Neuroprotective Effect of Human Mesenchymal Stem Cells in an Animal Model of Double Toxin-Induced Multiple System Atrophy Parkinsonism

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Multiple system atrophy (MSA) is an adult-onset sporadic neurodegenerative disorder of unknown etiology featuring parkinsonism, ataxia, and autonomic failure in any combination. Because disease progression in MSA is rapid and no drug treatment consistently benefits MSA patients in the long term, neuroprotective or regenerative strategies may be invaluable in the management of MSA patients. In this study, we investigated whether human mesenchymal stem cells (hMSCs) had a protective effect on MSA using an animal model of double-toxin-induced MSA parkinsonism (MSA-P). MSA-P was established with coinjections of MPTP and 3-NP; hMSCs were injected into the tail vein 1 day after the last toxin injection. Three groups of mice were compared (i.e., control, MPTP + 3-NP, and MPTP + 3-NP with hMSC treatment) through histopathological, behavioral, and Western blot analyses. In the substantia nigra (SN) and the striatum, 2.0% and 3.8% of total injected hMSCs were observed, respectively. Compared with double-toxin-treated mice, hMSC treatment in double-toxin-treated mice significantly increased survival of TH- and NeuN-immunoreactive cells in the SN and the striatum, with coincident improvement in motor behavior. Additionally, hMSC treatment significantly decreased double-toxin-induced microglial and astroglial activation in the SN and striatum. Western blot analysis showed that hMSC administration in double-toxin-treated mice increased the expression of p-Akt and Bcl-2 and decreased Bax and cytochrome c expression. This study demonstrates that hMSC treatment protected against loss of neurons in the SN and the striatum induced by double toxin exposure, which may be mediated by modulation of inflammatory and cell survival and death signaling-pathway as the hMSCs migrated from the peripheral circulation into the SN and striatum.

Key words: Multiple system atrophy (MSA); Mesenchymal stem cells (MSCs); Neuroprotection

INTRODUCTION

Multiple system atrophy (MSA) is a sporadic neurodegenerative disease of the central and autonomic nervous system. Pathologically, MSA includes striatonigral degeneration, olivopontocerebellar degeneration, astrogliosis, and microgliosis. Clinically, cardinal features include autonomic failure, parkinsonism (MSA-P), cerebellar ataxia, and pyramidal signs in any combination, of which autonomic failure is an integral component in the diagnosis of MSA (29). MSA is regarded as a unique entity within the spectrum of oligodendrogliopathy, with α-synuclein-positive glial cytoplasmic inclusions (GCI) being a pathological hallmark (30). Because the prognosis of MSA is fatal, many in vivo and clinical trials have been conducted to archive neuroprotective strategies in MSA. Among those, Stefanova et al. (23) demonstrated that MAO-B inhibitor had a disease-modifying activity in transgenic animal model of MSA. However, other clinical trials have failed to delay disease progression.

Mesenchymal stem cells (MSCs) are present in adult bone marrow and represent <0.01% of all nucleated bone marrow cells. MSCs are themselves capable of multipotency, with differentiation under appropriate conditions into chondrocytes, skeletal myocytes, and neurons (16,21,32). MSCs secrete various cytotrophic factors that, in turn, exert neuroprotective effects (3). Our previous study in both in vitro and animal model of Parkinson's disease using a proteasome inhibitor demonstrated that human MSCs (hMSCs) had a protective effect on progressive dopaminergic neuronal loss through a variety of mechanisms, such as antiapoptotic effects,

modulation of polyubiquitinated proteins, and anti-inflammatory actions in addition to possible transdifferentiating effect of hMSCs into dopaminergic neurons (20). Furthermore, we recently reported an open-label clinical trial of hMSCs in patients with MSA, which demonstrated that hMSC injection delayed progression of neurological deficits and improved cerebral glucose metabolism in cerebellum compared with untreated patients (14).

The animal model of MSA-P is based on the concept of inducing selective degeneration in nigral and striatal neurons by using 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and 3-nitropropionic acid (3-NP), which were previously used to mimic Parkinson's disease and Huntington's disease, respectively, in rodents (24). In this double lesion model, the MPTP is known to potentiate striatal damage and behavioral impairments induced by 3-NP intoxication and thus constitute a useful model of MSA-P. In the present study, we investigated whether hMSCs had a protective effect against neuronal loss in the substantia nigra (SN) and striatum using double-toxin-induced animal model of MSA-P.

MATERIALS AND METHODS

Animal Study

In total, 34 male C57BL/6 mice (16 weeks old) were used. Animals were divided into three groups: controls, MPTP + 3-NP-treated group, and hMSCs group (MPTP + 3-NP-treated animals followed by administration of hMSCs). For 9 days, 16-week-old male C57BL/6 mice were injected with MPTP (10 mg/kg, total dose 90 mg/kg, IP) and 3-NP (10 mg/kg × 4, 20 mg/kg × 4, 30 mg/kg × 4, 40 mg/kg × 4, and 50 mg/kg × 1, total dose 450 mg/kg; 12-h intervals; IP) (5). Control mice were injected with saline alone using the same administration schedule and method. One day after the last injection, hMSCs were injected into the tail vein (1×10^6 cells/200 μl). After the last drug injection, MPTP + 3-NP mice showed 36% mortality and we chose hMSC-injected group randomly from live mice. Behavioral ($n = 8$/group), histopathological ($n = 5$/group), and Western blot analyses ($n = 3$/group) were performed randomly among three groups of mice. Animal experimental protocols were approved by the Ajou University Institutional Animal Care and Use Committee.

Isolation of hMSCs

Bone marrow aspirates (10 ml) were obtained from the iliac crests of human donors. The mononuclear cell layer was isolated by Ficoll-Hypaque, washed with PBS, and plated in polystyrene 100-mm culture dishes. Cells were maintained in low-glucose Dulbecco modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Hyclone,

Irvine, CA, USA) and 1% penicillin/streptomycin (P/S; Sigma, St. Louis, MO, USA) in a humidified incubator maintained at 37°C with 5% CO_2. Nonadherent cells were removed after 24 h. When the primary cultures reached 80% confluence, cells were harvested using 0.25% trypsin and subcultured. At passage 6, hMSCs were injected into mice via tail vain.

