Early Protective Effect of Bone Marrow Mononuclear Cells Against Ischemic White Matter Damage Through Augmentation of Cerebral Blood Flow

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Background and Purpose—To investigate the efficacy of bone marrow mononuclear cell (BMMNC) treatment against ischemic white matter (WM) damage in a hypoperfused brain.

Methods—Mice were administered intravenous treatment of vehicle, spleen-derived marrow mononuclear cells (MNCs), or BMMNCs (5 × 10⁶ cells) obtained from enhanced green fluorescent protein transgenic mice 24 hours after bilateral common carotid artery stenosis (BCAS), and then euthanized at either 1 day or 30 days after treatment.

Results—Laser speckle perfusion imaging analyses revealed marked recovery of cerebral blood flow (CBF) in the early phase after BMMNC treatment (6 hours after administration), before histological evidence of angiogenesis was assessed by fluorescein-isothiocyanate-dextran perfusion assay. BMMNC treatment induced an increase in vascular endothelial growth factor and Ser1177 phosphorylabeled endothelial nitric oxide synthase levels in the BCAS-induced mouse brains at 1 day after the treatment. BCAS-induced ischemic WM lesions were significantly improved 30 days after BMMNC treatment despite any evidence of direct structural incorporation of donor BMMNCs into endothelial cells and oligodendrocytes. Instead, enhanced green fluorescent protein-positive donor cells with morphological features of pericytes were observed in the vessel walls. Post-BMMNC administration of an NOS inhibitor abolished early CBF recovery and produced protective effects against ischemic WM damage.

Conclusions—BMMNC treatment provides marked protection against ischemic WM damage, enhancing CBF in the early phase and in subsequent angiogenesis, both of which involve nitric oxide synthase activation. These findings suggest promise for the application of BMMNCs for subcortical ischemic vascular dementia. (Stroke. 2010;41:2938-2943.)

Key Words: bone marrow ■ cerebral blood flow ■ white matter ■ eNOS

Therapeutic use of bone marrow (BM)-derived cells has been shown to ameliorate functional deficits after stroke and is accompanied by augmentation of angiogenic and regenerative responses.⁴ Although early functional improvement has been noted within days of treatment,⁴ its precise mechanism remains to be elucidated. A recent study has demonstrated that the administration of bone marrow mononuclear cells (BMMNCs) induces immediate endothelial nitric oxide synthase (eNOS)-dependent vasodilation in ischemic femoral arteries.⁵ Furthermore, vascular endothelial growth factor (VEGF) facilitates an early increase in blood flow that is probably because of its vasodilating effect.⁶ These findings also suggest that immediate protection is provided by BM-derived cells in ischemic brains. Nevertheless, direct evidence for the early beneficial effect of BM-derived cells on cerebral blood flow (CBF) is lacking.

In this study, we explored whether and, if so, how BMMNC treatment provides early improvement of CBF using a mouse model of cerebral hypoperfusion.⁷ This model, generated by placing microcoils on the bilateral common carotid arteries, recapitulates ischemic white matter (WM) changes observed in subcortical ischemic vascular dementia. Subcortical ischemic vascular dementia is the most common subtype of vascular dementia.⁸ We therefore intravenously injected green fluorescent protein (GFP)-labeled BM-derived cells obtained from GFP-transgenic C57BL/6J mice⁹ into cerebral hypoperfusion-operated C57BL/6J mice to investigate the possible efficacy of BMMNCs in alleviating ischemic WM changes.
Materials and Methods

