

Early Transplantation of Human Cranial Bone-derived Mesenchymal Stem Cells Enhances

Functional Recovery in Ischemic Stroke Model Rats

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Abstract

We analyzed the cell characteristics, neuroprotective, and transplantation effects of human cranial bone-derived mesenchymal stem cells (hcMSCs) in ischemic stroke model rats compared with human iliac bone-derived mesenchymal stem cells (hiMSCs). The expressions of *brain-derived neurotrophic factor (BDNF)* and *vascular endothelial growth factor (VEGF)* as neurotrophic factors were analyzed in both MSCs. HiMSCs or hcMSCs were intravenously administered into ischemic stroke model rats at 3 or 24 h after middle cerebral artery occlusion (MCAO) and neurological function was evaluated. The survival rate of NG108-15 cells after 3 or 24 h oxidative or inflammatory stress and the neuroprotective effects of hiMSCs or hcMSCs-conditioned medium (CM) on 3 or 24 h oxidative or inflammatory stress-exposed NG108-15 cells were analyzed. The expressions of *BDNF* and *VEGF* were higher in hcMSCs than in hiMSCs. HcMSCs transplantation at 3 h after MCAO resulted in significant functional recovery compared with that in the hiMSCs or control group. The survival rate of stress-exposed neuroblastoma × glioma hybrid cells (NG108-15) was lower after 24 h stress than after 3 h stress. The survival rates of NG108-15 cells cultured with hcMCSs-CM after 3 h oxidative or inflammatory stress were significantly higher than in the control group. Our results suggest that hcMSCs transplantation in the early stage of ischemic stroke suppresses the damage of residual cells and leads to functional recovery through the strong expressions of neurotrophic factors. This is the first report demonstrating a functional recovery effect after ischemic stroke following hcMSCs transplantation.

Introduction

Brain injury followed by an ischemic stroke may eventually lead to death in severe cases or considerable functional impairment, which might hamper the performance of regular activities in later stages of life. In recent years, due to advances in medication therapy and surgical treatment, including endovascular treatment, the number of treatment options available for ischemic stroke has increased. However, satisfactory functional recovery is achieved only in a limited number of cases. Therefore, stem cell therapy could be an effective and novel treatment option for central nervous system (CNS) injuries, including damage caused by ischemic stroke.

Several studies have demonstrated that transplantation of human mesenchymal stem cells (hMSCs) could improve functional recovery after ischemic stroke in rodents,¹⁻⁸⁾ a non-human primate model,⁹⁾ and humans.^{10,11)} Several neurotrophic factors released from the MSCs result in neuroprotection, nerve regeneration, and angiogenesis, which are considered to be the primary therapeutic effects of MSC transplantation in patients with CNS disorders.^{1,3-5,8,12-15)}

Human MSCs can be derived from various tissues, such as bone marrow,^{1,3-5,7,8)} skin,¹⁶⁾ muscle,¹⁶⁾ adipose tissue,^{2,17)} placenta,¹⁸⁾ umbilical cord,¹⁹⁾ and peripheral blood.⁶⁾ However, hMSCs transplanted into ischemic stroke models or patients were generally harvested from iliac bone (hiMSCs), originating from the mesodermal layer.^{1,3-5,7,8)} According to some studies, the transplantation of dental pulp-derived stem cells (DPSCs) originating from the neural crest in ischemic stroke model rats and spinal cord injury model rats enhances functional recovery,²⁰⁻²²⁾ and it has been confirmed that DPSCs exhibit higher neurotrophic factor expression levels than bone marrow-derived MSCs.²⁰⁾ We previously established MSCs from human cranial bone

(hcMSCs) and reported that hcMSCs expressed neural crest markers at significantly higher levels than hiMSCs and exhibited a greater tendency to undergo neuronal differentiation, in an *in vitro* study.²³⁾ In addition, it has been demonstrated that MSCs established from rat cranial bone abundantly secrete neurotrophic factors.²⁴⁾ To the best of our knowledge, no report has explored the effects of transplanting hcMSCs on cerebral ischemic injury in an *in vivo* study.