Behavioral Test

The pole test was performed according to a previous study (17). Each mouse was placed on the top of a vertical wooden rough-surfaced pole (1-cm diameter; 50-cm height). On the day prior to testing, mice were habituated to the apparatus by placing them at the top of the pole and allowing them to descend five times. The total time that it took each mouse to reach the base of the pole and place all four paws on the floor was recorded. For each session of five descents, the best performance was recorded as the total time. If the mouse was unable to turn completely downward, fell off, or slipped down the pole, a default value of 120 s was recorded. The pole test was performed at baseline, then at days 2, 4, 6, and 8 during double toxin injection and at days 1, 10, and 20 after hMSC administration.

Tissue Preparation

For immunohisotochemistry, mice were perfused with a saline solution containing 0.5% sodium nitrate and heparin (10 U/ml) and were fixed with 4% paraformaldehyde dissolved in 0.1 M PB (~50 ml/mouse) at 30 days after the first injection. Brains were removed from the skulls, postfixed overnight in buffered 4% paraformaldehyde at 4°C, and stored in a 30% sucrose solution for 1–2 days at 4°C until they sank. Coronal sections (30 μm) were obtained and stored in tissue stock solution (30% glycerol, 30% ethylene glycol, 30% 3× distilled water, 10% 0.2 M PB) at 4°C until use. For Western blotting, the mice were euthanized 30 days after the first injection of double toxins, and the SN and striatum were rapidly dissected from the brains and frozen at −70°C.

Immunohistochemistry

The 30-μm coronal brain sections were rinsed twice in PBS and incubated in 0.2% Triton X-100 for 30 min at room temperature (RT). They were rinsed three times and blocked with 0.5% bovine serum albumin (BSA) in 1× PBS. After blocking, sections were incubated at 4°C overnight with the following primary antibodies: mouse anti-tyrosine hydroxylase (TH; 1:2000; Pel-freez, Rogers, AR, USA), mouse anti-NeuN (1:500; Chemicon, Billerica, MA, USA), mouse anti-nuclear matrix (NuMA; 1:100; Calbiochem, San Diego, CA, USA), mouse anti-calbindin-D-28K (Cal; 1:3000; Sigma-Aldrich, St.

Louis, MO, USA), rabbit anti-Iba-1 (1:1000; Wako, Richmond, VA, USA), and rabbit anti-GFAP (1:1000; Chemicon, San Diego, CA, USA). Following overnight incubation, the brain sections were rinsed three times with 0.5% BSA in 1× PBS (10 min/rinse) and incubated with the appropriate biotinylated secondary antibody and avidin-biotin complex (Elite Kit; Vector Laboratories, Burlingame, CA, USA) for 1 h at RT. Bound antibodies were visualized by incubating the sections with 0.05% diaminobenzidine-HCl (DAB) and 0.003% hydrogen peroxide in 0.1 M PB. The brain sections were rinsed with 0.1 M PB for DAB inhibition. Immunostained cells were analyzed by bright-field microscopy.

Western Blot Analysis

Brain regions of the striatum and SN were dissected and homogenized in ice-cold lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothretol, 0.1 mM phenylmethylsulfonyl fluoride plus protease inhibitor cocktail; Sigma-Aldrich). Tissue homogenate was centrifuged ($14,000 \times g$, 20 min, 4°C), and supernatant was transferred into fresh tubes. Proteins were analyzed using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (i.e., 50 μg) were loaded in each lane with loading buffer containing 0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 10% mercaptoethanol, and 0.002% bromophenol blue. Samples were boiled for 5 min before gel loading. Proteins were transferred electrophoretically to polyvinylidiene difluoride membranes (Millipore, Bedford, MA, USA). Membranes were washed in Tris-buffered saline solution with 2.5 mM EDTA (TNE) and then blocked in TNE containing 5% skim milk for 1 h. Membranes were incubated overnight at 4°C with the following primary antibodies: Akt, p-Akt, cytochrome C (1:1000; Cell Signaling, Danvers, MA, USA), Bax, BCL-2 (1:1000; Stressgene, Ann Arbor, MI, USA), and β-actin (1:500; Imgenex, San Diego, USA). After washing, the membranes were incubated with secondary antibodies (1:2000; Amersham, Piscataway, NJ, USA) for 1 h at RT and washed again. The blots were finally developed with ECL Western blotting detection reagents (Amersham). For semiquantitative analysis, the densities of the immunoblot bands were measured average of each group ($n = 3$) by computer imaging (Image J; NIH, Bethesda, MD, USA).

Stereological Cell Counts

Total SN and striatum cell number was estimated using an optical fractionator and unbiased stereology of stained cells, as previously described, with some modifications (12). This sampling technique is not affected by tissue volume changes and does not require reference volume determination (31). The sections used for counting covered the entire SN and striatum from the rostral tip of the pars compacta back to the caudal end of the pars reticulate. This generally yielded eight to nine sections in a series. Sampling was performed using an Olympus BX51 microscope in conjunction with the Olympus CAST-Grid system (Olympus Denmark A/S, Denmark), which was connected to the stage. Information regarding the *z*-axis distance was defined in the software. The SN and striatum were delineated at 1.25× objective. A counting frame (60%, 35,650 μm²) was placed randomly on the first counting area and systematically moved though all counting areas until the entire delineated area was sampled. Actual counting was performed using a 40× oil objective. Guard volumes (i.e., 4 μm from the top and 4–6 μm from the bottom of the section) were excluded from both surfaces to avoid the problem of lost cap, and only the profiles that came into focus within the counting volume (with a depth of 10 μm) were counted. The total number of stained cells was calculated according to the optical fractionator formula (31).

