Intra-Arterial Bone Marrow Mononuclear Cells in Ischemic Stroke
A Pilot Clinical Trial

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Background and Purpose—Bone marrow mononuclear cell (BM-MNC) intra-arterial transplantation improves recovery in experimental models of ischemic stroke. We aimed to assess the safety, feasibility, and biological effects of autologous BM-MNC transplantation in patients with stroke.

Methods—A single-blind (outcomes assessor) controlled Phase I/II trial was conducted in patients with middle cerebral artery stroke. Autologous BM-MNCs were injected intra-arterially between 5 and 9 days after stroke. Follow-up was done for up to 6 months and blood samples were collected for biological markers. The primary outcome was safety and feasibility of the procedure. The secondary outcome was improvement in neurological function.

Results—Ten cases (BM-MNC-treated) and 10 control subjects (BM-MNC-nontreated) were consecutively included. Mean National Institutes of Health Stroke Scale before the procedure was 15.6. Mean BM-MNCs injected were 1.59 × 10⁶. There was no death, stroke recurrence, or tumor formation during follow-up, although 2 cases had an isolated partial seizure at 3 months. After transplantation, higher plasma levels of beta nerve growth factor (β-nerve growth factor) were found compared with control subjects (P = 0.02). There were no significant differences in neurological function at 180 days. A trend to positive correlation between number of CD34+ cells injected and Barthel Index was found (r = 0.56, P = 0.09).

Conclusions—Intra-arterial BM-MNC transplantation in subacute ischemic stroke is feasible and seems to be safe. Larger randomized trials are needed to confirm the safety and elucidate the efficacy of BM-MNC transplantation.

Clinical Trial Registration-URL—www.clinicaltrials.gov. Unique identifier: NCT00761982.

Key Words: cell transplantation ◼ cerebral infarct ◼ stem cells

Cell-based therapy is a potential new approach in the treatment of ischemic stroke. Promising studies have shown improvement of neurological recovery in the acute phase of animal stroke models. Several biological effects such as attenuation of neuronal death, modulating microglial, reducing proinflammatory responses, and increasing neangiogenesis have been invoked.¹ However, very few clinical studies have been done in patients with stroke.²–⁴

We aimed to assess the safety, feasibility, and biological effects of autologous bone marrow mononuclear cell (BM-MNC) transplantation in a pilot single-blind (outcomes assessor) Phase I/II controlled clinical trial in middle cerebral artery (MCA) ischemic stroke.

Materials and Methods

Twenty patients (10 cases and 10 control subjects) with severe MCA ischemic stroke were prospectively enrolled. Criteria for inclusion were age between 18 and 80 years, an MCA ischemic stroke with a National Institutes of Health Stroke Scale score ≥ 8, and treatment window within 5 to 9 days of stroke onset. Trial profile and further inclusion and exclusion criteria are listed in online-only Data Supplement.

Transplantation was done 5 to 9 days after stroke onset. Fifty milliliters of bone marrow was obtained by puncture in the posterior iliac crest. Before any manipulation, 1 mL was separated for cell counting, flow cytometry, and bacterial culture. The aspirate was centrifuged on a Ficoll density gradient to isolate the mononuclear cells. On the same day, BM-MNCs were injected in the M1 segment of the infarct-related MCA at low pressure.
The first 10 consecutive patients included were considered the active group. No bone marrow aspiration or sham injection was performed in the control group.

Clinical evaluation, biochemical blood analysis, and recording of adverse events were performed the day after transplantation and 1, 3, and 6 months. Neurologists assessing the patients were unaware of the treatment allocation. MRI was performed the day after transplantation and at 6 months.

In 8 active cases and 9 control subjects, blood samples were drawn before and 4, 8, and 90 days after transplantation for determination of granulocyte-macrophage colony-stimulating factor and \( \beta \)-nerve growth factor serum level by means of enzyme-linked immunosorbent assay (DY256R&D Systems and Searchlight, reference: 84991 Aushon; respectively).

The primary outcome was safety and feasibility of the procedure, including assessment of adverse events during hospitalization and follow-up visits. The secondary outcome was the improvement in neurological function.

This study was done in accordance with the Declaration of Helsinki and approved by the National Ethics Committee. Written informed consent was obtained.

**Statistical Analysis**

We used univariate analysis for comparisons between both groups. Spearman rank correlation coefficient was used to assess the association between the cells injected and neurological status. Trophic factors serum levels at each time point were analyzed by repeated-measures analysis of variance with Bonferroni correction for multiple comparisons. We used SPSS 16.0 with \( P<0.05 \) considered statistically significant.

**Results**

Baseline characteristics were similar in both groups (see the online-only Data Supplement). All were severely disabled at inclusion (mean National Institutes of Health Stroke Scale score of 15.6 in BM-MNC group versus 15.0 in the control group, \( P=0.82 \)).