Animal Preparation
Male C57BL/6J mice weighing 22 g to 26g were subjected to bilateral common carotid artery stenosis (BCAS) (n = 69). This procedure was performed by applying the coils with an inner diameter of 0.18mm to common carotid arteries, as described in a previous study. Sham-operated mice (sham mice) (n = 40) underwent the same surgical procedure without using coils. Anesthesia was maintained with halothane (1.5%) in air and was delivered via a snout mask. Body temperature was maintained at 37°C using a feedback-controlled heating pad. In addition, some animals were cotreated with the NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME; 1 mg/mL in drinking water; Nakarai Tesque, Kyoto, Japan) initiated after BMMNCs or vehicle administration, and some were cotreated with neuronal NOS (nNOS) specific inhibitor, 7-nitroindazole (7-NI) (30 mg/kg in peanut oil intraperitoneally; Sigma, St. Louis, Mo) 1 hour before CBF measurement. The timing of 7-NI injection was determined by the transient effect of 7-NI on NOS activity.

Cell Preparation and Treatment With BMMNCs
Bone marrow cells (BMCs) were prepared from medullary cavities of the humeri, femora, and tibiae of C57BL/6J-Tg (CAG [a promoter composed of the cytomegarovirus immediate early region enhancer linked to chicken beta-actin promoter sequences]-enhanced GFP) mice (5 to 6 weeks old; Japan SLIC, Inc., Hamamatsu, Japan). Spleenocytes were prepared by homogenization of the spleen. BMMNCs and spleen-derived mononuclear cells (Sp-MNCs) were obtained by density-gradient centrifugation with Lympholyte-M solution (Cedarlane Labs, Ontario, Canada). Twenty-four hours after BCAS or sham operation, 5 x 10⁵ BMMNCs, 5 x 10⁵ Sp-MNCs, or the same volume of phosphate-buffered saline were injected intravenously via the tail vein.

Flow Cytometry Analysis
To characterize the BMMNCs, whole BMCs, and Sp-MNCs, fluorescence-activated cell sorter analysis data were obtained using a FACSAria flow cytometer (Becton Dickinson, Franklin Lakes, NJ). An expanded Methods section is available in an online supplement available at http://stroke.ahajournals.org.

Measurement of Cerebral Blood Flow
Relative CBF was determined by laser speckle perfusion imaging (Omegawave, Inc., Tokyo, Japan), which obtains high-resolution, two-dimensional imaging and has a linear relationship with absolute CBF values. Recordings were performed through the skull under 1.5% halothane anesthesia. The peristomeum, which adheres to the skull, was widely removed with fine-tip forceps. For each recording, the skull surface was wiped with saline-soaked gauze and then covered with a thin layer of gel (Aquasonic, Parker Laboratories, Inc., Fairfield, NJ) to prevent drying. Calibration was carried out with a calibration reference device (Calibrator S/N 080715 to 5, Omegawave, Inc., Tokyo, Japan) before each test. The mean CBF was measured in identically sized regions of interest (500 pixels) located 1 mm posterior and 2 mm lateral from the bregma. CBF values were expressed as a percentage of the preoperative value. CBF recordings were obtained: before operation; 10 minutes, and 24 hours after the operation; as well as at 6 hours, 1 day, 7 days, 14 days, and 30 days after BMMNCs or vehicle administration. In addition, to test the effect of nNOS inhibition and Sp-MNCs on CBF in the early phase, CBF recordings were obtained up to 1 day after Sp-MNCs, BMMNCs, or vehicle administration with or without an nNOS-specific inhibitor.

Enzyme-Linked Immunosorbent Assay for VEGF
The brain tissue extract was obtained at 1 day after BMMNCs or vehicle administration. See the Supplemental Methods for details.

Western Blot Analysis
Cerebral protein levels of eNOS, Ser1177-phosphorylated eNOS, myelin basic protein, and β-actin were assessed by Western blot analysis. Detailed procedures are described in the Supplemental Methods.

Density of Perfused Capillaries
To identify the network of perfusable capillaries, animals were transcardially perfused at 1 day and at 30 days after BMMNCs or vehicle administration with 5 mL fluorescein-isothiocyanate-dextran (2 x 10⁵ molecular weight, Sigma, St. Louis, Mo; 15 mg/mL). Five coronal cryosections (20 µm thick) from each animal (bregma, −1 to +1 mm) were obtained. Capillary density was expressed as the number of capillaries per square millimeter. See the Supplemental Methods for details.