Statistical Analysis

Comparisons between groups were made using the Student *t*-test (paired) or one-way analysis of variance (ANOVA, nonparametric) followed by a Dunnet post hoc test. Values of $p < 0.05$ were considered statistically significant. Data were expressed mean ± SD. Statistical analyses were performed using commercially available software (version 10.0; SPSS Inc., Chicago, IL, USA).

RESULT

Characterization of hMSCs

Fluorescence-activated cell sorting analysis confirmed that hMSCs expressed CD105 and CD73, positive markers for hMSCs. Furthermore, hMSCs did not express CD45 and CD34, negative markers for hMSCs (Fig. 1A). Immunofluorescent labeling showed that hMSCs were positive for CD105 and negative for CD34 (Fig. 1B).

Recovery of Motor Behavior by hMSC Treatment

The total time it took a mouse to descend a pole and place all four paws on the floor during the pole test was significantly increased in double-toxin-induced MSA-P mice compared with the control group ($p < 0.005$) (Fig. 2). Compared with the double-toxin treatment alone, hMSC administration in double-toxin-treated mice resulted in a significant decrease in the total time to descend the pole. This significant difference was maintained for 10 days after hMSC administration ($p < 0.05$) (Fig. 2).
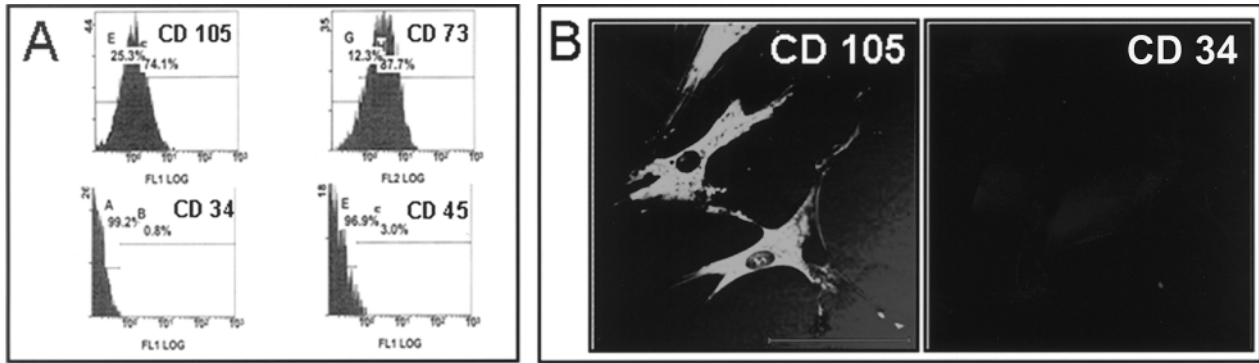
Figure 1. Flow cytometric analysis (A) and immunofluorescent labeling of human mesenchymal stem cells (B) Scale bar: 100 μm.

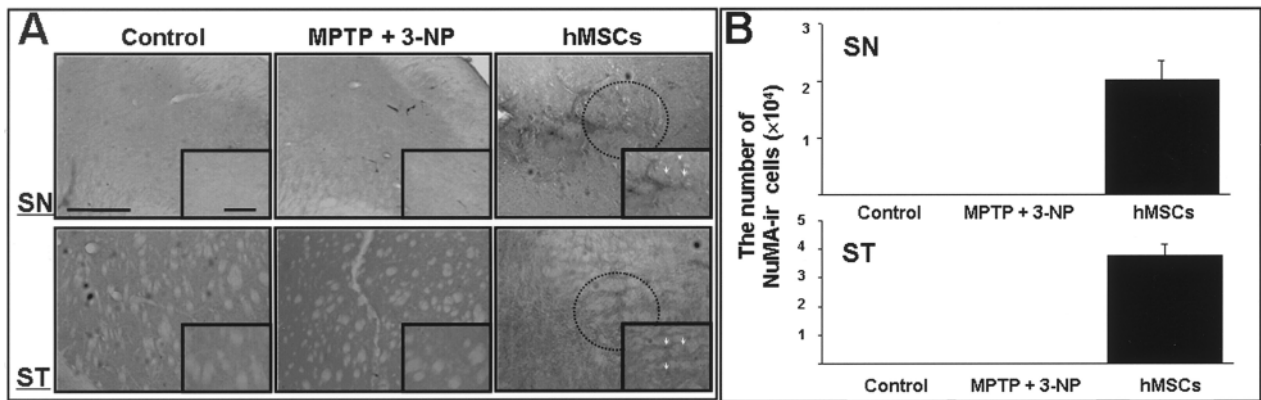*Detection of hMSCs in the SN and Striatum*
of Double-Toxin-Treated Mice

To determine whether transplanted hMSCs survived, we attempted to identify hMSCs in the SN and stratum in double-toxin-treated mice using human-specific NuMA immunostaining. In controls and in animals treated with double toxins alone, there were no NuMA-ir cells in the SN and striatum (Fig. 3A, B). In contrast, NuMA-ir cells were observed following hMSC administration in double-toxin-treated mice (Fig. 3A), and the number of NuMA-ir cells in the SN ($20,120 \pm 825$) and striatum ($37,859 \pm 25$) corresponded to about 2.0% and 3.8% of a total of 1×10^6 injected hMSCs (Fig. 3B).