BM-MNC transplantation was done at 6.4 (±1.3) days after stroke onset. Cell viability was 92.8% and a mean \( 1.59 \times 10^6 \) BM-MNCs were intra-arterially injected. Of them, \( 3.38 \times 10^6 \) were CD34+ cells. All cultures were negative.

Rate of infusion through the microcatheter was 0.5 to 1 mL/min.

There were no serious adverse events during the BM-MNC transplantation procedure. No significant hemodynamic or respiratory changes occurred during transplantation. No worsening on neurological function was detected in any patient during hospitalization or at follow-up visits. Diffusion-weighted MRI did not show new ischemic lesions after transplantation.

During follow-up, the main adverse events are listed in the online-only Data Supplement. Two BM-MNC-treated patients had an isolated partial seizure (at 3 months) during follow-up, considered as a serious adverse event. In both patients an antiepileptic drug was started with no seizure recurrence. No death, stroke recurrence, or tumor formation was observed during the follow-up period.

There were no significant differences in neurological function compared with the control group. At 6 months, a greater nonsignificant proportion of BM-MNC-treated patients had modified Rankin Scale scores ≥2 (20% versus 0%, \( P=0.47 \); Figure 1).

Although no correlation between the functional status and the amount of transplanted BM-MNCs was detected, there was a trend toward a better outcome when higher numbers of CD34+ cells were injected, especially in the Barthel Index at
1 month after transplantation ($r=0.57$, $P=0.09$; online-only Data Supplement).

Higher significant levels of $\beta$-nerve growth factor appeared during the first week in BM-MNC-treated patients than in control subjects: $\beta$-nerve growth factor levels after 4 days were $10.3\pm 3.1$ versus $8.5\pm 2.9$ ($P=0.68$) and after 8 days were $12.8\pm 2.7$ versus $3.9\pm 2.5$ ($P=0.029$). Granulocyte-macrophage colony-stimulating factor levels did not change significantly after transplantation (Figure 2).

Discussion

In animal models of stroke, BM-MNCs enhance endogenous neurogenesis, angiogenesis, axonal sprouting, and synaptic plasticity and neurological improvement.2 However, few clinical studies have been done in patients with stroke.2,3 In this trial, BM-MNC intra-arterial transplantation was feasible and there were no adverse events as a consequence of the procedure or deaths, strokes, or tumor formation on follow-up. Two BM-MNC-treated patients had an isolated partial seizure by the third month after stroke that was considered a serious adverse event and required antiepileptic drug treatment. Although a clear relationship could not be established, it warrants careful surveillance in future studies. Intra-arterial injection was safe and diffusion-weighted MRI after transplantation showed no new ischemic lesion.

The optimal time window is not well known. In the stroke rat model, only rats receiving bone marrow stem cells 7 days after MCA occlusion exhibited decreased ischemic lesion volume.5 It is plausible that earlier treatment could produce a greater effect on remodeling after stroke. In a recent study,2 patients were treated with BM-MNCs within 24 to 72 hours after stroke onset; however, these patients are usually neurologically unstable. In fact, 2 of 10 patients required hemicraniectomy after transplantation. We chose a 5- to 9-day time window because patients with stroke at the early subacute phase have a more stable neurological deficit but still have an active process of remodeling, and conclusions regarding the safety are more reliable. In another study,4 patients with moderate to severe MCA stroke were treated within 3 to 7 days, showing no procedure-related adverse events, with 40% good clinical outcome.

Some biological effects were found. BM-MNCs contain hematopoietic progenitor stem cells that express CD34. Hematopoietic progenitor stem cells mediate neuroprotection in stroke animal models.6 Interestingly, there was a trend toward a better outcome with higher number of CD34+ cells injected. Therefore, we hypothesize that higher number of CD34+ cells transplanted could produce better neurological outcomes. Nerve growth factor contributes to brain plasticity and increases at 4 and 7 days after injection of mesenchymal stem cells in the stroke rat model.7 In this trial, higher $\beta$-nerve growth factor levels were detected after transplantation compared with control subjects, supporting the hypothesis of a secretory function of BM-MNCs.

Our study has several limitations. Although a modest nonsignificant improvement in modified Rankin Scale was seen, conclusions regarding efficacy could not be taken, because this study was not designed to detect efficacy. Second, this study was not randomized. However, clinical evaluations were blinded to treatment allocation that allowed assessing the safety of the procedure compared with control subjects. Moreover, we could evaluate the changes of trophic factors serum level to try to elucidate the biological effects of BM-MNCs.

In summary, our results suggest that autologous BM-MNCs intra-arterial transplantation in subacute MCA ischemic stroke is feasible and safe. Higher plasma $\beta$-nerve growth factor level was detected after injection of BM-MNCs, probably related to secretory function of BM-MNCs.

