MRI monitoring of endogenous stem cell therapies in animal models of stroke

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Introduction: In animal models of stroke, endogenous neural precursor cells can be activated with growth factors such as epidermal growth factor (EGF) and erythropoietin (EPO) [1], leading to increased neurogenesis, generation of new tissue at the lesion site, and behavioural recovery. To translate similar therapies into the clinic, it is critical to evaluate, non-invasively and at multiple time points, this process of tissue repair and regeneration. In this study, we used a rat model of stroke to demonstrate the feasibility of using MR to distinguish between regenerating and pathological tissues when using endogenous stem cell therapies.

Methods: *Stroke induction and treatment*: Focal ischemia was induced in Wistar rats (males, 200g) using a devascularization model [2]. An opening was made in the skull over the motor and sensory cortices. A sterile saline-soaked cotton swab was used to wipe the pia and attached blood vessels from the cortical surface, creating focal ischemia. MR-compatible infusion cannulae were implanted into the contralateral lateral ventricle, and connected to an osmotic minipump that delivered vehicle (artificial cerebral spinal fluid plus 0.1% bovine serum albumin), or epidermal growth factor (EGF, 10 µg/ml) for 7 days. On Day 8, the EGF pump was exchanged with one containing erythropoietin (EPO, 1365IU/ml). This EGF+EPO cocktail has been shown to enhance differentiation and numbers of newly generated neurons [3, 4]. This treatment results in the formation of a tissue plug at the site of injury, which is not seen in untreated controls.
MRI. Rats were anesthetized (isoflurane), placed in an MR-compatible head restraint and scanned at 3T (GE Signa) using a custom-designed surface coil. Imaging was performed starting at 1 day until 56 days post-stroke. Imaging frequency was varied among cohorts to probe the effect of anesthesia in the animal model of stroke. Three 3D fSPGR sequences (flip angles: 2°, 10°, and 15°; 40×40mm FOV; 1mm slice thickness; matrix 128×128; 16 NEX) were used to calculate average T_1 in the lesion [5]. Semi-quantitative T_2 estimates were calculated using a FSE sequence at the same location and resolution (TE = 35, 75ms; TR = 2500ms; ETL 4; 2 NEX). Total scan time was approximately 1 hour.
Histology: Animals were sacrificed 1 day after their last imaging session at different times post-stroke (7, 14, 28, and 56 days). Brains were removed, sectioned, and stained with DAPI for cell nuclei and NeuN for neuronal nuclei.

Results: In the majority of treated animals (80%) the tissue plug formed within 14 days post-treatment; structural regrowth was accompanied by functional recovery. Analysis of the average $R_1(1/T_1)$ of the lesion volume as a function of cell count (DAPI) is illustrated in Fig. 1. The data indicates that the regrown tissue plug has an average R_1 that resembles that of normal tissue in the contralateral cortex. A much lower average R_1 is observed in untreated animals where the lesion remains a cavity. A similar trend is observed with R_2 in Fig. 2 (Error bars omitted for clarity). T_1-weighted MRI and corresponding T_2 map (56 days post stroke) and histology of representative animals in untreated and successfully treated groups is shown in Fig. 3. Successfully treated animals had a tissue plug with T_2 values that resembled that of normal cortical tissue, whereas untreated groups failed to grow tissue in the lesion. NeuN stain revealed neurons in the tissue plug for animals that were treated. These neurons are absent in the untreated cavity. MR imaging was able to predict the outcome of the treatment as early as two weeks post stroke (much earlier than functional recovery, Fig.4). In a limited number of animals (4 out of 33) the treatment was not successful (no tissue plug formation). In these animals the T_1 and T_2 relaxation of lesions were larger than in the regenerating brains.

Summary: We have demonstrated that MRI can be a powerful tool in the evaluation of stroke recovery. Tissue plug growth in the lesion site has MR characteristics (T_1 and T_2) similar to that of normal brain tissue, and differs distinctly from the cavity. Moreover, MRI is able to predict the outcome of the treatment as early as 2 weeks post stroke. Future experiments will investigate whether changing the dosage of drug delivered affects the recovery process.

References:
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