Histological Analysis of Administrated hMSCs
*in the Double-Toxin-Induced MSA-P Model*

Brain tissue was prepared for immunohistochemical analysis 4 weeks after the first MPTP and 3-NP coinjections. Immunohistochemical analysis showed that administration of both toxins induced a significant decline in the number of TH-ir cells in the SN and NeuN-ir cells in the striatum (Fig. 4A). Neuronal loss, as quantified by stereological analysis, revealed that TH-ir and NeuN-ir cells decreased by approximately 48% and 29%, respectively (both $p < 0.001$) (Fig. 4B). However, hMSC administration significantly reduced neuronal loss in the double-toxin-treated SN and striatum (Fig. 4A). Stereo-
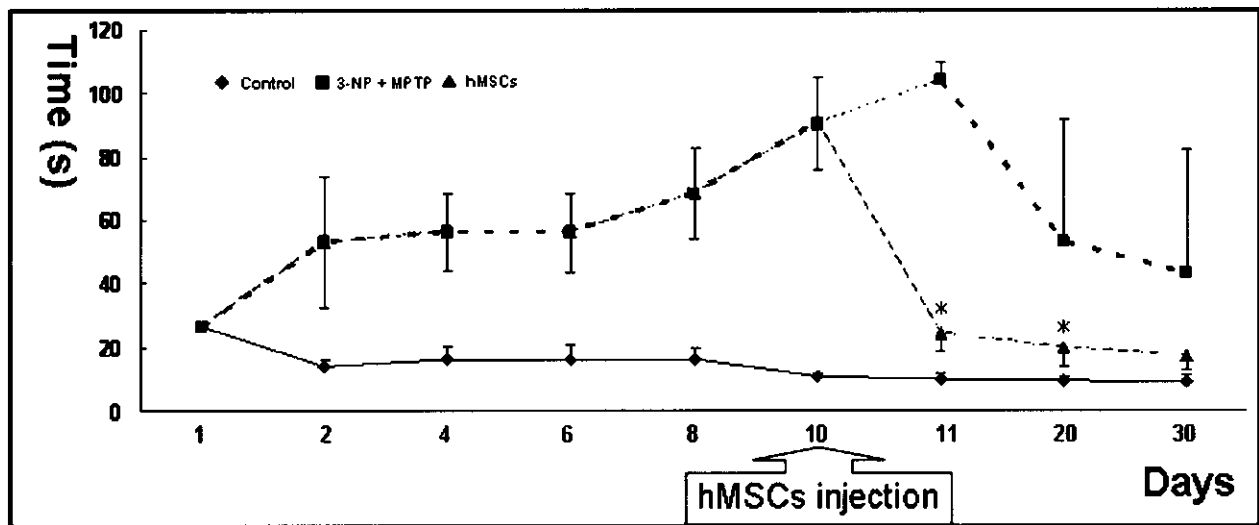
Figure 2. Motor behavior testing. The total time it took a mouse to descend a pole and place all four paws on the floor was significantly increased in double-toxin-treated mice compared with controls ($n = 8$; $p < 005$). Compared with double-toxin treatment alone, hMSC administration in double-toxin-treated mice significantly decreased the total descent time; this significant difference was maintained for 10 days after hMSC administration ($n = 8$; $*p < 0.05$).

Figure 3. Detection of hMSCs in double-toxin-treated mice. There were no NuMA-ir cells in controls or double-toxin-treated animals. However, NuMA-ir cells were observed in the substantia nigra (SN) and striatum (ST) of animals treated with hMSCs (A). The number of NuMA-ir cells in the SN and ST was $20,120 \pm 825$ and $37,859 \pm 25$, respectively, which corresponded to about 2.0% and 3.8% of a total of 1×10^6 injected hMSCs (B; $n = 5$). Scale bar: 100 μm.
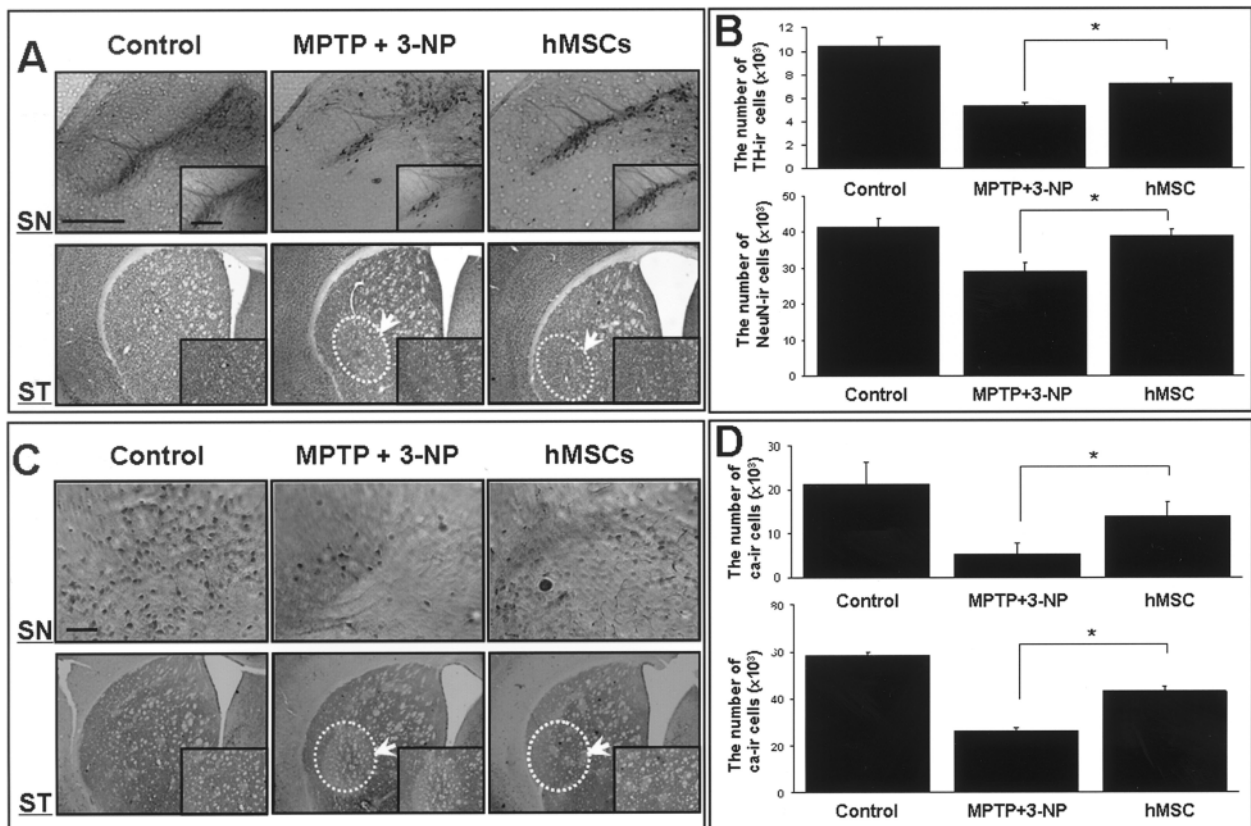
Figure 4. Effects of cell therapy with hMSCs on animals treated with MPTP and 3-NP. Immunohistochemical analysis showed that hMSC treatment significantly decreased the decline in the number of TH-ir and NeuN-ir cells in the substantia nigra (SN) and striatum (ST) of double-toxin treated animals (A). Stereological analysis revealed that the number of TH-ir and NeuN-ir cells was significantly higher in the hMSC-treated group than in the group treated with double toxin alone (B; $n = 5$; $*p < 0.05$). Functional neurons immunostained by Calbindin-D were also significantly increased in the SN and ST of double-toxin-treated mice after administration of hMSCs (C). Stereological analysis revealed that the number of Calbindin-ir cells was significantly higher in the hMSC-treated group than in the group treated with double toxin alone (D; $n = 5$; $*p < 0.05$). Scale bar: 100 μm.

