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

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Background

- Immunoselection of human mesenchymal stem cells from stromal precursor antigen 3 (STRO-3) 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 mesesenchymal 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 Rowett Nude (RNU) rats (Taconic IBU051001C, 300 g) underwent a modified permanent middle cerebral artery occlusion (MCAO) procedure (Tamura *et al*. 1981) resulting in a focal, right-sided cerebral infarction.
- According to pre-defined groups (n=12/group), rats received an intravenous (IV) injection into the lateral tail vein of vehicle at 24 h after MCAO or Mesoblast's human MPCs at 6 h, 12 h, 24 h, 48 h or 7 days after MCAO.
- Dose of MPCs was 1 million MPCs (3.63 million MPCs/kg) (170 µl of $6x10^6$ 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*. 1989) were carried out on forelimb (whisker, visual, tactile and proprioceptive placement) and on hindlimb (dorsal tactile, lateral tactile and proprioceptive placement). Each scored as follows: immediate response, 0.0 points; response within 2 s, 0.5 points; response within 2–3 s, 1.0 point; response >3 s, 1.5 points; no response, 2.0 points. Decrease (upward) in score reflects normalization of function.
- Body swing tests (modified from Borlongan *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=8/group) receiving MPCs or vehicle at 24 hr post-MCAO. On day 8 after MCAO, a T2-weighted whole-brain MRI anatomy scan was performed, as well as blood oxygen level dependent (BOLD) functional MRI (fMRI) following stimulus of forepaw or hindpaw (3 min baseline, 3 min forepaw; 3 min baseline, 3 min hindpaw). These animals were sacrificed on day 8.
- This work is done in alignment with the STAIR (Stroke Therapy Academic Industry Roundtable) and STEPS II (Stem Cell Therapeutics as an Emerging Paradigm for Stroke) initiatives.

Results

Forelimb Placement

- All MPC-treated groups had statistically significantly improved forelimb placement scores compared with the vehicle-treated group at day 28 after MCAO (24 hr group, $p<0.001$; others, $p<0.01$; Figure 1a).
- In all MPC-treated groups, a statistically significant improvement in forelimb placement score was evident at the first timepoint (Day 1, 3 or 14) following MPC administration.

Results

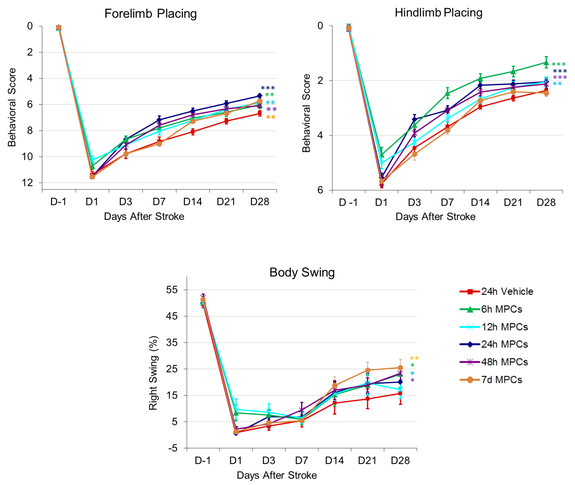

Figure 1. Effect of MPCs, administered 6 h to 7 days post-stroke in MCAO-treated rats, on (a) forelimb function; (b) hindlimb function; (c) body swing. *p<0.05; **p<0.01; ***p<0.001 for differences between groups at D28. D-1, pre-infarct; D1, D3 etc., days 1, 3 etc. post-infarct. Each limb placement score is the sum of subtest scores. MPCs, mesenchymal precursor cells; MCAO, middle cerebral artery occlusion

Hindlimb Placement

- All MPC-treated groups except the group receiving MPCs at 7 days 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 1b)

Body Swing

- All MPC-treated groups except the group receiving MPCs at 12 hr showed statistically significantly better body swing function than the vehicle-treated group at day 28 (7 day group, $p<0.01$; others, $p<0.05$; Figure 1c).