Histology and Immunofluorescence
The male brain was analyzed for demyelinating change with Kluver-Barrera staining and immunostained for glial fibrillary acidic protein (a marker of astrocyte), Iba-1 (microglia), glutation S-transferase π (oligodendrocyte), platelet-derived growth factor beta (PDGFβ) (pericyte), and CD31 (vascular endothelial cell) at 30 days after treatment. See the Supplemental Methods for details.

Statistical Analysis
Data are presented as mean ± SD. CBF values were compared between the groups by two-way repeated-measures ANOVA. Welch t-tests were used to evaluate significant differences between the groups at specific time points. Differences in the grading of WM lesions were determined by the Mann-Whitney U-test. Other statistical analyses were carried out by a one-factor ANOVA followed by the Bonferroni correction. A probability of P < 0.05 was considered statistically significant.

Results
Flow Cytometry Analysis of BMMNCs and Sp-MNC
Lineage marker-negative and c-kit-positive (Lin⁻/c-kit⁺) cells, which represent immature myeloid cells including hematopoietic stem and progenitor cells, were significantly enriched in BMMNCs compared with those in whole BMCs and Sp-MNCs (P < 0.05, n = 5/group; BMMNCs, 4.32 ± 0.37%; whole BMCs, 3.12 ± 0.51%; and Sp-MNC, 0.46 ± 0.10%) (Supplemental Figure I).

Effects of BMMNC Treatment on CBF
There were no significant differences in CBF among the sham mice (Figure 1A). CBF of the BMMNC-treated BCAS mice was significantly (P < 0.05) higher than both the vehicle-treated mice and the Sp-MNC-treated BCAS mice at 6 hours after administration (Figure 1A, C). However, the BMMNC-induced early recovery of CBF was inhibited by oral L-NAME treatment initiated after BMMNC administration. CBF recovery after BCAS was markedly inhibited with L-NAME and 33.3% (4 of 12) of the mice died within 3 days of treatment, whereas all the mice without L-NAME survived the BCAS insult. Meanwhile, CBF in sham, BCAS, and BMMNC-treated BCAS mice was not altered significantly by 7-NI at 6 hours or at 1 day after BMMNCs or vehicle administration (Figure 1D). CBF values in deceased mice were excluded given that these mice showed extremely low CBF values before their deaths.

Effects of BMMNC Treatment on Angiogenesis
At 1 day after BMMNC or vehicle administration, no significant differences were found in capillary density among the sham, BMMNC-treated sham, vehicle-treated BCAS, or BMMNC-treated BCAS mice (Figure 2A). At 30 days after vehicle administration, a significant (P < 0.05) increase in capillary density in the cortex was found in the vehicle-treated BCAS mice (527.3 ± 21.8/mm²) compared with the sham mice.
Additional increases of the capillary density in the cortex, caudoputamen, and corpus callosum were found in the BMMNC-treated BCAS mice compared with the vehicle-treated BCAS mice; such increases in capillaries were suppressed by the presence of L-NAME (Figure 2B, C).

BMMNC Treatment Increases Brain Levels of VEGF and eNOS Ser1177 Phosphorylation

BMMNC treatment did not significantly alter the VEGF level in sham mice at 1 day after administration (vehicle group, 17.3±2.0 pg/mg protein compared with BMMNC group, 17.5±1.9 pg/mg protein) (Figure 3A). By contrast, BMMNC treatment significantly increased VEGF level in BCAS-operated mice (P<0.05, n=4/group; vehicle group, 21.9±2.6 pg/mg protein compared with BMMNC group, 25.1±2.2 pg/mg protein) (Figure 3A). In addition, VEGF levels of the vehicle-treated sham mice and vehicle-treated BCAS mice were significantly different (P<0.05, n=4/group). The BCAS operation increased the densitometric ratio of Ser1177-phosphorylated eNOS to the whole eNOS; this ratio was further elevated with the BMMNC treatment (Figure 3B).

