Intravenous Administration of Human Bone Marrow Stromal Cells Induces Angiogenesis in the Ischemic Boundary Zone After Stroke in Rats

Jieli Chen, Zheng Gang Zhang, Yi Li, Lei Wang, Yong Xian Xu, Subhash C. Gautam, Mei Lu, Zhenping Zhu, Michael Chopp

Abstract—We tested the hypothesis that intravenous infusion of human bone marrow stromal cells (hMSCs) promotes vascular endothelial growth factor (VEGF) secretion, VEGF receptor 2 (VEGFR2) expression and angiogenesis in the ischemic boundary zone (IBZ) after stroke. hMSCs (1×10^6) were intravenously injected into rats 24 hours after middle cerebral artery occlusion (MCAo). Laser scanning confocal microscopy (LSCM), immunohistochemistry and ELISA were performed to assay angiogenesis and levels of human and rat VEGF in the host brain, respectively. In addition, capillary-like tube formation was measured using mouse brain-derived endothelial cells (MBDECs). Morphological and three dimensional image analyses revealed significant (P<0.05) increases in numbers of enlarged and thin walled blood vessels and numbers of newly formed capillaries at the boundary of the ischemic lesion in rats (n=12) treated with hMSCs compared with numbers in rats (n=12) treated with PBS. ELISA measurements showed that treatment with hMSCs significantly (P<0.05) raised endogenous rat VEGF levels in the IBZ from 10.5±1.7 ng/mL in the control group to 17.5±6.4 ng/mL in the hMSC-treated group. In addition, treatment with hMSCs increased endogenous VEGFR2 immunoreactivity. In vitro, when MBDECs were incubated with the supernatant obtained from cultured hMSCs, capillary-like tube formation was significantly (P<0.01) induced. However, hMSC-induced capillary-like tube formation was significantly (P<0.01) inhibited when the endothelial cells were incubated with the supernatant from hMSCs in the presence of a neutralizing anti-VEGFR2. These data suggest that treatment of stroke with hMSCs enhances angiogenesis in the host brain and hMSC-enhanced angiogenesis is mediated by increases in levels of endogenous rat VEGF and VEGFR2. (Circ Res. 2003;92:692-699.)

Key Words: middle cerebral artery occlusion ■ bone marrow stromal cell ■ angiogenesis ■ vascular endothelial growth factor ■ rats

In rats subjected to stroke and trauma, intravenously injected bone marrow stromal cells (MSCs) pass through blood brain barrier, migrate selectively and target damaged brain, and improve functional recovery.1–3 However, the mechanisms by which MSCs promote functional recovery are not clear. Stroke induces angiogenesis, and angiogenesis is associated with improved neurological recovery.4,5 Under normal circumstances after stroke, the contribution of angiogenesis to the brain capillary network is insufficient to support the brain plasticity required for functional recovery. MSCs secrete several growth factors including VEGF.6,7 Vascular endothelial growth factor (VEGF) has a prominent role in vascular formation.8 We have previously demonstrated that administration of recombinant human VEGF 165 (rh-VEGF165) to rats 48 hours after stroke significantly increased angiogenesis in the penumbra and improved functional recovery.4 Thus, it is reasonable to propose that functional benefit after treatment of stroke with human bone marrow stromal cells (hMSCs) may derive from cerebral angiogenesis. In the present study, we therefore tested the hypothesis that treatment of stroke with hMSCs enhances VEGF and thereby promotes angiogenesis in the host brain.

Materials and Methods

Animal Model

Adult male Wistar rats weighing 270 to 300 g were used in all our experiments. Rats were purchased from Charles River Breeding Company (Wilmington, Mass). All experimental procedures have been approved by the Care of Experimental Animals Committee of Henry Ford Hospital. Transient middle cerebral artery occlusion (MCAo) was induced using a method of intraluminal vascular occlusion modified in our laboratory.9 Briefly, the right common
carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were exposed. A length of 4.0–monofilament nylon suture (18.5 to 19.5 mm), determined by the animal weight, with its tip rounded by heating near a flame was advanced from the ECA into the lumen of the ICA until it blocked the origin of the MCA. Two hours after MCAo, animals were reanesthetized with halothane, and reperfusion was performed by withdrawal of the suture until the tip cleared the lumen of the ECA.