Infarct Volume

- Infarct volume measured with MRI imaging was significantly smaller in rats receiving MPCs 24 hr post-MCAO compared with vehicle-treated animals. (Figure 2). A mean 38 mm^3 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<0.01, Figure 3).
- In the group that received MPCs at 24 hrs, fMRI imaging during contralateral forepaw stimulation showed significantly greater activation of the primary motor cortex ipsilateral to the infarct (p<0.05, Figure 4a). There were no significant difference in activation of ipsilateral secondary motor cortex (Figure 4a), ipsilateral sensorimotor cortices (Figure 4b), or any contralateral cortices (data not shown).

Results

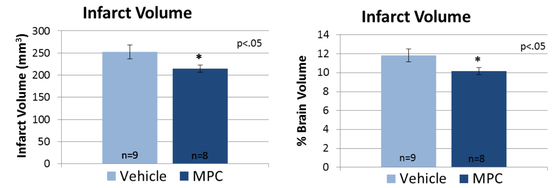

Figure 2. Effect of MPCs on infarct size (MRI measurement) in MCAO-treated rats. (a) Infarct volume, mm^3; (b) infarct as percentage of brain volume. MPCs, mesenchymal precursor cells; MCAO, middle cerebral artery occlusion

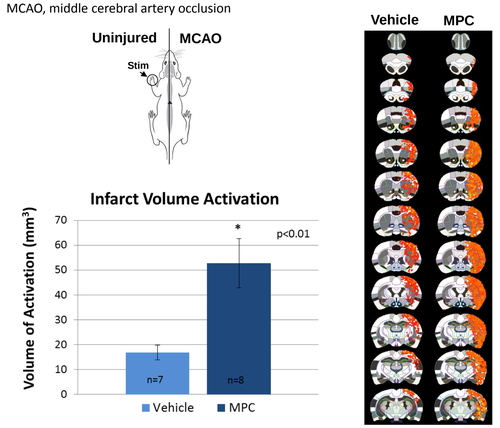

Figure 3. Neuronal activity in the infarct area following forepaw stimulation in MCAO-rats. Stimulation was contralateral to infarct; activation was measured ipsilateral to infarct. The volume of activation was significantly greater in the MPC-treated animals. MCAO, middle cerebral artery occlusion; MPC, mesenchymal precursor cell

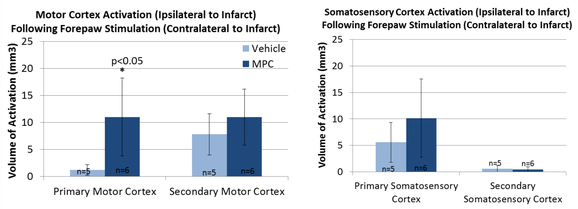

Figure 4. Volume of neuronal activation of: (a) motor cortex (b) somatosensory cortex, following forepaw stimulation in MCAO rats. Stimulation was contralateral to infarct; cortical activation was measured ipsilateral to infarct. MPC-treated animals displayed statistically greater neuronal activity in the primary motor cortex following forepaw stimulation. MCAO, middle cerebral artery occlusion; MPC, mesenchymal precursor cell

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.

Discussion (continued)

- 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 degenerative responses, including necrosis, inflammation, edema and apoptosis, give way over a period of14 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 and 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 h and 12 h groups had statistically better function than others at 24 h; 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 administered 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 treatment may enhance neuroplasticity and promote a reconstructive environment for improving neurological function as evidenced by the increased neuronal activity in the motor cortex.

Conclusions

- A single IV dose of human MPCs enhanced sensorimotor recovery in a rodent model of stroke.
- MPC administration after experimental stroke reduced infarct size and increased neuronal activity in the infarct area and motor cortex.

References

Borlongan CV *et al*. *Neuroreport* 1998;16:3615–21
De Ryck M *et al. Stroke* 1989;20:1383–90
See F *et al*.. *J Cell Mol Med* 2011;15:2117–29
Tamura A *et al*.. *J Cereb Blood Flow Met* 1981;1:53–60

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