logical analysis revealed that the number of TH-ir and NeuN-ir cells was significantly greater in the hMSC-treated group than in the MPTP + 3-NP-treated group, showing a 23% and 18% increase in the survival of TH-ir and NeuN-ir cells in the SN and striatum, respectively ($p < 0.05$) (Fig 4B). To evaluate functional neurons, cells in the SN and striatum were immunostained with Calbindin-D-28kD, a marker for calcium binding protein, which is important to maintain in synaptic transmission and axonal transport. Consistent with the increase of neuronal survival in the SN and striatum of double-toxin-treated mice after hMSC administration, the number of Calbindin-D-ir cells in the SN and striatum was significantly greater in the hMSC-treated group compared with the double-toxin-treated group ($p < 0.05$) (Fig. 4C, D).

Effects of hMSC Therapy on Modulation of Inflammation and Gliosis in Animals Exposed to Double Toxins

To determine the effects of hMSCs on modulation of inflammation and gliosis, the SN and striatum were immunostained with Iba-1 and GFAP, markers for activated microglia and activated astrocytes, respectively. A marked increase in Iba-1 and GFAP-immunoreactivity was observed in double-toxin-treated mice (Fig. 5); hMSC treatment in double-toxin-treated mice significantly decreased Iba-1 and GFAP immunoreactivity (Fig. 5A, C). Stereological analysis revealed that the number of activated microglia and astrocytes was significantly decreased in the SN and striatum of the hMSC-treated group compared with the double-toxin-treated group ($p < 0.01$ in microglia, $p < 0.001$ in astrocytes) (Fig. 5B, D).

Effect of hMSCs Treatment on Modulation of Cell Death Signaling Pathway

To determine the effects of hMSCs on cell survival and death signaling-pathway modulation, Western blot analysis was performed using brain tissue prepared at 4 weeks after the first MPTP and 3-NP coinjections (Fig. 6A, B). p-Akt expression was significantly reduced in double-toxin-treated mice compared with controls; however, hMSC administration in double-toxin-treated mice increased the expression of p-Akt. hMSC treatment significantly decreased Bax expression in double-toxin-treated mice, whereas the expression of Bcl-2 was significantly increased in double-toxin-treated mice after hMSC administration. In addition, hMSCs significantly decreased the expression of cytochrome c, which was elevated after double-toxin treatment.

DISCUSSION

The present study revealed that hMSC treatment significantly protected against neuronal loss induced by MPTP and 3-NP treatment in the SN and striatum with coincident improvement in motor behavior. Neuroprotective mechanisms exerted by hMSCs may be mediated by modulation of inflammatory and cell survival and death signaling-pathway as the hMSCs migrated from the peripheral circulation into the SN and striatum.

With advances in the understanding of MSA pathobiologies, it has been suggested that oligodendroglial degeneration resulting from α-synuclein inclusion formation contributes to secondary widespread neuronal degeneration. However, the initial trigger or aggravating mechanism underlying the abnormal accumulation and aggregation of α-synuclein in MSA remains unknown. In case-control epidemiological studies, occupational exposure to pesticides, insecticides, or solvents that interrupt mitochondrial electron transport is associated with increased risk of MSA (19,28). In animal studies, high-dose 3-NP administration also aggravated nigrostriatal and olivopontocerebellar degeneration in MSA transgenic mice using proteolipid protein promoters (25). Furthermore, we recently reported that 3-NP administration in transgenic mice led to oxidation-specific modifications of α-synuclein that were concomitant with an exacerbation of behavioral deficits and widespread neuronal and oligodendrocytic pathology in a number of brain regions implicated in MSA (27). These data support that derangement in mitochondrial function by mitochondrial neurotoxins, such as MPTP or 3-NP used in this study, may be a main mediator for progression of MSA pathology.

