



저작자표시-동일조건변경허락 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.
- 이차적 저작물을 작성할 수 있습니다.
- 이 저작물을 영리 목적으로 이용할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



동일조건변경허락. 귀하가 이 저작물을 개작, 변형 또는 가공했을 경우에는, 이 저작물과 동일한 이용허락조건하에서만 배포할 수 있습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

이학 석사학위 논문

**Mesenchymal Stem Cells Therapy
in Progressive Rat Model of
Parkinson's Disease**

아 주 대 학 교 대 학 원

의 학 과

박 현 정

Mesenchymal stem cells therapy in progressive rat model of Parkinson's Disease

지도교수 이 필 휴

이 논문을 의학 석사학위 논문으로 제출함.

2007년 2월

아 주 대 학 교 대 학 원

의 학 과

박 현 정

박현정의 이학 석사학위 논문을 인준함.

심사위원장 이 필 휴 인

심사위원 안 영 환 인

심사위원 이 광 인

아 주 대 학 교 대 학 원

2006년 12월 22일

Mesenchymal Stem Cells Therapy in Progressive Rat Model of Parkinson's Disease

by

Hyun Jung PARK

A Dissertation Submitted to The Graduate School of Ajou University
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Supervised by

Phil Hyu Lee, M.D., Ph.D.

Department of Medical Sciences
The Graduate School, Ajou University
February, 2007

ACKNOWLEDGEMENTS

우선 학위과정 내내 지도해 주시고 항상 염려해주신 든든하고 존경스러운 저희 이필휴 선생님을 비롯하여 작은 데이터 하나하나 꼼꼼히 체크해주신 안영환 선생님, 방오영 선생님, 이광 선생님, 남효석 선생님, 김세혁 선생님, 백만정 선생님, 박창석 선생님께 고개 숙여 감사를 표합니다.

그 동안의 학위과정 동안 옆에서 힘이 되어준 실험실 식구들...우영이, 아르미, 화정언니, 유정이, 선처리, 지여니 언니, 소유니, 창미, 민서니, 윤정이, 문옥언니, 경아.....그리고 특히나 내 옆에서 땀방 다 받아주며 힘들 때마다 손 꼭 잡아주면서 함께 나가준 진영이.... 귀찮게 이것저것 물어도 항상 기분 좋게 웃으면서 가르쳐준 멀리 있는 다용언니와 가까이 있는 근우오빠....정말 정말 고맙습니다...

뭔지도 잘 모르면서 항상 잘한다...힘내라.. 해준 내 푼마니 친구들... 미경, 지현, 해명... 그리고, 여기까지 올 수 있게끔 항상 기도해주시고 믿어주시고 응원해주신 올 엄마... 아빠.... 언니랑... 형부... 외할머니, 꼬모 그리고 정진, 반야, 바라, 밀타.. 많이 많이 감사하고 사랑합니다.

세상 중심에서 꽃을 피울 수 있는 그 날까지 더 열심히 노력하는 현정이가 되겠습니다.. 모두모두 다시 한번 감사드립니다..

- 이 논문을 위해 죽어간 쥐들에게도 감사하고 미안한 마음을...-

- ABSTRACT -

Mesenchymal Stem Cells Therapy in Progressive Rat Model of Parkinson's Disease

Parkinson's disease (PD) is a common progressive neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra (SN). In present study, we investigated the effect of human mesenchymal stem cells (hMSCs) therapy in vitro and progressive SD rat model of PD using MG-132, proteasome inhibitor. Immunocytochemical analyses in primary mesencephalic culture revealed that hMSC treatment increased dopaminergic neuronal survival compared to dopaminergic neuronal death in MG-132 treatment. Additionally, hMSCs treatment showed that there was a significant reduction of increased caspase 3 activity following MG-132 treatment. In Rats received systemic injection of MG-132, quantification of the extent of neuronal loss assessed by stereological analysis revealed that TH+ positive cell loss was $23\% \pm 7.2\%$ ($p < 0.05$, $n=5$ per group) at 8 weeks, 70 ± 9.5 at 10 weeks ($p < 0.01$, $n=5$ per group), and 92 ± 7.2 at 13 weeks ($p < 0.01$, $n=5$ per group). hMSCs treatment dramatically reduced a decline in the number of TH-immunoreactive cells in the SN of MG-132-treated rats, showing approximately 50% increase of survival in TH-immunoreactive cells in the SN compared to only MG-132-treated group ($p < 0.05$). Additionally, hMSC treatment in MG-132-treated rats revealed that increased OX-42 immunoreactivity induced by MG-132 was markedly decreased. Histological analysis showed that the number of NuMA-