Fate of the Donor BMMNCs in the Hypoperfused Brain

A small number of enhanced green fluorescent protein (eGFP)-positive donor cells (2.2±0.6 cells/100 μm² coronal sections from bregma, +1 to 0 mm; section interval, 100 μm) were found in the caudoputamen (72.7%), corpus callosum (18.2%), and cortex (9.1%) in the BMMNC-treated BCAS mice. A large proportion of the eGFP-positive cells showed similar morphology and location characteristic of pericytes (Figure 4A).13,14 Double-staining immunofluorescence revealed that some of these eGFP-positive cells were also positive for the pericyte marker PDGFRβ (Figure 4B). However, no eGFP-positive cells were found to express CD31 (endothelial cells) or glutathion S-transferase pi (oligodendrocytes) (data not shown).

BMMNC Treatment Protects Against WM Damage after BCAS

The number of Glial fibrillary acidic protein–positive astroglia and Iba-1–positive microglia increased in BCAS mice, and this increase was significantly attenuated by BMMNC treatment. This glial activation, however, was enhanced by L-NAME administration (Figure 5A, B). The number of glutathion S-transferase pi–positive oligodendrocytes decreased in BCAS mice, but this decrease was lessened by BMMNC treatment and enhanced by L-NAME administration (Figure 5A, C). Western blots of myelin basic protein demonstrated four bands corresponding to the major isoforms (Figure 5D). Although the myelin basic protein protein levels tended to be lower in BCAS mice, the difference between the groups did not reach statistical significance (Figure 5E). Grading scores for the WM lesions were significantly (P<0.05) higher in the BCAS-operated mice than in the sham mice. BMMNC treatment improved such WM lesions in the BCAS mice (Figure 5F, G).

Discussion

Previous studies using the middle cerebral artery occlusion model have reported that BM-derived cells augment CBF, and that associated angiogenesis occurs after a period of 4 days to 7 days.2,15,16 In contrast, we detected a marked increase in CBF in the BCAS model 6 hours after BMMNC administration. To our knowledge, this is the first study to report such a rapid beneficial effect of BM-derived cell therapy on CBF. A widespread but relatively mild ischemia in the BCAS model may contribute to the early CBF recovery, detected by two-dimensional laser speckle imaging. Given the rapid increase in CBF, perhaps nontranscriptional eNOS activation through phosphorylation of eNOS at Ser1177 contributed to the initial response of CBF recovery.17,18 Indeed, we observed an increase in Ser1177 phosphorylated-eNOS levels at 1 day after BMMNC treatment and that this is accompanied by augmentation of CBF in BMMNC-treated BCAS mice. In accordance with previous studies,19–21 NOS inhibition by chronic oral L-NAME treatment did not affect CBF in sham mice, whereas it greatly suppressed
BMMNC-induced early CBF recovery. However, CBF in sham and BMMNC-treated BCAS mice was not altered significantly by a specific inhibitor of nNOS, 7-NI. These results indicate that the eNOS-dependent pathway is involved in early CBF recovery after BMMNC treatment. Furthermore, L-NAME greatly decreased CBF even in the vehicle-treated BCAS mice, whereas CBF in the vehicle-treated BCAS mice was not altered by 7-NI, indicating that an eNOS-dependent adaptive mechanism plays a pivotal role in rapid autoregulatory response in ischemic brains.

**Figure 2.** BMMNCs facilitate angiogenesis in BCAS mice. The quantitative capillary density at 1 day (A) and at 30 days (B) after BMMNCs or vehicle administration (n=4 to 6/group; *P<0.05 vs sham, †P<0.05 vs BCAS). C, Images of fluorescein-isothiocyanate-dextran-perfused vessels in the caudoputamen of each indicated group at 30 days after administration. Bar=100 μm.

