Effects of intravenous administration of human bone marrow stromal cells after intracerebral hemorrhage in rats

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Object. The goal of this study was to investigate whether human bone marrow stromal cells (hBMSCs) administered by intravenous injection have a beneficial effect on outcome after intracerebral hemorrhage (ICH) in rats.

Methods. An ICH was induced in 54 adult male Wistar rats by a stereotactically guided injection of autologous blood into the right striatum. Intravenous infusion of the hBMSCs (3, 5, or 8 million cells) was performed 1 day after ICH, and for each dose group there was a control group that received injections of vehicle. Neurological function, which was evaluated using the Neurological Severity Score (NSS) and the corner turn test, was tested before and at 1, 7, and 14 days after ICH. After 14 days of survival, the area of encephalomalacia was calculated and histochemical labeling was performed.

For all three groups, there were no statistical differences in either the NSS or corner turn tests after 1 day. After 7 and 14 days, however, the three groups that received the hBMSCs showed significant improvement in functional scores compared with the control group. In addition, after 14 days there was significantly more striatal tissue loss in the placebo groups compared with each of the three treatment groups. The region of injury in the treated animals demonstrated a significantly increased presence of hBMSCs, immature neurons, neuronal migration, synaptogenesis, and newly formed DNA.

Conclusions. Intravenous administration of hBMSCs significantly improves neurological function in rats subjected to ICH. This improvement in the treated animals is associated with reduced tissue loss and increased local presence of the hBMSCs, mitotic activity, immature neurons, synaptogenesis, and neuronal migration.

KEY WORDS • bone marrow stromal cell • intracerebral hemorrhage • neural regeneration • rat

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PONTANEOUS ICH causes 10 to 20% of all strokes, but effective and standardized clinical treatment remains elusive.9 By the very nature of its pathophysiological features, spontaneous ICH results in a variety of neural injury mechanisms: direct mechanical injury, ischemia, toxicity, and apoptosis.10–12,28 No currently available medical therapy has shown a consistent or unambiguous benefit in terms of functional outcome. In recent years attention has been focused on the ability of undifferentiated pluripotent stem cells to improve experimental neurological conditions, including ischemic stroke, brain trauma, and spinal cord injury.6,17,19 Specifically, human embryonic neural stem cells have been used in a collagenase model of ICH to restore neurological function and demonstrate migration of the cells to the site of hemorrhage.13

Bone marrow contains a subpopulation of cells that can serve as tissue stem cells because they can be used as precursors of nonhematopoietic tissue. These pluripotent cells of bone marrow origin are referred to as MSCs.24 The MSCs have a capacity for self-renewal and differentiation in a variety of nonhematological tissues, and have the potential to be used for cell therapy.20–23 In the appropriate cellular microenvironments, MSCs are able to produce mesenchymal tissues, such as fibrous tissue, bone, cartilage, and muscle, and can differentiate specifically into adipocytes, osteoblasts, and chondrocytes.12,20 Of significance for the treatment of neurological disorders, MSCs pass through the blood–brain barrier to target sites of brain lesions under experimental conditions.16,17,29 In the neonatal mouse, MSCs migrate widely throughout the developing brain and have shown the capacity to differentiate into neurons and astrocytes.34 Cells of bone marrow origin infused systemically into rats preferentially migrate to ischemic cortex.7 In recent studies, hBMSCs have shown significant benefit in animal models of ischemic stroke and closed head injury.15,19 In these models of neural damage, the MSCs appear to have the capacity to induce endogenous brain-derived cells, likely derived from the SVZ, to participate in the restorative process. In light of the recognized ability of intravascularly delivered MSCs to treat neural injury and the potential application of stem cell technology to treat ICH, this experiment was conducted to test the hypothesis that hBMSCs improve functional outcome and reduce cellular injury after experimental ICH.
The BrdU-positive cell number was measured in the hBMSCs in PBS solution. Each experimental group had an equivalent control group, which was given the PBS vehicle solution but omitted the use of primary antibodies. Dead MSCs (J Chen and M Chopp, unpublished data) were used as controls in previous experiments in our laboratory and have demonstrated no effect. For mitotic labeling of newly formed DNA, all rats also received daily intraperitoneal injections of 100 mg/kg BrdU starting 24 hours after ICH. The NSS is a composite score in which motor, sensory, balance, and reflex measures are used to calculate a value ranging from 1 to 18, with the higher score implying greater neurological injury. The corner turn test is performed by placing the animal in a corner and observing the number of times (as a percentage) it turns right or left before exiting (the normal state is a value of 50%). All animals were killed after 14 days, and their brains were fixed in formalin and sliced into 2-mm-thick sections. Every 40th coronal section (cut at a thickness of 6 µm between the bregma +0.1 mm to –0.86 mm in each rat, for a total of six sections) was used for H&E and immunohistochemical staining.