The source of MSCs and the timing of MSCs transplantation both seem to affect the therapeutic outcome after transplantation. In fact, Ishizaka et al. have demonstrated timing-dependent cell distribution and functional recovery using a combination of neuroprotection, reactive astrocyte enhancement, and angiogenesis activities.²⁵⁾ In addition, Kawabori et al. have suggested the importance of optimal timing of MSC transplantation for functional recovery and engraftment of transplanted MSCs in ischemic stroke model rats.²⁶⁾ Toyoshima et al. have also examined the acute therapeutic time window of MSC transplantation and reported that extremely early transplantation of MSCs does not lead to functional recovery because the blood–brain–barrier may still be intact immediately after cerebral infarction.²⁷⁾ However, the optimal timing of hcMSCs transplantation in ischemic stroke model rats is still unclear. Therefore, the aim of the present study is to analyze the cytological features and neuroprotective effects of hcMSCs further and to clarify the effects of early transplantation of hcMSCs in ischemic stroke model rats.

Materials and Methods

All experiments involving the use and care of animals were performed in compliance with the National

Institutes of Health Guidelines. All studies were designed in accordance with the Animal Testing Committee Guidelines at Hiroshima University. In accordance with the Hiroshima University Hospital's guidelines, cranial bone marrow waste was collected from the temporal and sphenoid bones during craniotomy after obtaining informed consent from the patients.

Isolation and culture of hiMSCs and hcMSCs

Human iMSCs were purchased from Lonza Japan Ltd. We have previously reported the isolation and establishment of hcMSCs from human cranial bone and confirmed that they had features consistent with that of MSCs using flow cytometric analysis and a multi-lineage differentiation assay.²³⁾ In the present study, hcMSCs were established using a method previously described.²³⁾ The bone marrow samples were seeded onto 90-mm culture dishes (Sumitomo Bakelite Co.) containing Dulbecco's modified Eagle's medium (DMEM) with low glucose (Sigma-Aldrich Co.) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific HyClone), penicillin (100 units/ml), and streptomycin (100 µg/ml; both from Sigma-Aldrich Co.). The culture dishes were incubated at 37°C in a humidified atmosphere with 5% CO₂. The medium was changed every 3 days. Both hMSCs were passaged several times upon reaching 80% confluence. All hiMSCs and hcMSCs were derived from different individuals.

Reverse-transcription polymerase chain reaction (RT-PCR)

Cells that reached confluence in the growth medium were collected with PBS. Total RNA was extracted

using Nucleospin[®] RNA (MACHEREY-NAGEL GmbH & Co. KG) (hiMSCs: n = 4, hcMSCs: n = 3). We used the NanoDrop spectrophotometer (Thermo Fisher Scientific) to measure RNA concentration. In accordance with the manufacturer's instructions, we used the ReverTra Ace- α (Toyobo Co.) to generate and amplify cDNA from 1 μ g of total RNA. The 7500 Real-time PCR system (Applied Biosystems) was used to perform real-time PCR. We used *brain-derived neurotrophic factor (BDNF)* and *vascular endothelial growth factor (VEGF)* as neurotrophic factors. *Hypoxanthine phosphoribosyltransferase (HPRT)* was examined as a housekeeping gene.

Establishment of transient middle cerebral artery occlusion (MCAO) model and cell transplantation

Adult male Sprague–Dawley rats (body weight, 250–300 g) were housed at a room temperature of 23°C \pm 1°C, humidity of 50% \pm 10%, and a light/dark cycle of 12 h, with free access to food and water. The transient MCAO procedure was performed by an intraluminal thread occlusion method, as described previously.^{28,29)} The rats were anesthetized with isoflurane, and the right common carotid, external carotid, internal carotid, and pterygopalatine arteries were exposed via midline cervical incision. A 4-0 nylon thread coated with silicone (403745PK10; Docol Corp) was inserted from the cut stump of the external carotid artery toward the internal carotid artery until it blocked the origin of the right MCA. Following 2 h of occlusion, the thread was retracted and reperfusion was performed. During the procedure, rectal temperature was maintained at 37°C \pm 0.5°C using a thermostat-controlled heating pad (Bio Research Center Co.). Cerebral infarction area was confirmed by magnetic resonance imaging (MRI) at 24 h after MCAO, 2,3,5-triphenyltetrazolium chloride (TTC) staining at 24 h after MCAO, and hematoxylin–eosin (H–E) staining on day 35 after MCAO (Figure 1A–C). The rats were

divided according to the time of treatment administration after MCAO (3 or 24 h). At 3 or 24 h after MCAO, the rats were divided into the following three groups according to the treatment received: administration of only 300 μ l of PBS after MCAO (control (Ctrl) group, n = 7 and 7, respectively), hiMSCs (1×10^6 cells/300 μ l of PBS) (hiMSCs group, n = 8 and 5, respectively), and hcMSCs (1×10^6 cells/300 μ l of PBS) (hcMSCs group, n = 9 and 6, respectively). The cells were administered intravenously through the tail vein. Human iMSCs and hcMSCs from different individuals were passaged three times and used for transplantation. All the rats were immunosuppressed with cyclosporine-A (10 mg/kg, intraperitoneally) daily from the day before MCAO.^{6,7)}

MRI experiment

The MRI was performed using a 4.7 Tesla superconducting magnet system (BioSpec47/40USR; Bruker BioSpin) with a combination of transmit quadrature volume coil (154-mm inner diameter) and a 30-mm receiver surface coil. The rats were anesthetized with isoflurane, placed in a prone position inside the imaging coil, and monitored by breathing excursions of the thoracic wall. The diffusion-weighted images (DWIs) of coronal forebrain sections were obtained at the level of the caudate–putamen complex. The DWIs were obtained with the multi-shot echo-planar imaging technique with the following scanning parameters: TR: 2848 ms, TE: 48 ms, matrix: 128×128 , segments: 3, b-values: 0, 1000, and 2000 s/mm^2 , and NEX: 11, and incorporated chemical shift selective radiofrequency (RF) pulses to provide effective fat suppression. The other parameters were identical between the two sequences: 17 coronal slices, slice thickness: 1 mm, inter-slice gap: 0.25 mm, and field of view: 35×35 mm. The scan time of DWIs was 4 min 41 s. A region with a high-intensity signal in the

DWIs was diagnosed as a cerebral infarction lesion.

Neurological functional evaluation

Neurological function was evaluated using the previously reported modified neurological severity score (mNSS), which is a complex of motor, sensory, balance, and reflexes.¹²⁾ mNSS is graded on a scale from 0, reflecting a normal status, to 18, reflecting a large deficit. mNSS tests were performed before MCAO and 1, 2, 3, 4, 7, 14, 21, and 28 days after MCAO. In the present study, rats with mNSS of 9 to 13 points before transplantation were used.

Oxidative or inflammatory stress exposure and cell death assay on NG108-15 cells

To analyze the neural cell viability after ischemic damage *in vitro*, neuroblastoma × glioma hybrid cells (NG108-15; ECACC) were exposed to a mimic of oxidative stress created using H₂O₂ (Santoku Chemical Industries Co.) and a mimic of inflammatory stress created using lipopolysaccharide (LPS) (Wako Pure Chemical Industries) as an *in vitro* cerebral infarction model.^{24,30-33)} NG108-15 cells were seeded into 60-mm culture dishes (Corning Co.) and cultured in DMEM with high-glucose (Sigma-Aldrich Co.), 10% FBS (Thermo Fisher Scientific), penicillin (100 units/ml), streptomycin (100 µg/ml: both from Sigma-Aldrich Co.), and HAT supplement (Thermo Fisher Scientific). The culture dishes were incubated at 37°C in a humidified atmosphere with 5% CO₂. Cell survival rates after stress exposure for 3 or 24 h were analyzed and compared with those of the control group. NG108-15 cells were cultured in fresh medium for 48 h, after which the medium was changed

to fresh growth medium with 500 μM H_2O_2 or 200 ng/mL LPS in the stressed groups, as shown in Figure 2A. After exposure to stress for 3 or 24 h, cells were harvested, centrifuged, and suspended in PBS (n = 10). In the control group, the medium was changed to only fresh growth medium and cells were harvested, centrifuged, and suspended in PBS after 3 or 24 h (n = 10). Trypan blue staining was used to measure cell survival rates in a counting chamber.

Preparation of hiMSCs- and hcMSCs-conditioned medium (CM)

To analyze the neuroprotective effect of hiMSCs and hcMSCs after ischemic damage in an *in vitro* study, hiMSCs and hcMSCs were seeded on 60-mm culture dishes (Corning Co.) and cultured in growth medium. When the cells reached 80% confluence, the medium was changed to fresh growth medium. After 24 h, culture supernatants were collected as CM and stored at $-80\text{ }^\circ\text{C}$ after 0.2- μm filtration.

Neuroprotective effect of hiMSCs-CM or hcMSCs-CM on stress-exposed NG108-15 cells

The neuroprotective effect of hiMSCs- or hcMSCs-CM was examined based on the protocol shown in Figure 3A. After the exposure of NG108-15 cells to oxidative or inflammatory stress for 3 or 24 h, the medium was changed to fresh growth medium (absence of MSC culture and without FBS) (Ctrl), hiMSCs-CM, or hcMSCs-CM (with 500 μM H_2O_2 or 200 ng/mL LPS). After culturing with hiMSCs- or hcMSCs-CM for 24 h, cells were harvested, centrifuged, and suspended in PBS (n = 10). Trypan blue staining was used to measure cell survival rates in the counting chamber.

Statistical analysis

mNSS was analyzed using repeated-measures two-way analysis of variance with Bonferroni's test. The Mann–Whitney U test was used to analyze mNSS of the hcMSCs transplantation group on days 14 and 28, and survival rates of stress-exposed NG108-15 cells. To analyze the survival rate of oxidative or inflammatory stress-exposed NG108-15 cells cultured with hiMSCs- or hcMSCs-CM, the Kruskal–Wallis test with the modified Tukey test was applied. For all analyses, a p -value < 0.05 was considered significant. JSTAT software (Sato) was used to perform statistical analyses.

Results

Neurological functional recovery in MCAO model rats

To compare the transplantation effects of hiMSCs and hcMSCs in a stroke model rat, neurological functional recovery was assessed using mNSS. When MSCs were transplanted 24 h after MCAO, there were no significant differences among the three groups at all observation times (Figure 1D). When MSCs were transplanted 3 h after MCAO, mNSS in the hcMSCs group was significantly better than in the Ctrl group on days 2, 3, 4, 7, 14, 21, and 28, and significantly better than in the hiMSCs group on days 2, 4, and 7 (Figure 1E). In the hiMSCs group, there were no significant differences in mNSS compared with that in the Ctrl group. In addition, the mNSS of the hcMSCs group (transplantation at 3 h after MCAO) was significantly improved than that of the hcMSCs group (transplantation at 24 h after MCAO) on days 14 and 28 (Figure 1F, G).

The morphology of hiMSCs and hcMSCs

The cell morphology of both hiMSCs showed a spindle shape, with no characteristic difference being exhibited between hiMSCs and hcMSCs (Figure 4A).

Gene expression analysis of hiMSCs and hcMSCs

We used real-time PCR to analyze the neurotrophic factor-associated gene expression. The *BDNF* expression was not determined in all hiMSCs lines by our analysis, but was determined in all hcMSCs lines (Figure 4B). The *VEGF* expression of hcMSCs showed a higher tendency than that of hiMSCs (Figure 4C).

Survival rates of stress-exposed NG108-15 cells

The survival rate of oxidative stress-exposed NG108-15 cells was significantly lower in both the 3 and 24 h stress groups than in the Ctrl group (Figure 2B, C). The survival rate of NG108-15 cells upon exposure to 24 h of oxidative stress was significantly lower than that for the group with 3 h of exposure (Figure 2D). The survival rate of inflammatory stress-exposed NG108-15 cells in the 3 h stress group was not significantly different but that of the 24 h stress group was significantly lower than in the Ctrl group (Figure 2E, F). The survival rate of inflammatory stress-exposed NG108-15 cells was significantly lower in the 24 h stress-exposed group than in the 3 h stress-exposed group (Figure 2G).

Survival rate of oxidative or inflammatory stress-exposed NG108-15 cells cultured with hiMCSs-CM or hcMSCs-CM

The survival rate of NG108-15 cells cultured with hcMCSs-CM after exposure to oxidative or inflammatory stress for 3 h was significantly higher than in the Ctrl group (Figure 3B, C). The survival rate of NG108-15 cells cultured in hcMCSs-CM after exposure to stress for 24 h was not significantly higher under oxidative stress (Figure 3D), but was significantly higher under inflammatory stress, compared with that of the hiMSCs group or the Ctrl group (Figure 3E). The survival rate of NG108-15 cells cultured in hiMCSs-CM after 3 and 24 h of exposure to oxidative or inflammatory stress was not significantly higher than that of the Ctrl group (Figure 3B–E).

Discussion

The present study indicates that hcMSCs express higher levels of neurotrophic factors (*BDNF* and *VEGF*) than hiMSCs. Intravenous transplantation of hcMSCs in ischemic stroke model rats 3 h after MCAO enhanced neurological functional recovery than that in the hiMSCs and control groups. In an in vitro study, although hiMSCs-CM did not increase the survival rate of NG108-15 cells after exposure to stress for 3 or 24 h, hcMCSs-CM improved the survival rate of NG108-15 cells after 3 h of exposure to oxidative or inflammatory stress. In the present study, early transplantation of hcMSCs in ischemic brain injury improved functional recovery via neurotrophic factors, when compared with hiMSCs transplantation. This is the first report demonstrating the functional recovery effect after ischemic stroke by hcMSCs transplantation.

Human cranial bones, such as frontal bone, sphenoidal bone, and temporal bone originate from the neural crest, similar to teeth.³⁴⁾ We reported previously that hcMSCs expressed neural crest markers (*SLUG*, *SNAIL*, and *P75*) at significantly higher levels than hiMSCs.²³⁾ It has been reported that DPSCs express neural crest-associated genes.³⁵⁾ In addition, with regard to neurotrophic factors, Abiko et al. have observed that rat cranial bone-derived MSCs (rcMSCs) exhibit higher mRNA expression levels of neurotrophic factors, such as BDNF and nerve growth factor (NGF), than rat bone marrow-derived MSCs (rbMSCs).²⁴⁾ Moreover, Sakai et al. have revealed that the expression levels of human BDNF, glial cell line-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF) in DPSCs was higher than those in MSCs harvested from human iliac bone marrow.²⁰⁾ Our study revealed that hcMSCs expressed higher levels of neurotrophic factors than hiMSCs. The results suggest that hcMSCs originate from the neural crest, which may lead to the high levels of expression of neurotrophic factors.

The mechanism behind the therapeutic effect of MSC transplantation in CNS diseases, including ischemia is commonly accepted to involve neuroprotection, angiogenesis, neurogenesis, synaptogenesis, and immunomodulation by the secretion of several neurotrophic factors released from MSCs.^{1,3-5,8,12-15)} Chen et al. presented that hiMSCs cultured in supernatant derived from ischemic brain extracts can induce the production of BDNF, VEGF, NGF, and hepatocyte growth factor (HGF).³⁶⁾ In rat cerebral ischemic models, BDNF,^{3,37)} VEGF,^{8,38,39)} GDNF,^{4,40)} NGF,^{41,42)} HGF,³⁹⁾ and placental growth factor (PIGF)⁵⁾ have also been described to have beneficial effects on cerebral ischemia. It has been reported that the transplantation of rcMSCs in ischemic stroke model rats improved functional recovery compared with rbMSCs transplantation and that the expression

of BDNF and NGF in rcMSCs was higher than in rbMSCs.²⁴⁾ DPSCs were found to express abundant neurotrophic factors compared with hiMSCs²⁰⁾ and DPSC transplantation in model rats of ischemic stroke and spinal cord injury promotes functional recovery.²⁰⁻²²⁾ Our study revealed that the transplantation of hcMSCs in rat stroke models improved functional recovery and the expression of *BDNF* and *VEGF* in hcMSCs was higher than in hiMSCs. BDNF decreases the local levels of pro-inflammatory cytokines, such as TNF- α , and increases the local levels of anti-inflammatory cytokines, such as IL-10, in addition to the DNA-binding activity of NF- κ B⁴³⁾; it also promotes angiogenesis and neurogenesis in the cerebral ischemic area.^{22,44)} VEGF is also an important neurotrophic factor for the improvement of functional recovery after cerebral ischemia because it stimulates the proliferation of neural progenitors in the non-ischemic region, promotes neuroprotection and neurogenesis in ischemic lesions, and induces angiogenesis in the ischemic penumbra.^{45,46)} In the present study, the higher expression of neurotrophic factors in hcMSCs may lead to excellent functional recovery from ischemic cerebral damage. We consider that, in ischemic stroke, hcMSCs can be a more useful source in transplantation therapy than other MSCs.

In the present study, the effect on functional improvement could not be confirmed by the transplantation of hMSCs at 24 h after MCAO. However, the transplantation of hcMSCs at 3 h after MCAO improved functional recovery. In an *in vitro* study, the survival rate of NG108-15 cells rapidly decreased as oxidative or inflammatory stress exposure time increased, and hcMCSs-CM improved the survival rate of NG108-15 cells after 3 h of exposure to oxidative or inflammatory stress. Garcia et al. confirmed that neuronal necrosis progresses rapidly at 6 h or more after ischemic damage, as revealed by histological analysis,⁴⁷⁾ the same as in

our *in vitro* study. Some previous reports presented that intravenous transplantation of MSCs in ischemic stroke model rats reduced apoptosis in the penumbral zone of the lesion.^{1,15,48)} It was suggested that the transplantation of hcMSCs abundantly secreting neurotrophic factors before nerve cell necrosis progresses may rescue the residual nerve cells and result in good functional recovery after cerebral infarction. Although the intravenous transplantation of rcMSCs at 24 h after MCAO in ischemic stroke model rats has been reported to improve functional recovery,²⁴⁾ the transplantation of hcMSCs did not result in functional recovery in our study. Thus, it is necessary to determine the differences in the characteristics of MSCs depending on the species in addition to the differences in transplantation effects between allogeneic and xenogeneic transplantations. Further investigations on the effect of transplanting hcMSCs in patients with cerebral infarction are required.

Cell therapy employs either autograft or allograft. With respect to immunological factors, autograft is considered to be better than allograft. However, at present, hcMSCs require at least 5 weeks to secure a sufficient number of cells for transplantation; therefore, while performing cell therapy for cerebral infarction with autograft, administration is forced in the chronic phase. As a first step, we are planning a clinical study in which patients with severe cerebral infarction who require decompressive craniotomy are administered autologous hcMSCs cultured from their free bone flap in the chronic phase. After this clinical study, we aim to perform allograft so that cell therapy can be conducted as soon as possible after cerebral infarction as a final goal.