Our study demonstrated that hMSCs had neuroprotective properties against mitochondria-inhibiting double-toxin-induced neuronal cell loss, showing about a 20% increase in the survival of TH-ir and NeuN-ir cells in the SN and striatum. A significant improvement of motor behavior after hMSC treatment was in accordance with increased survival of these neuronal cells following hMSC treatment in double-toxin-treated mice, although functional recovery was not maintained in the end of the study period possibly due to the effect of spontaneous recovery in double-toxin-only-treated animals. The neuroprotective effects of MSCs seem to be mediated by complex mechanisms. First, our study has demonstrated that hMSCs can restore the balance between neuronal survival and apoptosis, which is disrupted by mitochondrial neurotoxins. In this study, hMSC treatment significantly increased the expression of the cell survival factor p-Akt in double-toxin-treated mice. p-Akt activation is modulated by growth factors and prevents apoptotic cell death signaling pathways (22). Although we did not investigate the potential factors that induced pAkt activation, MSCs are known to increase the production of various neurotrophic factors, such as NGF, BDBF, or NT-3 (2,10), which may modulate pAkt activation in
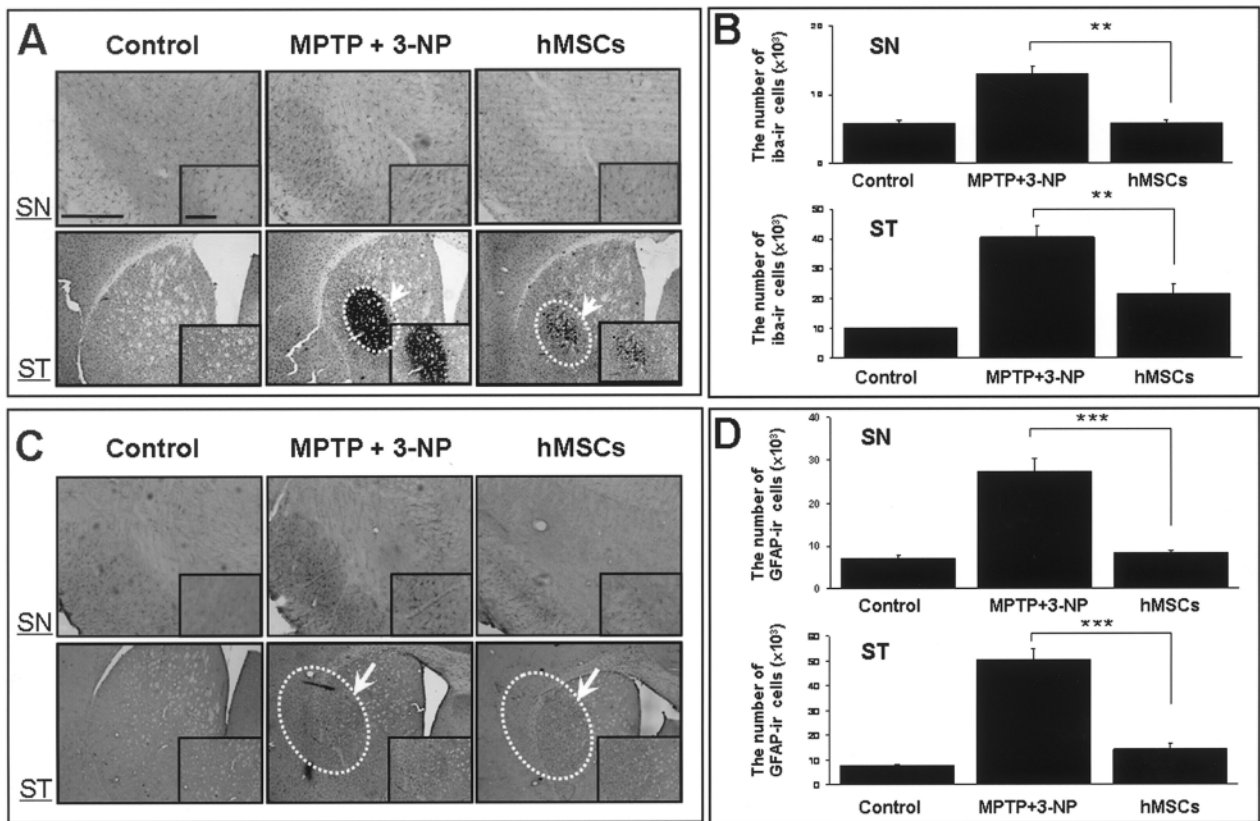
Figure 5. Effects of cell therapy with hMSCs on modulation of inflammation and gliosis in animals treated with double toxin. Combined MPTP and 3-NP treatment led to microglial activation and gliosis in the substantia nigra (SN) and striatum (ST); however, hMSC treatment significantly attenuated activation of microglia (arrow head) and gliosis (arrow) in double-toxin-treated SN and ST (A, C). Stereological analysis revealed that the number of activated microglia and astrocytes was significantly lower in the hMSC-treated group than in the group treated with double toxin alone (B, D; $n = 5$; **$p < 0.01$, ***$p < 0.001$). Scale bar: 100 µm.
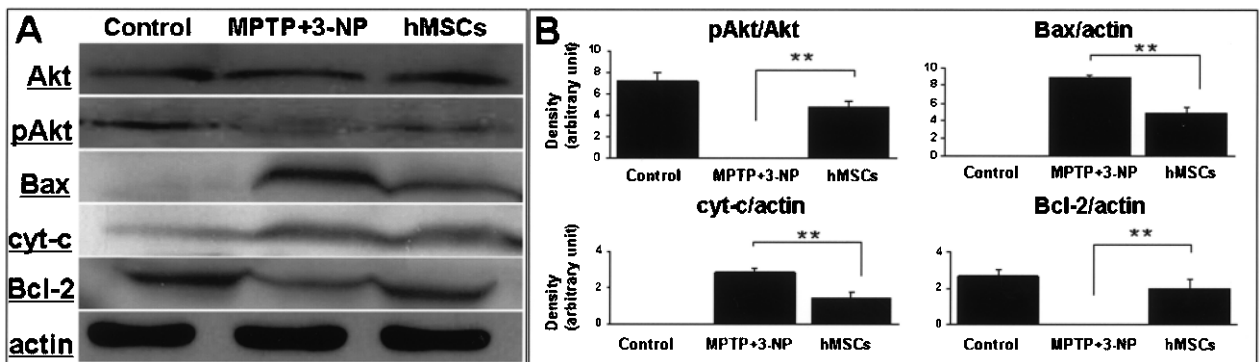
Figure 6. Effects of cell therapy with hMSCs on modulation of cell survival and death-signaling pathways. Western blot analysis, performed 4 weeks after first double-toxin injection, showed that the p-Akt expression was significantly decreased in double-toxin-treated mice compared with controls. However, hMSC administration in double-toxin-treated mice increased p-Akt expression. hMSC treatment significantly decreased Bax expression in double-toxin-treated mice, whereas hMSC treatment significantly increased the expression of Bcl-2 in these mice. In addition, hMSCs significantly decreased the expression of cytochrome c, which was elevated after double-toxin treatment. (A, B; $n = 3$; **$p < 0.01$).

this study. Along with upregulation of cell survival signaling pathways by hMSCs, hMSCs also modulated expression of pro- and antiapoptotic proteins toward suppressing apoptotic cell death signaling, and thus prevented the release of cytochrome c from mitochondria.