The percentage of striatal tissue loss in one section was calculated using an image analysis system (Data Translation, Marlboro, MA). The area of preserved striatum on the side of the hemorrhage was subtracted from the area of the contralateral striatum, thus reckoning the degree of encephalomalacia or tissue loss from the injury in that brain section. A percentage value was then calculated by dividing the amount of cell loss by the total area of the contralateral striatum.

Well-established immunohistochemical analytical methods were used, which consisted of staining both control and treatment groups with synaptophysin, TUJ1, DCX, mAAb 1281, and BrdU. Synaptophysin (1:40 mAb, Clone SY38) is a marker of presynaptic plasticity and synaptogenesis.26 TUJ1 (1:400 mAb Class III beta-tubulin isotype) is a developmental neuronal marker, and DCX is a marker of neuronal migration,2 whereas mAAb 1281 (1:300 primary mouse anti-human nuclei mAb) is specific for all human cell types and is used to identify hBMSCs.26 On the other hand, BrdU (100 mg/kg) is a marker for newly formed DNA and is generally accepted as an expression of cell division and new cell growth. Control experiments consisted of staining coronal brain tissue sections as outlined earlier, but omitted the use of primary antibodies.

Quantification of TUJ1, DCX, and Synaptophysin

For semiquantitative measurements of TUJ1, DCX, and synaptophysin, six slides from the block (bregma +0.1 mm to –0.86 mm) were used. Synaptophysin was measured in the striatum, and TUJ1 and DCX were measured at the SVZ. Synaptophysin, TUJ1, and DCX were digitized under a 20 × objective lens (Olympus BX40; Olympus Optical Co., Tokyo, Japan) by using a 3-CCD color video camera (model DFC-970MD; Sony Corp., Tokyo, Japan) interfaced with an MCID image analysis system (Imaging Research, Inc., St. Catharines, ON, Canada). Data are presented as a percentage of area, in which the TUJ1-, DCX-, and synaptophysin-immunopositive areas in each field were divided by the total areas in the field (628 × 480 µm2). The BrdU-positive cell number was measured in the boundary around the lesion. Quantitative data for mAAb 1281 are presented as the total number of mAAb 1281–immunoreactive cells within contralateral and ipsilateral areas of each slide.

Statistical Analysis

Statistical evaluations of functional scores, area of ICH-related tissue damage, and histochemical results were performed using the independent Student t-test.

Results

All 54 animals survived the 14-day experimental period.
There was no apparent difference between the control group and any of the experimental groups in the results of NSS and corner turn tests 1 day after ICH. Nevertheless, after 7 days both tests showed significant improvement in results for the rats injected with hBMSCs compared with controls. The results of the neurological assessment according to the NSS for all groups can be seen in Fig. 1 left, whereas the data for corner turn tests are shown in Fig. 1 right.

The data for striatal tissue loss are shown in Fig. 2. The area of tissue loss as a percentage of the normal hemisphere was as follows (given as the mean ± standard error of the mean for all values): control, 30 ± 1%; 3 million hBMSCs, 23 ± 2.6% (p = 0.002); 5 million hBMSCs, 23 ± 2.5% (p = 0.003); and 8 million hBMSCs, 23 ± 3.8% (p = 0.002). As can be seen, there is virtually no difference between any of the treatment groups, with all of them showing significant improvement over the control group 2 weeks after ICH.

The mAb 1281, BrdU, synaptophysin, TUJ1, and DCX histochemical staining data shown in Fig. 3 suggest that there was a significant increase in the positive-staining cells in the region of ICH for all treatment groups compared with the controls. Specifically, for DCX, TUJ1, and synaptophysin, the area of positive-staining cells was significantly increased in treated animals compared with controls. Labeling of mAb 1281 was seen in the treated animals in the region of the ICH, verifying that the injected hBMSCs did reach the site of injured brain preferentially compared with the contralateral hemisphere. Staining for BrdU was significantly increased in the boundary zone around the ICH, implying localized new cell formation in the rats treated with hBMSCs compared with control animals. The BrdU colocalized with TUJ1, as seen in Fig. 4, suggesting the presence of newly formed immature neurons. Control immunostaining, which omitted the primary antibodies, did not show positive-staining cells.

Discussion

In this study of experimental ICH, intravenously injected hBMSCs given 1 day posthemorrhage preferentially migrated to the site of injury by 2 weeks, and treated rats had improved functional scores and less parenchymal cell loss compared with control animals. There were significant increases in immature neurons, neuronal migration, synaptogenesis, and new cell formation in the striatum and SVZ near the site of the ICH in the animals receiving the hBMSCs. These data support the growing body of evidence that intravenously administered pluripotent cells, in this case derived from human bone marrow, can have significant reparative effects in conditions of neural injury.