positive cells was 1.7% of total injected hMSCs and the number of cell double-stained with NuMA and TH was 35.7 % of NuMA-positive cells. In addition, these cells seem to have a functional characteristic of neurons, demonstrating triple-stained with NuMA, TH, and synaptophysin. Behavioral analysis revealed that hMSC treatment in MG-132-treated rats had a tendency of increase in mean stay time on the rod at a 25 rpm ($p < 0.1$).

In conclusion, we have shown that hMSC treatment have a protective effect of dopaminergic neuronal cell death induced by MG-132 in vitro and in vivo. Complex mechanisms mediated by trophic effect of hMSCs and differentiation of hMSCs into functional TH-positive neurons may work in the neuroprotective process of hMSCs. In addition, neuroprotective strategies of hMSCs in this study may be clinically applicable.

Key words : Parkinson's disease (PD), MG-132, proteasome inhibitor, human mesenchymal stem cell

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	v
I . INTRODUCTION	1
II . MATERIALS AND METHODS	3
A. MATERIALS	3
1. Antibodies	3
B. METHODS	4
1. Isolation of human bone marrow MSCs	4
2. Administration of proteasome inhibitors and hMSCs	4
3. Primary culture of Mesencephalic tissue and proteasome inhibitor treatment ..	5
4. Tissue preparation	6
5. Immunocytochemistry and Immunohistochemistry	7
6. Immunofluorescence double-labeling	7
7. Gas chromatography-mass spectrometry.....	8
8. Western blot analysis	8
9. Rotarod test.....	9
10. Stereological cell counts.....	10
11. Statistical analysis.....	10

III. RESULTS	12
1. Characterization of hMSCs.....	12
2. Effect of hMSC on dopaminergic neurons in vitro.....	12
3. Progressive decline in the number of TH-immunoreactive cells in MG-132- treated rats	14
4. Effect of cell therapy with hMSC on animals treated with MG-132.....	15
5. Histological analysis of transplanted hMSCs in animal model of PD induced by MG-132	17
6. Behavioral assessment.....	19
IV. DISCUSSION	20
V. CONCLUSION	25
REFERENCES	26
국문요약	28

LIST OF FIGURES

Fig. 1. Timeline and summary of treatment regimens and analyses	4
Fig. 2. Characterization of hMSCs	12
Fig. 3. Effect of hMSC on dopaminergic neurons in vitro.....	13
Fig. 4. Progressive decline in the number of TH-immunoreactive cells in MG-132-treated rats.....	14
Fig. 5. Effect of cell therapy with hMSC on animals treated with MG-132	16
Fig. 6. Histological analysis of transplanted hMSCs in animal model of PD induced by MG-132 Characterization of hMSCs.....	18
Fig. 7. Behavioral assessment of transplanted hMSCs in animal model of PD induced by MG-132 Characterization of hMSCs.....	18

I. INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by a selective loss of dopaminergic neurons and the presence of Lewy bodies, proteinaceous inclusions that contain alpha-synuclein, synphilin-1, components of the ubiquitin proteasomal pathway and parkin in substantia nigra (SN) (Moore. et al., 2005). Recently, various genes responsible for familial PD have been identified and these have led to an increased understanding of its molecular pathogenesis (Healy. et al., 2004, Morris. et al., 2005). Of those, mutations in the parkin gene are relatively common in familial PD, with mutations found in 50% of familial early-onset cases compatible with recessive inheritance (Healy et al., 2004, Schrag. et al., 2006). Parkin has E3 ligase activity which is an important part of the cellular machinery that covalently tags target proteins with ubiquitin through the ubiquitin-proteasome system (Hattori. et al., 2004). Therefore, loss of function of parkin from mutations is thought to result in the improper targeting of its substrates for proteasomal degradation leading to inadequate protein degradation and consequently unfolded protein accumulation.

