION OF THE 1.28 PPM AFTER TRANSPLANTATION OF PURIFIED NEURAL PROGENITOR CELLS IN THE BRAIN

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Introduction

Transplantation of neural progenitor cells (NPCs) is a promising treatment for neurodegenerative disorders. However, evaluation of the long term effects of NPC transplantation requires elaborate noninvasive methodology. Recently, Manganas et al. proposed that the transplanted NPCs can be detected *in vivo* at the 1.28 ppm (Manganas, et al., 2007). The signal is not present in differentiated neural cells such as neurons and astrocytes. The method may be useful for tracking the transplanted NPCs on a long term basis. That is, when the cells remain progenitors, they can be detected by the 1.28 ppm. If the cells differentiate into neurons or astrocytes, the MR spectra should reflect these changes, such as decreases of the 1.28 ppm signal and alterations of *N*-acetyl-aspartate (NAA) levels with time. To examine this hypothesis, the present study used purified NPCs to study the changes of the 1.28 ppm and NAA over a three month period following the transplantation of cells.

Materials and methods

The NPCs were derived from human embryonic stem cells sorted by Sox1. One-dimensional ¹H-NMR spectra of the aqueous suspensions of the NPCs containing 10% D₂O as a field frequency lock were measured using an 800 NMR spectrometer. In the experiments, the temperature was maintained at 35°C and pH at 7.25. The spectra were acquired with a Free Induction Decay (FID, 32,768 points in a spectral width of 8389.3 Hz, readout time of 1.95 seconds, repetition time of 2 seconds and 128 averages). To minimize the large water peak, the water signal was pre-saturated with a low power radiofrequency (RF) pulse. Before Fourier transform, FIDs were line broadened to 1.0 Hz with an exponential weighting function. ¹H-NMR spectra were phase and baseline corrected for distortions and referenced to 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS) at 0.00ppm. The NPCs were transplanted into the striatum and monitored by localized *in vivo* proton MRS using a point-resolved spectroscopy sequence (TR/TE = 3000/136 or 18ms, NEX = 256, and voxel volume = 2*2*2 mm³) to reveal the metabolic changes of NPCs *in vivo*. The peak areas of the 1.28 ppm signal, NAA, creatine (Cr) from the spectra with long TE were resolved by singular value decomposition (SVD) based signal processing, and the ratios of brain metabolites relative to Cr were measured. For MRI, T₂-weighted imaging (T₂WI) and diffusion weighted imaging (DWI) were acquired. T₂WI used a spin echo sequence with TR= 4500 ms, TE=70 ms, NEX = 8, field of view = 2.56 cm x 2.56 cm, matrix size = 256x 256. DWI used a Stejskal-Tanner spinecho sequence with TR = 1500 ms, TE = 34 ms, diffusion gradient duration = 3.5 ms, diffusion gradient separation = 15 ms, b value = 1100mm²/s along the z direction, NEX= 8, field of view = 2.56 cm x 2.56 cm, matrix size = 256x 128.

Results and conclusions

High resolution ¹H-NMR verified the presence of the 1.28 ppm peak in the purified NPCs sorted by Sox1 (Figure 1A). The NPCs were then transplanted into the striatum, as appeared in Figure 1B (T₂WI) and 1C (DWI). On the first day following transplantation, a significant increase in the signal at the 1.28 ppm was observed, accompanied by a slight decrease in NAA. At the 4th week, the 1.28 ppm peak decreased and the NAA level also dropped. At the 8th and 12th week, the 1.28 ppm peak gradually returned to the baseline while the NAA signal also recovered to the original level. Figure 2 shows the original spectra acquired with short TE from one rat. Figure 3 shows the average data of 4 rats, based upon the spectra acquired with long TE processed by SVD.

The temporal pattern of changes in the 1.28 ppm signal suggests that the NPCs were detectable in the early time point after transplantation. Afterwards, the transplanted NPCs lost their progenitor identity so that they became undetectable on the spectrum. Immunohistology confirmed that the transplanted cells have differentiated into neural cells and migrated away. The temporal pattern of changes in the NAA level is intriguing. The decreases observed within the first month is particularly elusive. We suspect that the deposit of NPCs diluted the NAA concentration of the brain tissue in the measured voxel. As the NPCs gradually reached a stability associated to committed cells, such as matured neurons, the NAA level then returned to the original levels. The underlying mechanisms associated with the NAA turnover remains to be elucidated.

Figure 1

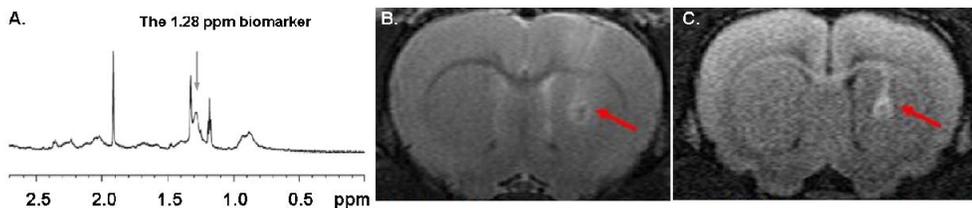


Figure 3.

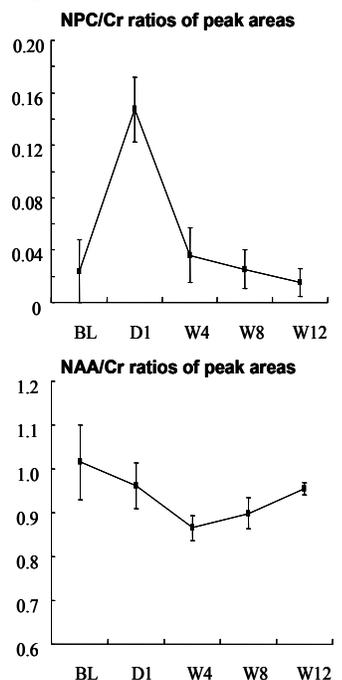
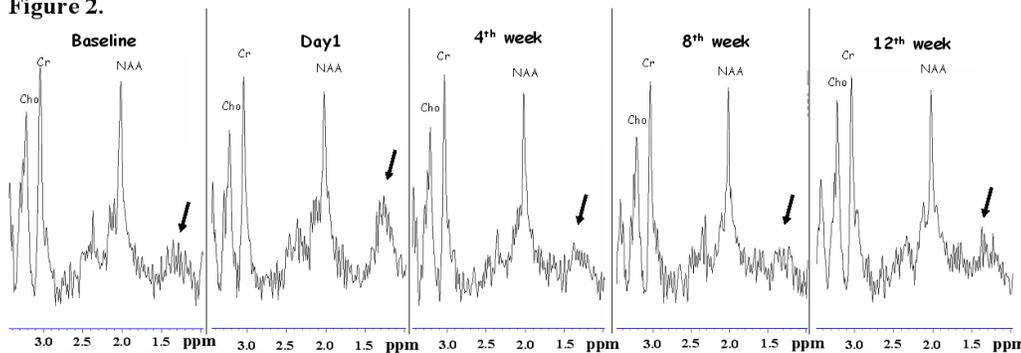


Figure 2.



References: Manganas et al., 2007. Science. 318,980-5.