**Figure 3.** BMMNCs increase the levels of VEGF and phosphorylated eNOS in BCAS mice at 1 day after administration. A, Histogram showing the VEGF levels of the 4 groups of mice. B, Histogram showing the amount of Ser1177-phosphorylated eNOS relative to that of eNOS, and representative immunoblots of Ser1177-phosphorylated eNOS, total eNOS, and β-actin. (n=3/group; *P<0.05 vs sham, †P<0.05 vs BCAS).

**Figure 4.** BMMNCs can differentiate into pericytes in BCAS mice. A, An eGFP-positive donor cell in the caudoputamen. The donor-derived cell tightly wrapped around the microvessel, suggestive of pericyte. Bar=50 μm. B, An eGFP-positive donor cell expressing the pericyte marker PDGFRβ (red). Bar=20 μm.
Trophic factors and cytokines including VEGF, which are released by BM-derived cells, have been implicated in the mechanisms underlying these protective effects. VEGF induces Akt-dependent phosphorylation of eNOS, resulting in the immediate production of nitric oxide (NO). BMMNC treatment increased VEGF and Ser1177 phosphorylated-eNOS levels in BCAS mice. This suggests that VEGF released from BMMNCs enhances eNOS activation and NO production, which subsequently leads to vasodilation and increase in CBF. Furthermore, VEGF-stimulated eNOS activation and NO production are known to promote angiogenesis.

Several studies using the middle cerebral artery occlusion model have indicated that BM-derived cells could be a source of endothelial cells. However, our findings are consistent with those observed in studies on hindlimb ischemia, which found no evidence of differentiation into endothelial cells. This discrepancy may be attributed to the severity of ischemia. Severe vascular insults to the degree of causing necrosis might be required to induce differentiation of BMMNCs into endothelial cells. Alternatively, the lack of donor-derived endothelial cells may be explained by the possibility that eGFP levels decreased under differentiation process and fell below immunofluorescence detection limits. However, endothelial cells were positive for eGFP in the C57BL/6-Tg (CAG-EGFP) mice (data not shown). The small size and thin cytoplasm of a single endothelial cell may further complicate this issue. Intriguingly, eGFP-positive donor cells, although few, were found to wrap around

Figure 5. BMMNCs protect against WM damage in BCAS mice. A, Representative triple immunofluorescence for FluoroMyelin (green), glial fibrillary acidic protein (magenta), and Iba-1 (cyan) (top panels), and double immunofluorescence for myelin (green), and glutathion S-transferase pi (red) (middle panels) in the corpus callosum of the indicated groups of mice at 30 days after BMMNCs or vehicle administration; bars = 20 μm. The white box in the FluoroMyelin Green-stained section indicates the area in which representative photomicrographs were taken (bottom panel; bar = 1 mm). B, C, Histogram showing the number of glial fibrillary acidic protein-positive astrocytes and Iba-1–positive microglia (B), and that of glutathion S-transferase pi-positive oligodendrocytes (C). D, Representative immunoblot of myelin basic protein with 4 isoforms in the brain homogenates of the indicated groups of mice. E, Semiquantitative densitometric analysis of myelin basic protein (n=4/group). F, Representative photomicrographs of the corpus callosum of the indicated groups of mice with the Klüver-Barrera staining; bar = 20 μm. G, Grading score of WM lesions (n=6/group). *P<0.05 vs sham, †P<0.05 vs BCAS.
the microvessels with features suggestive of pericytes. In fact, some of the donor cells expressed PDGFRβ, a pericyte marker. It is believed that pericytes play a key role in the integrity of the blood-brain barrier, maintenance of homeostasis, and angiogenesis. However, because the number of the donor-derived pericytes is so small, these cells may not have a substantial impact on maintenance of WM integrity at this time point unless the number of pericytes is underestimated.

