MRI Monitoring of Stem Cells Transplantation in Traumatic Brain Injury Mice and its Therapeutic Potential

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Target Audience: Researchers, Clinicians and Students

Introduction: Tissue replacement by stem cell transplants is a promising approach to treat various diseases like traumatic brain injury. Promising neuroprotective drugs that were identified as being effective in pre-clinical animal models have all failed in phase II/III clinical trials. Cell based therapy is one of the most effective method in TBI to treat neurodegeneration. After transplantation, tracking of stem cells is very necessary for its homing to the site of injury[1]. MRI is the most commonly used imaging modality for *in vivo* tracking, because it is non-invasive, generates high-resolution images and for longitudinal studies. Cellular MRI combines the ability of MRI contrast agents providing dynamic assessment of cell migration into target tissues. The MSCs may have the potential to differentiate into respective neuronal cells after transplantation and helpful in therapeutic application. The recovery in TBI model have analyzed by its functional behavior.

Aim and Objective: 1. Optimization of labelling efficiency of mMSCs with negative contrast agents.
2. Monitoring of labelled stem cells by using MRI.
3. Therapeutic activity of MSCs and its functional outcome

Material & Methods: The iron oxide negative contrast agents were synthesized and coated with reduced dextran by alkaline co-precipitation method. Mesenchymal stem cells were isolated from bone marrow of Balb/C mice and cultured in DMEM-LG medium with 15% FBS. Stem cells were labelled with different concentration Dextran-Fe_3O_4 and Protamine sulfate transfection agent with different incubation time[2]. Labelling optimization was carried out by MTT, Trypan blue, T_2-relaxometry studies and Prussian blue staining. Traumatic brain injury in mice was carried out by free falling weight drop method. Experimental setup like this (8 mice/group): 1. Control, 2. TBI mouse, 3. Control + labelled stem cells, 4. TBI mouse + labelled stem cells. Labelled and unlabelled stem cells ($1x10^6$) were administered intravenously and tracking serially in 7T Bruker Biospec animal MRI after 1hr, day 1, 3, 5, 7, 10, 14 and finally day 21. The sequence used in 7T MRI were MSME_T_2_map, MGE_T_2*_map and magnetic resonance spectroscopy (MRS) for analysis of T_2 time, T_2* time and different metabolites in that injured region respectively. The parameters were as follows: MSME_T_2_map (TR=3500ms, TE= 13-208ms with a gap of 13ms, slice thickness/interstice distance= 0.7/0.7mm, Average=1, slices= 15), MGE_T_2*_map (TR=1500ms, 12 TE/echo spacing= 4.0-81.0/7.0ms, slice thickness/interstice distance= 0.7/0.7mm, Average=1, slices= 7, matrix size= 256/192), MRS (TR=2500ms, TE=20ms, No. of scans= 512, line width= 8-12 Hz). The post processing and semi-quantitative data analysis were analyzed in control and injured area in same mouse by using Paravision-5.1. The behavioural outcomes like depression, cognition and grip strength were analyzed by behavioural test, which is the functional outcome after recovery[3].

Results and Discussion: Stem cells labelling were optimized with Dextran-Fe_3O_4 (50µg/ml) and Protamine sulfate (2.0µg/ml) at an incubation period of 6 hours by taking cell viability, toxicity and relaxometry into consideration. Labelling of stem cells with nanoparticles was confirmed by Prussian blue staining. Stem cells were monitored serially by MRI, where we found a significant decrease in signal intensity and T_2* relaxation time on day-3. These findings indicate that optimum number of transplanted MSCs have homed to the site of injury. This is also validated by immunohistochemistry in which we found the PHH26 labelled cells present at injury area (Fig-1). The injured area inside the mice have decreased from Day-7 and almost diminished at day-21. The increasing signal intensity and T_2* relaxation time indicate that the injured cells might be replaced and generation of new neural cells by differentiation of MSCs (Fig-2). The improvement in cognitive index, grip strength and locomotory activity in TBI+stem cells mice on day-14 indicates a good functional outcome (Fig-3). The behavioural activities strongly validate the MRI data.

Conclusions: Lower toxicity, good viability and efficient T_2* relaxation was found at an incubation period of 6hr. The magnetic nanoparticles in conjugation with MSCs act as a probe in MRI to monitor the stem cells homing to the injury site. There is a low amount of stem cells homed after transplanted in tail vain intravenously. The MSCs may differentiate into neural cells by differential activity or may replace the injured cells by immunomodulation. T_2/T_2* values were significantly less in TBI and increase in transplanted stem cells might be due to repairing activity of MSCs, which is also validated by its functional outcome.

Fig-1: Immunohistochemistry- After transplantation of MSCs on day-3

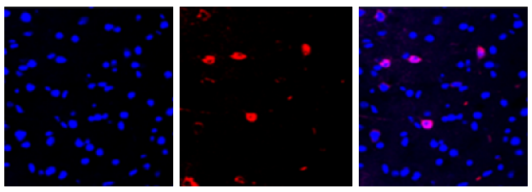


Fig-3: Behavioral test in control, TBI, Control+Stem cells and TBI+Stem cells on day-21 after transplantation of MSCs

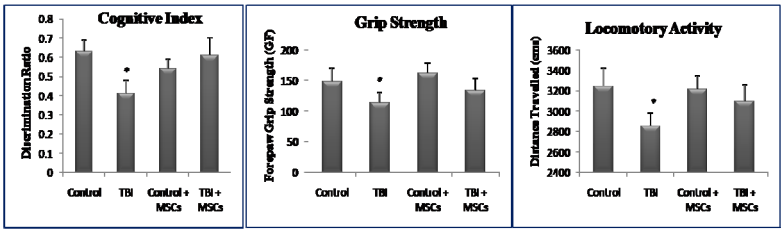
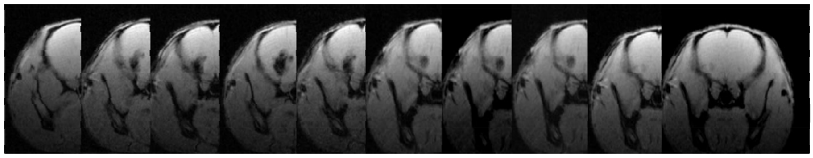


Fig-2: MGE-T_2* imaging of before and after transplantation of MSCs at different time points



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