Second, hMSC treatment had anti-inflammatory and antigliotic effect, showing significantly decreased activation of microglia and astrocytes in the double-toxin-treated SN and striatum. As in PD, microglial reaction and inflammatory processes also participate in the cascade of neuronal degeneration in MSA. In human MSA, neuropathological studies suggest that the mode of microglial activation is system specific, consistent with the known pattern or system degeneration in MSA, and is significantly correlated with the burden of GCI in the extrapyramidal motor and cerebellar input systems (8). A similar pattern of microglial activation was also observed in MSA patients using [11C](R)-PK11195 PET (6). Additionally, we reported that 3-NP administration in MSA transgenic mice produced marked microglial activation and gliosis (27). It has been suggested that MSCs can not only inhibit nearly all cells participating in the immune response cell–cell contactdependent mechanism, but can also release a variety of soluble factors that may be involved in the immunosuppressive activity of MSCs (9,13,18). Furthermore, we recently demonstrated in vitro and in vivo evidence that hMSCs have a neuroprotective effect on dopaminergic neurons through anti-inflammatory actions, where soluble factors released from MSCs, such as IL-6, IL-10, and TGF-β may regulate the microglial response to inflammatory stimulants (11). Accordingly, our data suggest that the neuroprotective properties of hMSCs via anti-inflammatory effects were also evident in an animal model of MSA.

MSCs characteristically migrate towards injured brain area in various animal models of ischemia and PD, possibly in response to signals that are upregulated under injury condition (7,15). Chemokines released from damaged brain cells and their receptors, such as stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4, may play an important role in migration of MSCs (4,26). SDF-1 is widely expressed in the brain, including cortex, cerebellum, basal ganglia, and SN pars compacta (1). Damage in the SN and striatum induced by MPTP and 3-NP may increase the expression of SDF-1 and CXCR4, leading to recruitment of MSCs to these regions. In this study, the number of surviving hMSCs in the SN and striatum 20 days after hMSC administration was approximately 2.0% and 3.8% of the total number of injected hMSCs, respectively. These migrated cells may contribute to modulate the microenvironmental cascade of the neurodegenerative process in the SN and striatum.

In conclusion, we have shown that hMSC treatment has a protective effect against neuronal death induced by double mitochondrial neurotoxins in the SN and striatum. Modulation of inflammatory actions and cell survival and death signaling pathways by hMSCs may work in the neuroprotective process. To be clinically applicable in patients with MSA, further study to evaluate the long-term beneficial effect of hMSCs using transgenic mice of MSA is needed.

REFERENCES

 1. Banisadr, G.; Skrzydelski, D.; Kitabgi, P.; Rostene, W.; Parsadaniantz, S. M. Highly regionalized distribution of stromal cell-derived factor-1/CXCL12 in adult rat brain: Constitutive expression in cholinergic, dopaminergic and vasopressinergic neurons. Eur. J. Neurosci. 18:1593–1606; 2003.
 2. Blandini, F.; Cova, L.; Armentero, M. T.; Zennaro, E.; Levandis, G.; Bossolasco, P.; Calzarossa, C.; Mellone, M.; Giuseppe, B.; Deliliers, G. L.; Polli, E.; Nappi, G.; Silani, V. Transplantation of undifferentiated human mesenchymal stem cells protects against 6-hydroxydopamine neurotoxicity in the rat. Cell Transplant. 19:203–217; 2010.
 3. Caplan, A. I.; Dennis, J. E. Mesenchymal stem cells as trophic mediators. J. Cell. Biochem. 98:1076–1084; 2006.
 4. Chamberlain, G.; Fox, J.; Ashton, B.; Middleton, J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25:2739–2749; 2007.
 5. Fernagut, P. O.; Diguet, E.; Bioulac, B.; Tison, F. MPTP potentiates 3-nitropropionic acid-induced striatal damage in mice: Reference to striatonigral degeneration. Exp. Neurol. 185:47–62; 2004.
 6. Gerhard, A.; Banati, R. B.; Goerres, G. B.; Cagnin, A.; Myers, R.; Gunn, R. N.; Turkheimer, F.; Good, C. D.; Mathias, C. J.; Quinn, N.; Schwarz, J.; Brooks, D. J. [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. Neurology 61:686–689; 2003.
 7. Hellmann, M. A.; Panet, H.; Barhum, Y.; Melamed, E.; Offen, D. Increased survival and migration of engrafted mesenchymal bone marrow stem cells in 6-hydroxydopamine-lesioned rodents. Neurosci. Lett. 395:124–128; 2006.
 8. Ishizawa, K.; Komori, T.; Sasaki, S.; Arai, N.; Mizutani, T.; Hirose, T. Microglial activation parallels system degeneration in multiple system atrophy. J. Neuropathol. Exp. Neurol. 63:43–52; 2004.
 9. Karussis, D.; Kassis, I.; Kurkalli, B. G.; Slavin, S. Immunomodulation and neuroprotection with mesenchymal bone marrow stem cells (MSCs): A proposed treatment for multiple sclerosis and other neuroimmunological/neurodegenerative diseases. J. Neurol. Sci. 265:131–135; 2008.
10. Kim, H. J.; Lee, J. H.; Kim, S. H. Therapeutic effects of human mesenchymal stem cells on traumatic brain injury

in rats: Secretion of neurotrophic factors and inhibition of apoptosis. J. Neurotrauma 27:131–138; 2010.