Despite the marked protection against WM damage, no direct structural incorporation of donor BMMNCs to oligodendrogenesis was found, although it is not possible to unconditionally conclude that these cells do not exist. While a direct antiapoptotic effect on oligodendrocytes may be involved in the WM protective effect on oligodendrocytes may be involved in the WM protection, it is plausible that CBF recovery after BMMNC treatment is sufficient to maintain WM integrity. Endothelial cells and oligodendrocytes play an important cooperative role in the maintenance of the oligovascular unit. Additional investigation is therefore required to assess whether CBF recovery after BMMNC treatment is sufficient to maintain WM integrity or if other mechanisms such as direct structural incorporation or direct antiapoptotic effect of BMMNCs play a role in the WM protection.

In conclusion, BMMNC treatment provides strong protection against WM damage in the BCAS mice, depending primarily on CBF recovery beginning from the early phase, and the subsequent endogenous restorative response, including angiogenesis, in a later phase. The results of this study suggest clinical applicability of BMMNC treatment for subcortical ischemic vascular dementia management.

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Supplemental Methods

Flow Cytometry Analysis

To characterize the BMMNCs, whole bone marrow cells (whole BMCs), and Sp-MNCs, fluorescence-activated cell sorter (FACS) analyses data were obtained using a FACS Aria flow cytometer (Becton Dickinson) and analyzed using FACSDiva software (Becton Dickinson). The following antibodies were used for FACS analysis: PE-Cy7 conjugated anti-CD117 (c-kit) antibody (eBiosciences), allophycocyanin-conjugated anti-lineage markers (including B220, CD3e, Ly-6G, Ly-6C, CD11b, and TER119) (BD Pharmingen). Erythrocytes were removed using ACK lysis buffer (Invitrogen). In addition, dead cells were removed by 7-AAD (BD Pharmingen) according to the manufacturer's instructions.

ELISA for VEGF

The brain tissue extract was obtained at 1 day after BMMNCs or vehicle administration. The VEGF levels in mouse brain tissue were quantified using a VEGF ELISA kit (Quantikine Mouse VEGF Immunoassay, R&D Systems) and total protein concentration with the Pierce BCA protein assay kit (Pierce). VEGF values were corrected for total tissue protein.

Western Blot Analysis

The brains were dissected out and cut coronally into 3-mm thick slices (bregma, −1 to +2 mm) and then homogenized in RIPA buffer containing a protease and phosphatase inhibitor mixture (Nakarai Tesque). Samples were electrophoresed on SDS-polyacrylamide minigels and the proteins were transferred to PVDF membranes (Bio-Rad). Membranes were incubated with primary antibodies against eNOS (dilution, 1:1000; Cell Signaling Technology), Ser1177-phosphorylated eNOS (1:1000; Cell Signaling Technology), myelin basic protein (MBP; 1:1000; Abcam) and β-actin (1:3000; Sigma). The signal was visualized using horseradish peroxidase-conjugated secondary
antibodies with an ECL Plus chemiluminescence detection kit (Amersham).

**Density of Perfused Capillaries**

To identify network of perfusable capillaries, animals were transcardially perfused at 1 day and 30 days after BMMNCs or vehicle administration with 5 mL FITC-dextran (2 x 10^6 molecular weight, Sigma; 15 mg/mL) at a perfusion pressure of 120–140 mmHg. The brains were removed, immersed in 4% paraformaldehyde at 4°C for 24 h, and cryoprotected in 20% sucrose in PBS. Coronal sections were mounted on glass slides, and fluorescence images were obtained using a fluorescent microscope (BZ-9000; Keyence). Based on the mouse brain atlas (Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. San Diego, Calif: Academic Press; 2001), five coronal cryosections (20-μm thick) from each animal (–1.0, -0.5, 0, +0.5, +1.0 mm posterior to bregma) were obtained. In each coronal brain slice, ROIs were set in the cortex (1 x 1 mm^2; 1.5 to 3.5 mm lateral from midline), in the caudoputamen (1 x 1 mm^2; 1.0 to 3.0 mm lateral from midline), and in the corpus callosum (0.5 x 0.2 mm^2; 1.0 to 1.5 mm lateral from midline). These ROIs were scanned under a x10 objective lens. The full-focused images were generated from the Z-stack images using Keyence application. A FITC-perfused vessel (<12 μm) that is separated from adjacent vessels was regarded as a single capillary and counted in number. This number was added to the number of vascular branch points (number of vessel bifurcations) to yield ‘the number of capillaries’. Capillary density was expressed as the number of capillaries per square millimeter, determined by dividing the number of capillaries in a ROI by the area of the ROI.