It has been documented that after rats are subjected to stroke and/or trauma, MSCs delivered by intravenous and intracarotid routes will pass through the blood–brain barrier, migrate selectively, target damaged areas of the brain, and improve functional recovery. The mechanism by which MSCs promote this recovery is not fully understood. It is known that ischemic stroke promotes the formation of new blood vessels (angiogenesis) as part of an endogenous response to injury. In normal circumstances after a cerebral infarction, however, the actual contribution of angiogenesis to the brain capillary network and overall recovery is probably insufficient to support the brain plasticity required for significant functional recovery.

A postulated means of neurological recovery with administration of MSCs after ischemic stroke is the secretion or induction of parenchymal expression of several growth factors, including vascular endothelial growth factor, which play a prominent role in generating new vascular tissue in the brain’s ischemic boundary zone. Using a different model of pathological insult (closed head injury), Mahmood, et al., used hBMSCs to improve the functional outcome in adult rats. A conclusion reached in their study was that there is successful migration into the interconnecting extracellular matrix of the brain and selective concentration around the injury site after the intravenous injection of these cells. Their work also suggested that the donor hBMSCs express cellular proteins specific for neurons and the growth of nervous tissue. This study and other similar ones demonstrate a functional benefit after the intracerebral and intravenous transplantation of hBMSCs; however, it is unlikely that this benefit is due to donor cells replacing damaged neurons. Some of the transplanted cells express physical and biochemical characteristics of neurons and astrocytes, but their number is small and the probability of them being integrated into functional neural circuitry is extremely low. In the adult central nervous system, the SVZ retains cells capable of further differentiation, and migration of cells from this region may contribute to the functional recovery induced by the exogenous MSCs.

Although much research has been conducted in the general area of stem cell use to treat neurological diseases, no studies have specifically investigated the benefits of intravenous injection of hBMSCs by using the injection model of ICH. Jeong, et al., performed intravenous transplantation of human embryonic neural stem cells in a rat collagenase model of ICH. They found that the neural stem cells entered the region of injury and differentiated into neurons (10%) and astrocytes (75%), yielding improved functional scores, but did not significantly reduce the brain atrophy that occurred after ICH.

An advantage of the hBMSCs used in our study is the...
availability and nonfetal origin of the cells, but a concern may be their failure to differentiate into neural cells in vivo compared with implanted embryonic cells, even in the presence of neural injury. Nevertheless, the effectiveness of hBMSCs in the models of brain trauma and ischemic stroke documented thus far is more likely related to the ability to induce neurotrophic and vascular growth factors, including brain-derived neurotrophic factor, nerve growth factor, basic fibroblast growth factor, and vascular endothelial growth factor, and is not dependent on neural differentiation.\(^{15,16,19,30,31}\) Administration of hBMSCs in a model of ICH is both a novel application of this technology and a means of testing the mechanisms of neurological recovery after this type of cerebral injury.

The synaptophysin data indicate increased synaptogenesis or new neuronal connections. Results obtained with TUJ1 support an emergence of more immature neurons or neuronal precursors, whereas DCX data indicate the migration of neurons in the perilesional region. These data are supported by the significant presence of the injected hBMSCs (mAb 1281) near the site of brain injury compared with the contralateral, unaffected hemisphere. New cells are being formed locally, as demonstrated by the elevated BrdU labeling in the region of the ICH. Some BrdU immunoreactive cells colocalized with TUJ1, implying formation of new neurons in the region adjacent to the ICH. These findings are consistent with previous study data showing the regenerative potential of the periventricular region, particularly the SVZ, which in ICH is directly adjacent to the site of injury.\(^{18,25}\) The origin of the newly formed cells and the specific mechanism by which neurological function is improved by treatment with hBMSCs were not

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**Fig. 3.** Photomicrographs and bar graphs showing values for DCX, mAb (MAB) 1281, BrdU, synaptophysin, and TUJ1 immunohistochemical labeling data for the three treatment (injection of 3, 5, and 8 million hBMSCs [hMSCs]) and control groups of rats. Arrows indicate cells or regions with positive staining. Original magnifications × 100 (panel with BrdU staining) and × 200 (all other panels).
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Fig. 4. Photomicrograph showing BrdU colocalizing with TUJ1 in a subpopulation of cells near the injury zone. Arrow indicates cells with positive staining. Original magnification × 40.

determined in this study and will require additional investigation. Magnetic resonance imaging technology will be a valuable aid to track the hBMSCs (labeled with paramagnetic particles) in vivo to determine the time course of their migration in the brain.

Conclusions

Intravenous injection of hBMSCs at doses of 3, 5, and 8 million cells 1 day after experimental ICH improves neurologic function and is associated with a significant reduction in local tissue loss. By 14 days posttreatment, the injected human cells are found in high concentrations at the site of the hemorrhage. Immunohistochemical staining methods using markers of neuronal plasticity indicate that new cells are being recruited and formed from adjacent regions, the most likely being the SVZ.

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References


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