hMSC Transplantation
hMSCs were obtained from 20 mL aspirates from the iliac crest of one normal human donor. Each 20 mL of aspirate was diluted 1:1 with Hanks’ balanced salt solution (HBSS; Gibco) and layered over 10 mL of Ficoll (Ficoll-Paque; Pharmacia). After centrifugation at 2500 rpm for 30 minutes, the mononuclear cell layer was removed from the interface and suspended in HBSS. Cells were centrifuged at 1000 rpm for 10 minutes and resuspended in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% FBS. The cells were incubated at 37°C in 5% CO2 in flasks for 3 days and nonadherent medium (DMEM) supplemented with 10% FBS. The cells were harvested. They were then frozen for later use. Cells used in these experiments were harvested from 3 to 5 passages.

At 1 day after ischemia, randomly selected animals received either hMSCs (n=12) or phosphate buffered saline (PBS, n=12). Animals were anesthetized with 3.5% halothane and then maintained with 1.0% to 2.0% halothane in 70% O2 and 30% N2 using a face mask. Approximately, 1×106 hMSCs in 1 mL total fluid volume PBS were injected into a tail vein at 24 hours after stroke. We have previously shown that treatment of stroke in rat with dead MSCs or with rat liver fibroblasts provides no functional benefit compared with PBS-treated rats. Therefore, the present study uses PBS as the control treatment. Imunosuppressants were not used in any animals. All animals were euthanized at 14 days after MCAo.

To measure VEGFR1 (flt-1) and VEGFR2 (flk-1) expression in the ischemic brain after hMSC treatment, 6 rats received either 1×106 hMSCs (n=3) or PBS (n=3) at 1 day after stroke. These animals were euthanized at 3 days after treatment, and frozen sections were analyzed for VEGFR1 and VEGFR2 immunoreactivity.

Bromodeoxyuridine Labeling
Bromodeoxyuridine (BrdU, Sigma Chemical), the thymidine analog that is incorporated into the DNA of dividing cells during S-phase, was used for mitotic labeling. BrdU (50 mg/kg) was intraperitoneally injected daily for 14 consecutive days into ischemic rats starting 1 day after MCAo.

Three-Dimensional Image Acquisition and Analysis
To examine neovascularization in ischemic brain, fluorescein isothiocyanate (FITC) dextran (2×10⁶ molecular weight, Sigma; 1 mL of 50 mg/mL) was administered intravenously to the ischemic rats subjected to 14 days of MCAo. FITC-dextran remains dissolved and free in plasma. The brains were rapidly removed from the severed heads and placed in 4% paraformaldehyde at 4°C for 48 hours. Coronal sections (100 μm thick) were cut on a vibratome. The vibratome sections were analyzed with a Bio-Rad MRC 1024 (argon and krypton) laser-scanning confocal imaging system mounted onto a Zeiss microscope (Bio-Rad), as previously described. Seven 100-μm thick vibratome coronal sections at 2-mm intervals from bregma 8.2 mm to bregma –8.8 mm from each animal injected with FITC-dextran were obtained. Eight brain regions in the ipsilateral and contralateral hemispheres were selected within a reference coronal section (internal capsule 8.8 mm, bregma 0.8 mm). These regions were scanned in 512×512 pixel (279×279 μm²) format in the x-y direction using a 4× frame-scan average, and 25 optical sections along the z-axis with a 1-μm step-size were acquired under a 40× objective. Vascular branch points, segment lengths, and diameters were measured in three dimensions using software developed in our laboratory.