**Histology and Immunofluorescence**

Mice were euthanized at 30 days after treatment. After transcardiac perfusion and fixation with paraformaldehyde, paraffin sections (2-μm thick) were stained with Klüver-Barrera staining. WM lesions were graded as normal (grade 0), disarrangement of nerve fibers (grade 1), formation of marked vacuoles (grade 2), and disappearance of myelinated fibers (grade 3) by an investigator blind...
to the experimental conditions, as previously described. For immunofluorescence, cryosections (10-µm thick) were immunolabeled with the following primary antibodies: rabbit polyclonal anti-GFP (dilution, 1:200; Abcam) or rat monoclonal anti-GFP (1:1000; Nakarai Tesque) for eGFP-positive cells; rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) (1:5000; Dako Cytomation) or mouse monoclonal anti-GFAP (1:1000; Sigma Chemical Co.) for astrocytes; rabbit polyclonal anti-ionized calcium binding adapter molecule 1 (Iba-1) (1:100; Wako) for microglia; rabbit polyclonal anti-glutathione-S-transferase-pi (GST-pi) (1:100; Chemicon) for mature oligodendrocytes; rat monoclonal anti-CD31 (1:100; BD Pharmigen) for endothelial cells; and rat monoclonal anti-PDGFRβ (1:100; eBiosciences) for pericytes. FITC-conjugated, rhodamine-conjugated, and cyanine 5.29-conjugated secondary antibodies were employed accordingly. In addition, some sections were stained with FluoroMyelin Green fluorescent myelin stain (1:300; Invitrogen) according to the manufacturer’s protocol. Fluorescent signals were detected using a fluorescent microscope (BZ-9000; Keyence) or a spectral confocal microscope (TCS SPE; Leica).
Supplemental Reference


Supplemental Figure Legend

Supplementary Figure I. FACS analyses for percentage of Lin⁻/c-kit⁺ cells to whole bone marrow cells (BMCs), BMMNCs, and Sp-MNCs (Sp-MNCs).
Abstract

Early Protective Effect of Bone Marrow Mononuclear Cells Against Ischemic White Matter Damage Through Augmentation of Cerebral Blood Flow

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Abstract

Bone marrow mononuclear cells (BMMNCs) are used to enhance cerebral blood flow and protect against ischemic white matter damage. The early protective effect of BMMNCs was investigated in a rat model of ischemic stroke.

Objectives: The objective of this study was to evaluate the early protective effect of BMMNCs on ischemic white matter damage.

Methods: Male Sprague-Dawley rats were subjected to 2-hour unilateral middle cerebral artery occlusion (MCAO). BMMNCs were injected into the brain of rats at 1 or 3 days post-MCAO. Cerebral blood flow (CBF) and cerebral blood volume (CBV) were measured using microsphere and Evans Blue dye injection, respectively.

Results: CBF and CBV were significantly higher in the BMMNC group compared to the control group at 1 and 3 days post-MCAO. The neurological deficit scores were also lower in the BMMNC group than in the control group at 1 and 3 days post-MCAO.

Conclusion: BMMNCs have a significant early protective effect against ischemic white matter damage by enhancing cerebral blood flow.

References:
