Tracking of Labelled Stem Cells after Traumatic Brain Injury: A Serial, in vivo Magnetic Resonance Imaging Study

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Introduction: Utilization of stem cells for therapeutic approaches becomes a realistic alternative to conventional treatments. Applications in experimental models range from stem cell therapy to overcome the limited regenerative capacity of many organs to the utilization of cells to administer therapeutic agents to the targeted tissue. MRI is the most commonly used imaging modality for in vivo tracking of labeled stem cells, because it is noninvasive, generates high-resolution images, and does not rely on radioactive isotopes, which may be an important advantage for longitudinal studies. Cellular MRI combines the ability of MRI with contrast agents for labeling cells providing dynamic assessment of cell migration into target tissues. MRI contrast agents in nanomolar to micromolar concentrations can alter the relaxation rates of many nearby tissue water protons thereby making them conspicuous on post contrast enhanced MRI.

Aim and Objective:
1. The purpose of this study was to evaluate the efficiency and toxicity of labelling mMSCs with dextran-Fe_3O_4 and poly-L-lysine
2. Tracking of labelled stem cells by MRI.

Material & Methods: The dextran coated iron oxide nanoparticles were prepared by co-precipitation method. Ferrous and ferric chloride (1:2) was mixed with 2M HCl and coprecipitated in an excess of NaOH. Fe_3O_4 was put drop wise in degassed dextran by continuous stirring. After that sonication, dialysis and ultrafiltration were carried out for purification. Mice mesenchymal stem cells were isolated from femur and tibia of Balb 'C' mice. Cells were cultured in αMEM with 20% FBS by frequent medium changes and incubated at 37^0C and 5% CO_2 upto 3rd passage. mMSCs labelled with Dextran-Fe_3O_4 contrast agent and PLL transfection agent at different concentration and different incubation time. MTT, trypan blue and T_2-relaxometry study were carried out to know cytotoxicity, viability and relaxation time respectively. Labelled stem cells were analyzed by Prussian blue staining. Five Balb 'C' mice were taken for study and among them TBI were carried out in three mice. Experimental setup like this: 1. Control, 2. TBI mouse, 3. Mouse + labelled stem cells, 4. TBI mouse + unlabelled stem cells, 5. TBI mouse + labelled stem cells. mMSCs ($1x10^6$) were labelled with iron oxide nanoparticles and PLL transfection agent for 4hrs. Labelled and unlabelled stem cells were administered intravenously (i.v.) at tail region and tracking serially in 7T Bruker Biospec animal MRI on day 0, after 1hr, day 1, 3, 5, 7, 10 and finally day 14. The sequence used in 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= 0.8/0.7mm, Average=1, slices= 15), MGE_T_2*_map (TR=1500ms, 12 TE/echo spacing= 4.50/5.54ms, slice thickness/interstice distance= 0.8/0.7mm, Average=1, slices= 7, matrix size= 256/192), MRS (TR=2500ms, TE=20ms, No. of scans= 512, line width= 8-12 Hz).

Results and Discussion: Nanoparticles were squasi-spherical and core size of ≈18 nm by Transmission Electron Microscopy (TEM) analysis (Figure 1) and 41.83 nm by dynamic light scattering (DLS) with poly-dispersivity index 0.130 (Figure 2). T_2 values were decreased after increasing conc. of magnetic nanoparticles in samples by using RARE T_1T_2 sequence in 7T Bruker Biospec system. Mice mesenchymal stem cells were isolated and cultured in media up to 3rd passage for experimental uses (Figure 3). From the pilot study, Dextran-Fe_3O_4 contrast agent labelled at a final conc. of 50µg/ml and PLL (1.5 µg/ml) at incubation period of 4 hours. Labelling of stem cells with nanoparticles was confirmed by Prussian blue staining (Figure 4). There is no significant difference in cell toxicity (<5%) and viability (>95%) between labelled and unlabelled stem cells. MSME_T_2_map and T_2 value analysis are shown after labelled stem cells injected in control (Figure 5(a) and TBI mouse (Figure 6 (a). Decreasing T_2 value on day-5 as compared to day-0 in labelled control mice (Figure 5(b). In TBI mice water accumulated more on day-3 and also high T_2 value, but less on day-7 due to labelled stem cells and its therapeutic activity (Figure 6(b). The T_2* value analysis of left and right injury in mouse also decrease in between day 3&7 (Figure 7).

Conclusions: The nanoparticles synthesized in the present study exhibited very small particle size and efficient T_2 relaxation. Lower toxicity and good viability was found at an incubation period of 4hr. Prussian blue staining results show efficient labeling of nanoparticles and decrease in T_2/T_2* value at the injury site in between day 3 and 7 indicates homing of the stem cells to the site of injury.

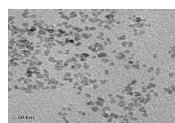


Figure 1: TEM image

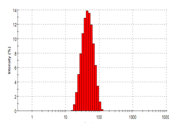


Figure 2: DLS image

Figure 3: mMSCs after 3rd passage

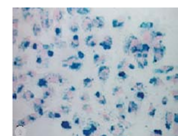
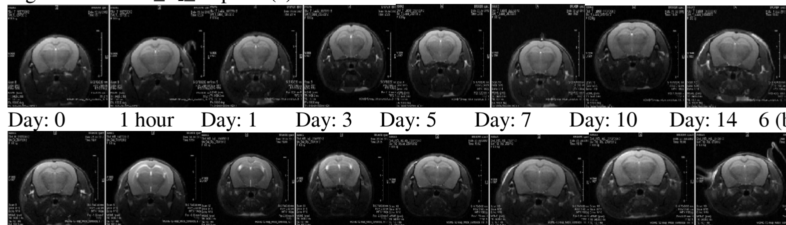


Figure 4: Prussian blue staining

Figure 5: MSME_T_2_map (a) Mice + Labelled stem cells



Day: 0 1 hour Day: 1 Day: 3 Day: 5 Day: 7 Day: 10 Day: 14

5 (b) T2 value analysis of Mice + Labelled stem cells

T2 values (msec)	Day- 0	Day- 1	Day- 3	Day- 5	Day- 7	Day- 10	Day- 14
Left side	67.9	61.5	61.1	53.6	60.5	64.2	64.5
Right side	67.5	66.7	62.8	57.4	60.6	65.0	63.7

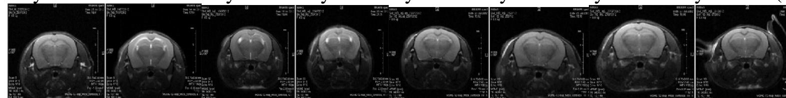


Figure 6 (a) TBI mice + Labelled stem cells

6 (b) T2 value analysis of TBI Mice + Labelled stem cells

T2 values (msec)	Day- 0	Day- 1	Day- 3	Day- 5	Day- 7	Day- 10	Day- 14
Left Injury	56.74	74.23	118.86	76.53	60.61	64.31	60.59
Right Injury	68.27	73.92	174.65	88.36	62.41	56.41	67.12

Figure 7: T2* value analysis of left & right injury (TBI mice + labelled stem cells)

T2* (msec)	Day- 0	Day- 1	Day- 3	Day- 5	Day- 7	Day- 10	Day- 14
Left Injury	22.89	22.56	20.67	20.06	16.97	18.88	23.12
Right Injury	29.88	26.03	21.64	22.53	20.32	23.78	30.57

References:-

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