Immunohistochemistry and Quantification
To identify grafted hMSCs, a mouse anti-human monoclonal antibody (MAB1281, Chemicon International, Inc.) was used at a titer of 1:300. To distinguish endogenous and exogenous VEGF immunoreactivity, a goat polyclonal antibody specific for human VEGF (Ab-1, CALBIOCHEM) and a goat polyclonal antibody against rat VEGF (P-20, Santa Cruz Biotechnology, Inc.) were used at a titer of 1:200 and 1:100, respectively. To identify cell types of VEGF immunoreactivity and the cellular phenotype of grafted hMSCs, double immunofluorescence labeling for von Willebrand factor (vWF), a marker for endothelial cells, or glial fibrillary acidic protein (GFAP), a marker for astrocytes, were performed. A monoclonal antibody against vWF (DAKO) and a polyclonal antibody against GFAP (DAKO) were used at a titer of 1:400 and 1:1000, respectively. Coronal sections were incubated with the antibody against VWF, GFAP at 4°C, and sections were then incubated with the anti-mouse or anti-rabbit immunoglobulin antibody conjugated to Cy5 (Vector). These sections were incubated with the antibody against VEGF, human cell nuclei (MAB1281), or BrdU at 4°C, and then with the anti-goat or anti-mouse immunoglobulin antibody conjugated to FITC. For BrdU immunostaining, DNA was first denatured by incubating brain sections (6 μm) in 50% formaldehyde 2× SSC at 65°C for 2 hours and then in 2N HCl at 37°C for 30 minutes. Sections were then rinsed with Tris buffer and treated with 1% of H2O2, to block endogenous peroxidase. Sections were incubated with a mouse monoclonal antibody (mAb) against BrdU (1:1000, Boehringer Mannheim) overnight and incubated with biotinylated secondary antibody (1:200, Vector) for 1 hour. BrdU (CY5) with MAB1281 (FITC) double staining was performed. Control experiments consisted of staining brain coronal tissue sections as outlined above, but omitted the primary antibodies.

To test whether hMSC treatment induces VEGFR1 and VEGFR2 expression in cerebral endothelial cells, flt-1 and flk-1 immunohistochemistry staining was performed on 20-μm thick frozen sections. A mouse monoclonal anti-VEGFR1 (flt-1, Santa Cruz Biotechnology) and anti-VEGFR2 (flk-1, Santa Cruz Biotechnology) were used at a titer of 1:100 and 1:100, respectively. To quantify flt-1 and flk-1 immunoreactive endothelial cells, numbers of endothelial cells and numbers of flt-1 and flk-1 immunoreactive endothelial cells in 10 vessels adjacent to the ischemic lesion were counted from each rat. Data are presented as percentage of flt-1 and flk-1 immunoreactive endothelial cells to the total endothelial cells in 10 enlarged vessels from each rat.
For semiquantification of VEGF immunoreactivity, five VEGF immunostained coronal sections at 100-μm interval were analyzed from each brain. Eight fields of view from the ischemic penumbra (cortex and striatum, Figure 1) in each section were digitized under a ×20 objective (Olympus BX40) using a 3-CCD color video camera (Sony DXC-970 MD) interfaced with MCID image analysis system (Imaging Research). All values were presented as a percentage of the maximum value obtained.

To quantify BrdU immunoreactive endothelial cells, numbers of endothelial cells and numbers of BrdU immunoreactive endothelial cells in 10 enlarged vessels adjacent to the ischemic lesion were counted from each rat. Numbers of endothelial cells and BrdU immunoreactive endothelial cells in the 10 vessels of the contralateral homologous area were also counted. Data are presented as percentage of BrdU immunoreactive endothelial cells to total endothelial cells in 10 enlarged vessels from each rat.

ELISA for VEGF
VEGF levels in rat brain tissue (n=4/group) were measured using commercially available kits specific for human VEGF (Human VEGF Immunoassay, Catalog number DVE00, Quantikine, R&D Systems) or rat VEGF (Mouse VEGF Immunoassay, R&D Systems), respectively, according to the manufacture’s instructions. The brain tissue extract was obtained from the ischemic boundary zone at 14 days after MCAo.

VEGF and VEGFR2 Expression
To test whether hMSC supernatant increases brain endothelial cell VEGF and VEGFR2 expression in vitro, brain endothelial cells were treated with or without 50% hMSC supernatant for 24 hours. VEGF and VEGFR2 immunohistochemistry staining was performed on slides containing cultured endothelial cells. DAPI was used as a nuclear counterstaining. All assays were performed in triplicate. VEGF and VEGFR2 (flk-1) reactive cells were counted in randomly selected 5 microscopic fields under ×10× objective. The percentage of VEGF and VEGFR2 (flk-1) reactive cells within the total number of DAPI positive cells were measured.