11. Kim, Y. J.; Park, H. J.; Lee, G.; Bang, O. Y.; Ahn, Y. H.; Joe, E.; Kim, H. O.; Lee, P. H. Neuroprotective effects of human mesenchymal stem cells on dopaminergic neurons through anti-inflammatory action. Glia 57:13–23; 2009.

12. Kirik, D.; Rosenblad, C.; Bjorklund, A. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp. Neurol. 152:259–277; 1998.

13. Krampera, M.; Pasini, A.; Pizzolo, G.; Cosmi, L.; Romagnani, S.; Annunziato, F. Regenerative and immunomodulatory potential of mesenchymal stem cells. Curr. Opin. Pharmacol. 6:435–441; 2006.

14. Lee, P. H.; Kim, J. W.; Bang, O. Y.; Ahn, Y. H.; Joo, I. S.; Huh, K. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin. Pharmacol. Ther. 83: 723–730; 2008.

15. Li, W. Y.; Choi, Y. J.; Lee, P. H.; Huh, K.; Kang, Y. M.; Kim, H. S.; Ahn, Y. H.; Lee, G.; Bang, O. Y. Mesenchymal stem cells for ischemic stroke: Changes in effects after ex vivo culturing. Cell Transplant. 17:1045–1059; 2008.

16. Makino, S.; Fukuda, K.; Miyoshi, S.; Konishi, F.; Kodama, H.; Pan, J.; Sano, M.; Takahashi, T.; Hori, S.; Abe, H.; Hata, J.; Umezawa, A.; Ogawa, S. Cardiomyocytes can be generated from marrow stromal cells in vitro. J. Clin. Invest. 103:697–705; 1999.

17. Matsuura, K.; Kabuto, H.; Makino, H.; Ogawa, N. Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. J. Neurosci. Methods 73:45–48; 1997.

18. Nauta, A. J.; Fibbe, W. E. Immunomodulatory properties of mesenchymal stromal cells. Blood 110:3499–3506; 2007.

19. Nee, L. E.; Gomez, M. R.; Dambrosia, J.; Bale, S.; Eldridge, R.; Polinsky, R. J. Environmental-occupational risk factors and familial associations in multiple system atrophy: A preliminary investigation. Clin. Auton. Res. 1: 9–13; 1991.

20. Park, H. J.; Lee, P. H.; Bang, O. Y.; Lee, G.; Ahn, Y. H. Mesenchymal stem cells therapy exerts neuroprotection in a progressive animal model of Parkinson's disease. J. Neurochem. 107:141–151; 2008.

21. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; Moorman, M. A.; Simonetti, D. W.; Craig, S.; Marshak, D. R. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147; 1999.

22. Saito, A.; Narasimhan, P.; Hayashi, T.; Okuno, S.; Ferrand-Drake, M.; Chan, P. H. Neuroprotective role of a proline-rich Akt substrate in apoptotic neuronal cell death after stroke: Relationships with nerve growth factor. J. Neurosci. 24:1584–1593; 2004.

23. Stefanova, N.; Poewe, W.; Wenning, G. K. Rasagiline is neuroprotective in a transgenic model of multiple system atrophy. Exp. Neurol. 210:421–427; 2008.

24. Stefanova, N.; Puschban, Z.; Fernagut, P. O.; Brouillet, E.; Tison, F.; Reindl, M.; Jellinger, K. A.; Poewe, W.; Wenning, G. K. Neuropathological and behavioral changes induced by various treatment paradigms with MPTP and 3-nitropropionic acid in mice: Towards a model of striatonigral degeneration (multiple system atrophy). Acta Neuropathol. 106:157–166; 2003.

25. Stefanova, N.; Reindl, M.; Neumann, M.; Haass, C.; Poewe, W.; Kahle, P. J.; Wenning, G. K. Oxidative stress in transgenic mice with oligodendroglial alpha-synuclein overexpression replicates the characteristic neuropathology of multiple system atrophy. Am. J. Pathol. 166:869–876; 2005.

26. Stumm, R. K.; Rummel, J.; Junker, V.; Culmsee, C.; Pfeiffer, M.; Krieglstein, J.; Hollt, V.; Schulz, S. A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: Isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J. Neurosci. 22:5865–5878; 2002.

27. Ubhi, K.; Lee, P. H.; Adame, A.; Inglis, C.; Mante, M.; Rockenstein, E.; Stefanova, N.; Wenning, G. K.; Masliah, E. Mitochondrial inhibitor 3-nitroproprionic acid enhances oxidative modification of alpha-synuclein in a transgenic mouse model of multiple system atrophy. J. Neurosci. Res. 87:2728–2739; 2009.

28. Vanacore, N.; Bonifati, V.; Fabbrini, G.; Colosimo, C.; De Michele, G.; Marconi, R.; Stocchi, F.; Nicholl, D.; Bonuccelli, U.; De Mari, M.; Vieregge, P.; Meco, G. Case-control study of multiple system atrophy. Mov. Disord. 20: 158–163; 2005.

29. Wenning, G. K.; Colosimo, C.; Geser, F.; Poewe, W. Multiple system atrophy. Lancet Neurol. 3:93–103; 2004.

30. Wenning, G. K.; Stefanova, N.; Jellinger, K. A.; Poewe, W.; Schlossmacher, M. G. Multiple system atrophy: A primary oligodendrogliopathy. Ann. Neurol. 64:239–246; 2008.

31. West, M. J.; Slomianka, L.; Gundersen, H. J. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat. Rec. 231:482–497; 1991.

32. Woodbury, D.; Schwarz, E. J.; Prockop, D. J.; Black, I. B. Adult rat and human bone marrow stromal cells differentiate into neurons. J. Neurosci. Res. 61:364–370; 2000.