In this study and previous reports, we found that cranial bone-derived MSCs secrete more abundant neurotrophic factors than iliac bone-derived MSCs; thus, they have a higher neuroprotective effect and

improved function recovery after cerebral infarction.²⁴⁾ Therefore, we consider cranial bone marrow to be more useful than iliac bone marrow as a source of cell therapy for cerebral infarction. Although the collection of skull bone marrow is more invasive than the collection of iliac bone marrow in a clinical setting, initially, we are considering to administrate hcMSCs which are cultured using the free bone flap after decompressive craniectomy to the patients with extensive cerebral infarction including middle cerebral artery perfusion area who need decompressive craniectomy to rescue their lives according to the recommendation by the guideline.⁴⁹⁾ Thus, patients do not have to undergo any additional invasive procedure to obtain hcMSCs from the cranial bone marrow. In the future, we believe that this could be clinically applied to patients other than those with severe cerebral infarction by investigating methods for collecting cranial bone marrow in a minimally invasive manner, such as by stereotactic procedures, and for efficiently establishing and culturing hcMSCs.

Conclusion

Our results provide novel evidence that the transplantation of hcMSCs in stroke model rats in an early phase improves functional recovery compared with hiMSCs transplantation, via a neuroprotective effect through the strong expression of neurotrophic factors.

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Conflicts of Interest Disclosure

All authors have no conflict of interest.

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Figure legends

Figure 1: Various images of brain infarction and neurological functional recovery

Brain infarction was confirmed by magnetic resonance imaging (MRI) (A), 2,3,5-triphenyltetrazolium chloride (TTC) staining (B), and hematoxylin–eosin (H–E) staining (C).

Results of neurological functional recovery in the rats transplanted at 24 h after middle cerebral artery occlusion (MCAO) (D) (Control (Ctrl) group: n = 7, human iliac bone-derived mesenchymal stem cells (hiMSCs) group: n = 5, human cranial bone-derived mesenchymal stem cells (hcMSCs) group: n = 6) or 3 h after MCAO (Ctrl group: n = 7, hiMSCs group : n = 8, hcMSCs group: n = 9) (E). †Ctrl group vs. hcMSCs group; and § hiMSCs group vs. hcMSCs group. Neurological function in the rats transplanted with hcMSCs (24 h (n = 6) or 3 h (n = 9)) at day 14 (F) or day 28 (G).

* $p < 0.05$; ** $p < 0.01$.

Figure 2: Oxidative or inflammatory stress exposure of NG108-15 cells

Schedule of stress exposure experiment using NG108-15 cells (A). The survival rate of oxidative stress-exposed NG108-15 cells at 3 h (B) (n = 10) or 24 h (C) (n = 10). Comparison of the survival rate in NG108-15 cells depending on oxidative stress exposure time (D) (n = 10). The survival rate of inflammatory stress-exposed NG108-15 cells at 3 h (E) (n = 10) or 24 h (F) (n = 10). Comparison of the survival rate in NG108-15 cells depending on inflammatory stress exposure time (G) (n = 10).

** $p < 0.01$.

Figure 3: The effects of human mesenchymal stem cells-conditioned medium (hMSCs-CM) on survival rate of stress-exposed NG108-15 cells

Schedule of the NG108-15 cell experiment using hMSCs-CM (A). The effect of hMSCs-CM against 3 h of oxidative stress (B) (n =10), 3 h of inflammatory stress (C) (n =10), 24 h of oxidative stress (D) (n =10), and 24 h of inflammatory stress (E) (n =10).

* $p < 0.05$; ** $p < 0.01$.

Figure 4: The morphology and gene expression of both human mesenchymal stem cells (hMSCs)

Both hMSCs showed a spindle shape, with no characteristic difference being exhibited between human iliac bone-derived mesenchymal stem cells (hiMSCs) and human cranial bone-derived mesenchymal stem cells (hcMSCs) (A). Gene expression of *brain-derived neurotrophic factor (BDNF)* (B), and *vascular endothelial growth factor (VEGF)* (C) (hiMSCs: n = 4, hcMSCs: n = 3).