Capillary-Like Tube Formation Assay
To examine if hMSCs induce angiogenesis, a capillary-like tube formation assay was performed using mouse brain-derived endothelial cells.14 Briefly, 0.8 mL of 4°C growth factor reduced Matrigel (Becton Dickinson) was added to prechilled 35-mm culture dishes and allowed to polymerize at 37°C for 2 to 5 hours. The endothelial cells (2×10⁴ cells) were incubated for 3 hours in (1) regular cell culture medium (DMEM), (2) 100% supernatant from 4-day-old hMSC culture, or (3) hMSC supernatant with a rat anti-mouse neutralizing antibody to VEGF receptor 2 (VEGFR2, DC101, 10 μg/mL, Imclone System). All assays were performed in triplicate. For quantitative measurements of capillary tube formation, Matrigel dishes were digitized under a 2.5× objective (Olympus BX40) for measurement of total tube length of capillary tube formation using a video camera (Sony DXC-970 MD) interfaced with MCID image analysis system (Imaging Research). Tracks of endothelial cells organized into networks of cellular cords (tubes) were counted and averaged in randomly selected 3 microscopic fields.15

Statistical Analysis
Cerebral microvessel angiogenesis was analyzed by calculating the total surface area of microvessels under a field of view (M. Lu, G.Z. Zhang, H. Soltanian-Zadeh, N. Oja-Tebbe, M.M. KHALIGHI, D. Morris, M. Chopp, unpublished data, 2002). Due to a lack of normality, ranked data were employed for the analysis microvessel surface area. Data in the two groups were first analyzed by a one-way ANOVA. All data are presented as mean±SD. A value of P<0.05 was taken as significant.

Results
Administration of hMSCs Induces Angiogenesis
Measurements of BrdU immunoreactive endothelial cells revealed few BrdU immunoreactive endothelial cells in the contralateral hemisphere (Figures 2A and 2D), which is consistent with fact that proliferation of the cerebral endothelial cells essentially ceases in the adult brain.16 In the ipsilateral hemisphere, however, there was a significant (P<0.05) increase in the number of BrdU immunoreactive endothelial cells in rats treated with PBS compared with the contralateral hemisphere (Figures 2B, arrow, and 2D). Treatment with hMSCs significantly (P<0.05) enhanced the number of BrdU immunoreactive endothelial cells in the ipsilateral hemisphere compared with the numbers in rats treated with PBS (Figures 2C, arrows, and 2D). BrdU immunoreactive endothelial cells primarily localized to enlarged cerebral vessels (Figures 2B and 2C). Enlarged thin-walled vessels are termed “mother” vessels and are markers of angiogenesis after cerebral ischemia.4,16 Treatment with hMSCs significantly (P<0.05) increased vascular perimeters in the ipsilateral hemisphere compared with perimeters in rats treated with PBS (Figure 2E).

To further examine angiogenesis, three-dimensional analysis was performed using software developed in our labora-
tory, which measures numbers of branch points, segment lengths, and diameters of vessels. Segments of capillaries were significantly (P<0.05) shorter in the hMSC-treated and MCAo-PBS groups (Figure 3 and Table) than in the homologous tissue in the contralateral hemisphere (Figure 3 and Table). Treatment with hMSCs (Figure 3 and Table) significantly (P<0.05) increased numbers of branch points in the boundary regions of ischemia compared with numbers in the homologous tissue in the contralateral hemisphere (Figure 3 and Table) and the ipsilateral hemisphere of rats treated with PBS (Figure 3 and Table). To avoid errors in statistical analysis for unpaired correlated data, three-dimensional cerebral vessels were further analyzed by calculating the total surface area of microvessels under a field of view. Vessel surface area in the ipsilateral hemisphere significantly (P<0.05) increased in rats treated with hMSCs compared with animals treated with PBS (Table).

Administration of hMSCs Increases Endogenous Brain Levels of VEGF and VEGFR2

Rats treated with PBS did not show any MAB1281 (a marker for human cells) immunoreactivity in brain tissue. In contrast, MAB1281 immunoreactive cells were detected in multiple brain regions of the ipsilateral hemisphere including cortex and striatum in rats treated with hMSCs, indicating specificity of this antibody for human cells (Figure 4A). The vast majority of hMSCs were localized to the ischemic boundary zone, with some cells within vessels (Figure 4B). Few hMSCs were detected in the contralateral hemisphere of brain. Scattered hMSCs (1% to 2%) showed vWF immunoreactivity. Likewise, less than 1% of BrdU immunoreactive cells were MAB1281-positive, indicating that the newly formed cells do not originate from the injected hMSCs.