Recently, McNaught and colleague reported that systemic injection of proteasome inhibitor induces a chronic progressive neurodegenerative disorder in rats that closely recapitulates the features of PD in pathological, neurochemical and behavioral aspects (Reaney. et al., 2006). Furthermore, they suggested that this model could be a useful tool for testing putative neuroprotective therapies for PD. There are a variety of proteasome inhibitors, lactacystin, PSI, epoxomicin and MG-132. These proteasome inhibitors consistently caused a significant loss of viability of dopaminergic neurons, although selective vulnerability to dopaminergic

neurons was different among these proteasome inhibitors with the least selectivity in PSI (McNaught. et al., 2004).

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 (Pittenger. et al., 1999, Woodbury. et al., 2000). The application of MSCs in neurodegenerative diseases is seldom studied. Jin and colleague (Jin. et al., 2002) reported that intracerebral transplantation of MSCs had significant effects on the progression of neurological deficits and life span in a knockout mouse model of Niemann-Pick disease, a lysosomal storage disorder showing progressive ataxia and Purkinje cell loss. Mazzini and colleague (Mazzini. et al., 2003) in a study of MSC transplantation into spinal cord of patients with amyotrophic lateral sclerosis reported that most patients showed a slowing down of the linear decline or increasing muscle strength. Li and colleague (Li. et al., 2001) reported that using lesional model of PD by 6-hydroxydopamine, MSCs injected intrastrially survive, express dopaminergic protein tyrosine hydroxylase (TH) immunoreactivity, and promote functional recovery. However, there were no reports of hMSCs application in similar clinical setting of PD with the viewpoint of neuroprotective strategies. The aim of this study was to investigate whether cell therapy with MSCs has a protective effect on progressive dopaminergic neuronal loss in vitro and in vivo system using MG-132. In addition, we also evaluated whether transplanted MSCs have the capacity of differentiation to TH-positive neuron and functional recovery.

II. MATERIALS AND METHODS

A. MATERIALS

1. Antibodies

Antibodies and dilutions used in this study include: mouse anti-TH (1:2000 dilution for brain tissue ; 1:7500 dilution for cell culture, Pel-freez, USA), rabbit anti-TH (1:2000 dilution for brain tissue; 1:7500 dilution for cell culture, Chemicon), mouse anti-Nuclear Matrix (NuMA, 1:100 dilution, Calbiochem), rabbit anti-Synaptophysin (Syn, 1:200 dilution, invitrogen), rabbit anti- α -synuclein (α -syn, 1:200 dilution, SIGMA), mouse anti-ubiquitin (Ub, 1:200 dilution, SANTA CRUZ), Ox-42 (1:200, Seratec, Raleigh, NC, USA), D105/CD34 for immunochemistry, immunofluorescence, and mouse anti-ubiquitin (Ub, 1:1000 dilution, SANTA CRUZ) for Western Blotting.

The different primary antibodies were codetected by immunofluorescence, using goat anti-mouse IgG Alexa Fluor-488 (green) and goat anti-rabbit IgG Alexa Fluor-594 (red) (1:200, Molecular probes).

B. METHODS

1. Isolation of human bone marrow MSCs (hMSCs)

Bone marrow aspirates (10ml) were obtained from iliac crests of human donors. The aspirates were with 10ml of PBS. The mononuclear cell layer was isolated by Ficoll-Hypaque, washed in PBS, and plated in polystyrene plastic 100mm culture dishes and were cultivated in low-glucose Dulbecco modified eagles' medium (Gibco-BRL, Grand Island, NY) containing 10% fetal bovine serum (Hyclone, Irvine, CA) and 1% penicillin/streptomycin (Sigma, St. Louis, MO) in a humidified incubator at 37°C under 5% CO₂. Nonadherent cells were removed after 24hr. When these primary cultures of MSCs reached 80% confluence, the cells were harvested using 0.25% trypsin and subcultured. At passage 6 of hMSCs, cells were injected by tail vein.

2. Administration of Proteasome Inhibitors and hMSCs

Male Sprague–Dawley (SD) rats (200-220g) were used for the study. The proteasome inhibitor, MG-132 was freshly dissolved in sterile dimethylsulfoxide (DMSO). Rats were injected subcutaneously with MG-132 on six occasions on alternate days over 2 weeks (Monday, Wednesday, and Friday). Control rats were injected with DMSO using the same administration method.

