

Brain tissue regeneration following activation of endogenous stem cells in animal model of stroke

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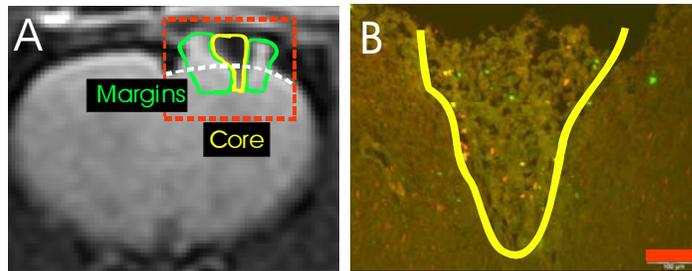
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Introduction. In animal models of stroke, endogenous neural precursor cells can be activated with growth factors such as epidermal growth factor and erythropoietin (1), leading to increased neurogenesis, generation of new tissue at the lesion site, 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 stem cell based therapies.

Methods. *Stroke induction and treatment:* Focal ischemia was induced in Wistar rats (males, 200g) using a devascularization model (3). 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 $\mu\text{g}/\text{ml}$) 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 (4,5). This treatment results in the formation of a tissue plug at the site of injury, which is not seen in vehicle controls.

MRI. Rats were anesthetized (ketamine-xylazine), placed in an MR-compatible head restraint and scanned at 3T (GE Signa) using a custom-designed surface coil. Images were acquired starting at 7 days and up to 60 days post-lesion. Two 3D fSPGR sequences (flip angles: 2° and 10°; 4cm FOV; 1mm slice thickness; matrix 128x128; 16 NEX) were used to generate T_1 maps (6). Semi-quantitative T_2 estimates were calculated using a fSE sequence at the same location and resolution (TEs: 11, 44 ms; TR 2500ms; ETL 4; 2 NEX). Total scan time was approximately 1 hour. Regions-of-interest (ROIs) divided the plug into 3 regions below and 3 regions above the cortical surface (Fig A). A reference ROI was placed in normal gray matter (GM).

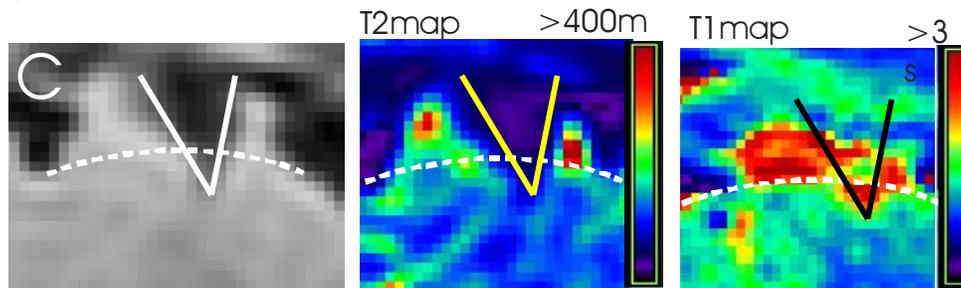
Results. Figure A shows a T_2 -weighted coronal image of the tissue plug in an EGF-treated animal at 3 weeks post-stroke. The white dotted line shows the top of the brain, and the margins and core of the plug are outlined. The plug was ~3000 μm wide and the core was ~300-600 μm wide at this point. Immunohistochemical analysis (Fig B) showed that by 3 weeks post-stroke, neurons (red), some of which were derived from endogenous precursors in the subependyma lining of the lateral ventricles (yellow) had migrated to the site of ischemia. Figure B shows the bottom portion of the tissue plug core and margins (scale bar=100 μm).



The tissue plug is shown enlarged in Figure C (left panel). Corresponding T_2 and T_1 maps are shown in the centre and right panels; the plug core is indicated by the straight lines on the images.

T_2 results: Compared to T_2 values in normal GM (140 \pm 23ms), the plug core showed uniformly shorter T_2 values (51 \pm 19ms). T_2 in the plug margins was more heterogeneous: T_2 values ranged from 50-400ms in the margins of the tissue plug that extended above the cortical surface. T_2 values in tissues below the cortical surface were similar to normal GM (146 \pm 28ms).

T_1 results: T_1 of normal GM was 1.7 \pm 0.1s, and differed from the T_1 measured in much of the tissue plug. Below the cortical surface, T_1 values were on average longer and more variable than normal GM (2.0 \pm 0.5s, range 1.3-4s). Above the cortical surface, they were much longer than normal GM, in both plug margins and core (mean 2.8 \pm 1s, range 0.8-3.6s).



Summary and Conclusions. By 3 weeks post-stroke, the tissue plug in growth factor treated animals was beginning to take on some characteristics typical of gray matter. The effect was more pronounced by 5 weeks post-stroke, where the extent of tissue recovery was significantly larger, and the plug core was still visible. At 3 weeks, T_2 in the plug margins in cortex was equivalent to normal GM, but T_1 was still slightly longer and more variable. Above the cortical surface, both T_2 and T_1 were longer than normal GM, likely reflecting reduced cell density. The centre core of the plug was characterized by very short T_2 and long T_1 values compared to GM. Histopathological assessment shows that the plug core is highly heterogeneous. It is therefore, not surprising to observe abnormal T_1 and T_2 values in the core. The results of this study clearly demonstrate that it is feasible to monitor and characterize tissue regeneration in this cell-based therapy to treat stroke.

References: (1) Cheng V, et al. (2004), Soc for Neurosci Abstract, 391.5. (2) Gray J. A., et al., Cell Transplant 2000;9:153. (3) Gonzalez C. L., Kolb B. Eur J Neurosci 2003;18:1950. (4) Shingo T., et al. J Neurosci 2001;21:9733. (5) Craig CG, et al. J Neurosci 1996;16:2649. (6) Cheng, H.L., MRM, 2005, in press.