To examine whether administration of hMSCs increases brain levels of VEGF, ELISAs for endogenous rat VEGF and exogenous human VEGF were performed. Treatment with hMSCs significantly (P<0.05) increased endogenous rat VEGF levels in the ischemic boundary regions from 10.5±1.7 ng/mL in the control group to 17.5±1.6 ng/mL in the hMSC-treated group. Human VEGF level was not detected in ischemic brain extracts. Parallel to ELISA results, immunostaining for endogenous rat VEGF showed increased VEGF immunoreactivity within the ischemic boundary in rats treated with hMSCs (Figures 4C and 4D). Many rat VEGF immunoreactive cells exhibited GFAP immunoreactivity (Figures 4E through 4G). Semiquantitative analysis revealed that rat VEGF immunoreactivity was significantly (P<0.05) increased in rats treated with hMSCs compared

Three-Dimensional Quantitative Measurements of Vascular Structure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contralateral</th>
<th>MCAo</th>
<th>MCAo+hMSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, μm</td>
<td>4.2±0.1</td>
<td>4.3±0.1</td>
<td>5.2±0.1†</td>
</tr>
<tr>
<td>Length, μm</td>
<td>32.8±1.6</td>
<td>25.5±1.2*</td>
<td>28.1±1.8*</td>
</tr>
<tr>
<td>No. of branch points (density)/0.002 mm³</td>
<td>134.3±7.6</td>
<td>131.6±11</td>
<td>177.7±11.4†</td>
</tr>
<tr>
<td>Total vessel surface area (μm²)/0.002 mm³</td>
<td>66 958±4626</td>
<td>44 558±5997</td>
<td>76 847±4601†</td>
</tr>
</tbody>
</table>

*P<0.05 compared with contralateral; †P<0.05 compared with MCAo. n=3 per group.
with the immunoreactivity in the control rats (Figure 4H). However, immunostaining with the anti-human VEGF antibody exhibited a few VEGF-positive cells in the ischemic brain. Furthermore, treatment with hMSCs significantly ($P<0.05$) increased the number of flk-1 immunoreactive endothelial cells in the ipsilateral hemisphere compared with the number in control rats (Figures 4I through 4K), whereas the number of fli-1-positive cells did not significantly increase (14.8±2.1% in hMSCs versus 11.4±1.6% in control). This suggests hMSC treatment after stroke induces endogenous cells to express VEGF and VEGFR2.

**hMSCs Upregulate VEGF and VEGFR2 Expression in Brain Endothelial Cells**

To further examine the effect of hMSCs on expression of VEGF and its receptors in endothelial cells, mouse-brain endothelial cells were treated with or without 50% hMSC supernatant for 24 hours. Consistent with in vivo findings, incubation with hMSC supernatant significantly ($P<0.05$) increased the number of VEGF (Figures 5A through 5C) and flk-1 (Figures 5D through 5F) immunoreactive cells compared with the number in control group.

**hMSCs Induce VEGF, Which Mediates Angiogenesis In Vitro**

To examine whether hMSCs induce angiogenesis, a capillary-like tube formation assay was performed. A significant ($P<0.05$) increase in capillary-like tube formation was detected when the endothelial cells were incubated with 100% hMSC supernatant (Figures 6B and 6D) compared with the endothelial cells incubated with regular cell culture medium (Figures 6A and 6D). However, when the endothelial cells were incubated with 100% supernatant from hMSCs in the presence of a rat anti-mouse neutralizing antibody to VEGFR2, capillary-like tube formation was significantly ($P<0.05$) inhibited compared with the supernatant alone (Figures 6C and 6D). The blockade of the VEGFR2 with anti-VEGFR2 antibody did not completely abolish hMSC-induced capillary-like tube formation (Figure 6D).

