Intravenous Human Mesenchymal Precursor Stem Cells Improve Functional Recovery and Neuronal Activity in a Rat Stroke Model


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Background

- Immunoscreening of human mesenchymal stem cells from stramal precursors region 1 (STROM1) prior to culture expansion yields mesenchymal precursor cells (MPCs) with improved proliferative capacity, gene expression for early stem cell markers and differentiation efficiency (See et al. 2011).
- Administration of human mesenchymal stem cells has been reported to improve sensorimotor function in animal models of stroke. We conducted a study to investigate how the timing of Mesoblast human MPC administration intravenously post-ischemia could improve behavioral recovery in a nude rat model of ischemic stroke. An imaging study, including fMRI, was conducted to investigate potential mechanisms for functional improvement.

Methods

- 72 male Rnu nude (RNU) rats (Taconic IBU051001C, 300 g) underwent a 2 h MCAO. All MPCs were administered intravenously post-MCAO. A separate imaging study was performed with rats (n=8/group) receiving vehicle, or vehicle + 1.25×106 MPCs (3.63 million MPCs/kg) (170 µl of 6×106 cells/ml preparation); concentration and viability of cells were confirmed prior to injection.
- Behavioral evaluations were performed by an investigator blinded to treatment on the day before MCAO (day −1) and 1, 3, 7, 14, 21 and 28 days post-MCAO. On study days, when applicable, evaluations were performed prior to test article administration.
- Limb placement tests (modified from De Ryck et al. 1998) were carried out on forelimbs (whisker, visual, tactile and proprioceptive placement) and on hindlimbs (dorsal tactile, lateral tactile and proprioceptive placement). Each score was as follows: immediate response, 0 points; response within 2 s, 0.5 points; response within 3–5 s, 1 point; response within 5–10 s, 1.5 points; response >10 s, 2 points. Decrease (upward) in score reflects normalization of function.
- Body sway tests (modified from Borton et al. 1998) were scored as percentage of 30 swings in a rightward direction. Increase in score represents normalization of function.
- A separate imaging study was performed with rats (n=6/group) receiving vehicle or vehicle + 1.25×106 MPCs (3.63 million MPC/kg). An fMRI protocol was used to assess neuronal activity.
- This work is done in alignment with the STAR (Stroke Therapy Academic Industry Roundtable) and STAIR (Stem Cell Therapeutics as an Emerging Paradigm for Stroke) initiatives.

Results

- All MPC-treated groups except the group receiving vehicle at 7 days after MCAO displayed statistically significant improvement in hindlimb placement scores at day 28 compared to the vehicle-treated group (12 hr group, p<0.01; others, p<0.001; Figure 1a).
- All MPC-treated groups except the group receiving vehicle at 12 hr showed statistically significantly better body sway function than the vehicle-treated group at day 28 (7 day group, p<0.01; others, p<0.05; Figure 1b).
- Infarct Volume
- Infarct volume measured with MRI imaging was significantly smaller in rats receiving MPCs 24 h post-MCAO compared with vehicle-treated animals. (Figure 2). A mean 38 mm3 reduction in infarct volume was recorded in the MPC group vs. vehicle, and a 17% relative reduction (1.9 % point absolute reduction) in infarcted brain volume (both p<0.05).
- Functional Imaging
- Neuronal activation in the infarct area during contralateral forepaw stimulation was significantly higher in MPC-treated rats than in vehicle-treated rats (p<0.02; Figure 3).
- In the group that received MPCs at 24 h, fMRI imaging during contralateral forepaw stimulation showed significantly greater activation of the primary motor cortex ipsilateral to the side of ischemic injury in the MPC-treated group (Figure 4a), ipsilateral sensorimotor cortex (Figure 4b), or any contralateral cortices (data not shown).

Discussion

- Current approved therapies for ischemic stroke need to be administered shortly after the onset of stroke (e.g. within 3 hours), the limitations of this small time window mean only 3–5 % of stroke patients receive thrombolytic therapy. Hence, there is a large unmet need for therapies that can be administered within a time window longer than 3 hours. To determine if clinically relevant times could be used with MPC therapy, we chose to study various time points of administration from 6 hours to 7 days.

Conclusions

- Timing of stem cell administration following stroke is potentially important in view of the sequence of events taking place during the hours and days post-infarct. In general, initial degradative responses are followed by ischemia, edema and apoptosis, give way over a period of 14 days to regenerative responses, including axonal sprouting, angiogenesis, neurogenesis and neuroplasticity. It is unclear if early mechanisms (12 hr) differ from late (day 7) mechanisms, e.g. anti-inflammatory vs. pro-inflammatory or neuroprotective vs. neuroregenerative and neurorestorative.
- We found that administration of MPCs at any time up to 7 days post-infarct could give rise to functional improvements in a rat model of focal ischemia. We did not investigate the effects, and therefore cannot comment on possible therapeutic benefits of administration after day 7 of the event.
- We saw evidence, from forepaw limb placement tests, that administration of MPCs improved function without delay following administration: the 6 and 12 hr groups had statistically better function than the vehicle group, the 24 h and 48 h groups improved by day 3, the 7 day group by day 14.
- Taken together, these observations support the use of MPCs delivered up to 7 days following ischemia.
- Imaging data, from rats treated with MPCs 24 h after ischemia, suggest that MPC administration at this time was able to reduce infarct volume by day 8, which could explain the greater functional improvements. Furthermore, greater neuronal activation was observed in the infarct region and greater neuronal activity was observed in the motor cortex of MPC-treated rats. We hypothesize that MPCs may provide a neuroprotective effect in sparing cortical tissue; as well, MPC treated animals displayed statistically greater neuronal activity in the primary motor cortex following forepaw stimulation. This may promote a neuroprotective effect in sparing cortical tissue; as well, MPC treated animals displayed statistically greater neuronal activity in the primary motor cortex following forepaw stimulation. This may promote a neuroprotective effect in sparing cortical tissue; as well, MPC treated animals displayed statistically greater neuronal activity in the primary motor cortex following forepaw stimulation. This may promote a neuroprotective effect in sparing cortical tissue; as well, MPC treated animals displayed statistically greater neuronal activity in the primary motor cortex following forepaw stimulation.

References

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