References

Supplemental Methods

Inclusion Criteria

Patients were enrolled in the study only if they met all of the following inclusion criteria:

- Symptoms and signs of clinically definite middle cerebral artery (MCA) acute stroke.
- Time of stroke onset is known and treatment can be started between day 5 and 9 of onset.
- DWI-MRI has reliably shown relevant acute MCA ischemic lesions
- Extracranial duplex/transcranial Doppler and magnetic resonance angiography (MRA) must confirm intra/extracranial arteries permeability.
- The stroke is severe (NIH Stroke Scale >= 8 before procedure).
- An age range of 18-80 years old.
- Written informed consent has been obtained.

Exclusion Criteria

Patients meeting any of the following criteria were excluded from study enrolment:

- Patients out of inclusion age range.
- Lacunar infarction.
- Patients with cancer.
- Patients with present or previous malignant disease during the last 5 years, except for basal cell carcinoma.
- Hematological causes of stroke.
- Severe co-morbidity.
- Hepatic or renal dysfunction.
- The patient is female and of childbearing potential (unless it is certain that pregnancy is not possible) or breast feeding.
- Patient is likely to be unavailable for follow-up.
- Patient with evidence of life threatening infection of life threatening illness.
- Patient was already dependent in activities of daily living before the present acute stroke.
Figure S1. Trial profile.

Acute MCA Stroke

Screening
TCD and MRI/MRA
Standard treatment for acute stroke

BM-MNC group
(n=10)

Bone marrow aspiration and BM-MNC injection

Follow-up at 1 day, 30 and 90 days

Final visit at 6 months
(n=10)

Control group
(n=10)

Follow-up at 1 day, 30 and 90 days

Final visit at 6 months
(n=10)

Follow-up visits:
- Clinical and functional evaluation
- Laboratory studies
- Adverse Event review

Brain MRI
Table S1. Baseline characteristics.

<table>
<thead>
<tr>
<th></th>
<th>BM-MNC group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>66.9 (13.9)</td>
<td>67.4 (12.7)</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Sex (Men)</strong></td>
<td>5 (50%)</td>
<td>7 (70%)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Vascular risk factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (60%)</td>
<td>9 (90%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>4 (40%)</td>
<td>5 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2 (20%)</td>
<td>6 (60%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Stroke characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left MCA</td>
<td>6 (60%)</td>
<td>5 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Intravenous thrombolysis</td>
<td>4 (40%)</td>
<td>2 (20%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Baseline NIHSS score</td>
<td>15.6 (2.9)</td>
<td>15.0 (4.2)</td>
<td>0.82</td>
</tr>
<tr>
<td>Time from stroke to inclusion</td>
<td>6.4 (1.3)</td>
<td>6.2 (1.7)</td>
<td>0.58</td>
</tr>
<tr>
<td>(days)</td>
<td></td>
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</table>

Data are number (%) or mean (SD).
Table S2. Adverse events.

<table>
<thead>
<tr>
<th></th>
<th>BM-MNC group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Urinary infection</td>
<td>2 (20%)</td>
<td>3 (30%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>1 (10%)</td>
<td>1 (10%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Depression</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3 (30%)</td>
<td>2 (20%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Partial seizure</td>
<td>2 (20%)</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Data are number (%).
Table S3. Correlation between number of BM-MNCs and CD34+ cells injected and functional status after transplantation.

<table>
<thead>
<tr>
<th></th>
<th>BM-MNCs</th>
<th>p</th>
<th>CD34+ cells</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td><strong>NIHSS score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>-0.38</td>
<td>0.32</td>
<td>-0.53</td>
<td>0.14</td>
</tr>
<tr>
<td>90 days</td>
<td>-0.16</td>
<td>0.66</td>
<td>-0.51</td>
<td>0.14</td>
</tr>
<tr>
<td>180 days</td>
<td>0.67</td>
<td>0.85</td>
<td>-0.25</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Barthel index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>0.12</td>
<td>0.73</td>
<td>0.57</td>
<td>0.09</td>
</tr>
<tr>
<td>90 days</td>
<td>0.18</td>
<td>0.61</td>
<td>0.48</td>
<td>0.16</td>
</tr>
<tr>
<td>180 days</td>
<td>0.12</td>
<td>0.75</td>
<td>0.46</td>
<td>0.18</td>
</tr>
<tr>
<td>mRankin score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>0.12</td>
<td>0.74</td>
<td>-0.28</td>
<td>0.43</td>
</tr>
<tr>
<td>90 days</td>
<td>-0.12</td>
<td>0.74</td>
<td>-0.51</td>
<td>0.13</td>
</tr>
<tr>
<td>180 days</td>
<td>-0.20</td>
<td>0.58</td>
<td>-0.48</td>
<td>0.16</td>
</tr>
</tbody>
</table>