Cellular MRI of neural stem cell therapy in a rat glioma model

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Introduction

Cellular MR imaging was conducted to trace the migration of labelled neural stem cells (NSCs) *in vivo* in rat brain within an experimentally induced glioma model. Murine neural stem cells (MHP36 cell line) were labelled with the bimodal contrast agent, gadolinium rhodamine dextran (GRID), *in vitro* prior to implantation. NSCs were grafted into the contralateral hemisphere of the glioma or sham-operated region. Three groups of animals were included in the study; glioma with intracranial injection of NSCs (glioma/NSCs), glioma with sham-operation (glioma/sham), and sham-operation with intracranial injection of NSCs (sham/NSCs). Longitudinal MR scanning was performed to dynamically visualise the migration and infiltration of GRID-labeled neural stem cells into tumour tissue in order to evaluate the potential of NSCs as a therapeutic vehicle in treatment of gliomas.

Material and methods

Intracranial gliomas were induced by injection of 10^5 CNS-1 glioma cells into the brain of syngeneic male adult Lewis rats. Sham operated animals received an injection of 1 mM N-acetyl-L-cysteine (Sigma). Grafting of GRID-labeled stem cells or a sham-operation was performed on day 8. All rats were MR scanned at 4 time-points: a baseline prior to glioma implantation; day 6 prior to NSC implantation; and at day 10 and; day 17 to assess NSC migration. All sequences had identical slice positioning, FOV (3.5x3.5 cm) and no slice gap to allow multiplanar reconstruction. A spin echo multi echo (SEME) T2-weighted sequence with TR=2000ms, TE=23, 46 and 69 ms, NEX= 4, matrix = 128x128, slice thickness 0.6 mm, number of slices = 30, was followed by a GE sequence: TR=1225ms, TE=6, 11, 16, 21, 26 and 31 ms, α = 33 º, and NEX = 2. Mean-echo T2- and T2*-images were created by a non-weighted arithmetic summation of the magnitude images from the multiple echoes. A spin echo T1-weighted sequence with TR=831ms, TE=23 ms, NEX = 6 was acquired pre- and post-injection of Gd-DTPA-BMA trough the tail vein. The tumour-doubling time was calculated by estimating the tumour volume from the enhanced tumour mass in post-contrast images. All animals were sacrificed by intracardial perfusion of 4% PFA after the last MR scanning. The brains were cut using a crystat (50 μm) and stained for macrophages using an antibody against CD68.

Results and discussion

MR data acquired of tumour animals after grafting of GRID-labeled stem cells revealed several small regions in the corpus callosum with signal drop in T2-weighted images (see Figure 1). No signal change was detected in the corpus callosum of sham operated rats. This indicates that the alternation of signal intensity in glioma/NSCs rats is induced by a migration of GRID-labeled NSCs towards the tumour region. The tumour doubling time (Vd) was higher in tumour bearing animals that received NSCs comapared to sham operated tumour animals (Vd = 2.04 +/- 0.16 (SEM) days Vd = 1.56 +/- 0.22 (SEM) respectively, P = 0.056), possibly reflecting a therapeutic effect of NSCs. A comparison of the signal intensity in the main tumour mass in glioma/sham operated and glioma/NSCs rats revealed hypointense areas in the glioma region in T2-weighted images in both groups. Immunohistopathology corroborated that the location of clusters of red fluorescent cells in the glioma/NSCs group (see Figure 1) corresponded to signal attenuations on T2-weighted MR images. In both glioma groups, there was a high infiltration of macrophages in tumour tissue, although MR hypointensities did not always correspond to areas with GRID-labelled cells as indicated by the sham-operated animals.

Conclusions

In vivo cellular MR imaging made it possible to track migrating GRID-labeled stem cells along the corpus callosum. However, the infiltration of NSCs into tumour tissue was difficult to evaluate purely based on the MR images. Corroborative immunohistopathology was requireed to confirm that hypointense regions in the tumour regions in the glioma/NSCs group were indeed caused by the presence of GRID-labeled cells. Increased tumour-doubling time in rats which received GRID-labeled stem cells also indicates a therapeutic effect of NSCs. NSCs therefore possess a therapeutic potential to treat gliomas, but further refinement of cellular MRI methodology is needed to allow a reliable tracking of these therapeutic vehicles in vivo.

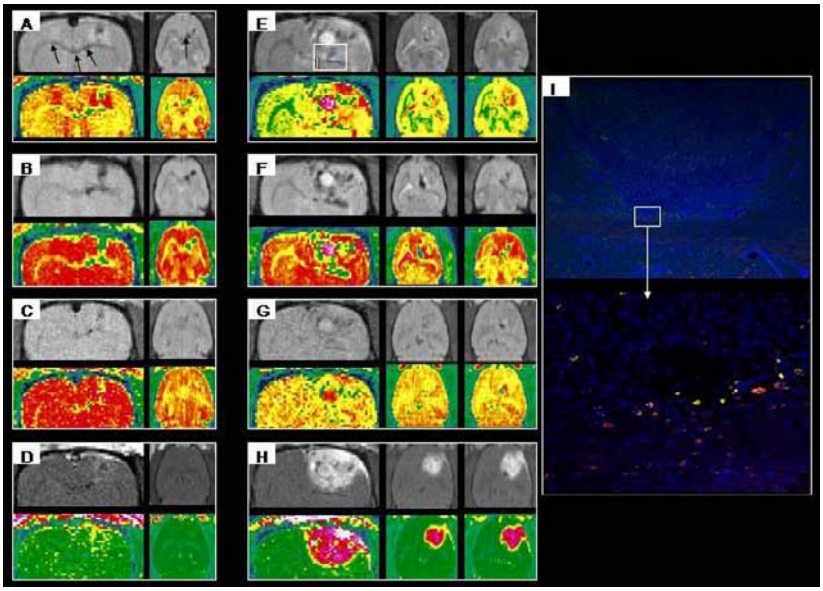

Figure 1: MR images (greyscale and colorized) and immunohistopathology of a glioma/NSCs rat. **A-D** and **E-H** are the same animal imaged at day 10 and 17 respectively. **A** and **E** - T2-weighted images in coronal and axial slice orientation. A signal drop in corpus callosum indicated by arrows (**A**) suggests migrating GRID-labelled cells. **B** and **F -** T2* weighted. **C** and **G** - T1 weighted pre-contrast and **D** and **H** - T1 post-contrast images. Histological corroboration of the migrating NSCs and the tumour of the inserted box on **E** are presented in **I**. Macrophages (green-labelled cells) and GRID-labelled NSC (in red) have infiltrated the tumour mass and tumour border