**Discussion**

Our data demonstrate that treatment of stroke with hMSCs significantly enhances angiogenesis in ischemic brain, and hMSC-enhanced angiogenesis is likely mediated by increases in endogenous rat VEGF and VEGFR2 expression. The present investigation was undertaken to identify potential mechanisms underlying the promotion of neurological functional recovery induced by hMSC treatment of stroke. Angiogenesis is associated with improved neurological recovery from stroke. Stroke patients with a higher cerebral blood vessel density make better progress and survive longer than patients with lower vascular density. Newly formed vessels improve tissue perfusion around the ischemic boundary zone and enhancement of angiogenesis promotes functional recovery in rats after stroke. hMSC-enhanced angiogenesis in ischemic brain, therefore, likely contributes to improvement of neurological functional recovery. Although proliferation of cerebral endothelial cells ceases in the adult brain, stroke induces neovascularization within the ischemic boundary zone. Newly formed vessels are derived from enlarged thin wall vessels by sprouting and by invagination. Our findings that treatment with hMSCs significantly increased vascular perimeter and numbers of capillaries are consistent with previous reports on dynamic formation of new vessels in ischemic brain. In addition, our observations that treatment with hMSCs increased numbers of proliferated endothelial cells and a few hMSCs (~1%) exhibit endothelial phenotype support the concept that formation of new vessels arises from the proliferation and migration of endothelial cells from the adjacent tissue and from circulating endothelial progenitor cells. To our knowledge, this is the first study using...
three-dimensional image analysis to demonstrate that hMSCs promote angiogenesis in adult brain after stroke.

hMSC treatment of ischemic stroke activates the endogenous cell expression of both VEGF and its receptor VEGFR2. VEGF exerts biological function via at least two related receptor tyrosine kinases, VEGFR1 and VEGFR2. The binding of VEGF to VEGFR2 elicits activation of signaling cascades that trigger the biological effects of VEGF. VEGFR2 has been shown to be essential for endothelial progenitor cell proliferation and differentiation. Blocking VEGFR2 inhibits angiogenesis in adult mice. Data in the present study demonstrate that treatment with hMSCs significantly increases rat brain levels of VEGF and VEGFR2 but not human VEGF levels. Distribution of increased VEGF corresponds with the localization of hMSCs in the host brain. Therefore, these data suggest that hMSCs increase endogenous levels of VEGF and VEGFR2. Our in vivo findings were confirmed...
by in vitro data showing that incubation of endothelial cells with hMSC supernatant increases endothelial cell expression of VEGF and VEGFR2. Furthermore, hMSC-induced capillary-like tube formation is inhibited by a neutralized antibody against VEGFR2. Collectively, we propose that administration of hMSCs enhance expression of VEGF in astrocytes and endothelial cells and VEGFR2 in endothelial cells, and that enhanced VEGF interacts with increased VEGFR2 in endothelial cells, which consequently promotes angiogenesis in adult ischemic brain.

Although the present study was not designed to investigate mechanisms of how hMSCs upregulate endogenous VEGF and VEGFR2, a possible tie between the exogenous hMSC and parenchymal cell local production of growth factors may be related to the ability of MSCs to produce a wide variety of trophic factors and cytokines, some of which activate the production of VEGF and VEGFR2. For example, hMSCs secrete bFGF,20–22 and bFGF promotes expression of VEGF and VEGFR2.23,24 We have also demonstrated that when hMSCs are cocultured with injured or rat ischemic brain, an array of trophic factors are produced, including bFGF.22 Thus, we speculate that hMSCs, by producing cytokines and trophic factors, indirectly upregulate the production of endogenous VEGF and VEGFR2, which promote angiogenesis in the ischemic brain.

The mechanisms of action of bone marrow stromal cells to treat neural injury and stroke stand in sharp contrast to those of stem and progenitor cells. Stem and progenitor cells, being totipotent cells, when placed in injured brain, are designed to replace dead and injured tissue.25,26 Embryonic stem cells placed in brain have been shown to differentiate into parenchymal cells and to migrate to sites of injury. Bone marrow stromal cells also likely contain a subpopulation of stem-like cells, which can differentiate into brain cells.27 However, these cells are a minor subpopulation of the hMSCs we use and do not contribute to the restoration of function, and only a very small percentage of the hMSCs assume parenchymal cell phenotype.10 We use MSCs not to replace tissue, but to augment function and to enhance plasticity of remaining tissue, particularly tissue in the boundary zone of the ischemic lesion. Thus, hMSCs when injected intravenously enter brain and evoke the expression of growth and trophic factors from the endogenous cells, primarily astrocytes and endothelial cells. The hMSCs are essentially catalysts for the production angiogenic remodeling of injured brain by means of VEGF and its receptor, VEGFR2.

The present study shows that treatment with MSCs enhances angiogenesis by increasing endogenous levels of VEGF and VEGFR2, suggesting that administration of MSCs provides a microenvironment to activate endogenous restorative mechanisms of ischemic brain. Therefore, treatment with MSCs may offer an additional avenue for stroke therapy.

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