These animals were divided into 3 groups. Group 1 was a control group of male rats (n=18) treated with subcutaneously DMSO. Group 2 was a group of male rats (n=18) treated with MG-132 (3mg/kg) subcutaneously dissolved in DMSO for 2weeks. Group 3 was a group of male rats (n=18) injected human Mesenchymal stem cells once (1×10^6) at 1 week for 3weeks via tail vein, two weeks after last administration of MG-132. The detailed experimental schedule was

illustrated in figure 1.

3. Primary culture of mesencephalic tissue and proteasome inhibitor treatment

Dopaminergic neurons were cultured from SN of 14-day-old embryonic SD rats (Chung et al., 2001; Cho et al., 2003; Han et al., 2003). The tissues incubated in Ca^{2+} -, Mg^{2+} - free Hanks' balanced salt solution (CMF-HBSS) for 10min and a 0.01% Trypsin solution in CMF-HBSS for 9 min at 37°C. Cultures were rinsed twice in RF (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 6mg/ml glucose, 204µg/ml L-glutamine, 100U/ml penicillin/streptomycin(P/S)) for trypsin inhibition and then dissociated into single cells by trituration. Dissociated cells were plated on 12-mm round aclar plastic coverslips or cultureslides pre-coated with 0.1mg/ml poly-D-lysine and 4µg/ml laminin and seeded in 24-well culture plates at a density of 1.0×10^5 cells/coverslip or slide. Cells were incubated in a humidified incubator at 37°C, 5% CO_2 for 24 hr. At two day-old in vitro cultures (DIV), the culture medium was replaced with chemically defined serum-free medium (DM) composed of Ham's nutrient mixture (F12-DMEM) and with 1% ITS (insulin, transferrin, selenium), glucose, L-glutamine and P/S. At DIV 4, the cultures were pre-treated with MG-132 (10µM). Primary culture of mesencephalic tissue was co-cultured with 3×10^5 hMSCs/ well (using transwell) 24 hr after MG-132 treatment.

4. Tissue preparation

For immunohistochemistry, the animals were perfused with a saline solution containing 0.5% sodium nitrate and heparin (10 U/ml) and fixed with 4% paraformaldehyde dissolved in 0.1M PB (both at almost 200 ml/rat) 8, 10, 13 weeks after MG-132, respectively. The brain was removed from the skull, post-fixed overnight in buffered 4% paraformaldehyde at 4°C and stored in a 30% sucrose solution for 1 to 2 days at 4°C until they sank. They were then sectioned on a sliding microtome to obtain a 30-µm-thick coronal section or after embedded in paraffin, 4µm sections from SN were immunofluorescenced. The 30-µm-thick coronal sections were stored in tissue stock solution (30% glycerol, 30% etholene glycol, 30% 3rd D.W., 10% 0.2M PB) at 4°C until required. For Western blotting and high-performance liquid chromatographic-mass spectrometer (HPLC), animals were euthanized 13weeks after MG-132 treatment, and the SN area was rapidly removed from the brains and frozen at -70°C.

5. Immunocytochemisrty and Immunohistochemistry

The 30-µm-thick coronal brain sections and co-cultured cells were rinsed twice in PBS and incubated in 0.2% Triton X-100 for 30 min at room temperature (RT). They were rinsed thrice with 0.5% bovine serum albumin (BSA) in 1× PBS for blocking. After blocking, they were incubated overnight at RT or 4°C with primary antibodies; the primary antibodies for TH (1:2000 for immunohistochemistry, 1:7500 for immunocytochemistry; Pel-freez, St. Rogers, AR, USA), Ox-42 (1:200, Seratec, Raleigh, NC, USA). After 24 hr, primary culture of mesencephalic tissue and brain sections were rinsed thrice in 0.5% BSA in 1× PBS (10 min each rinse) and incubated with appropriate biotinylated secondary antibody and avidin-

biotin complex (Elite Kit; Vector Laboratories, Burlingame, CA, USA) for 1 hr at RT. The bound antiserum was visualized by incubating with 0.05% diaminobenzidine-HCL (DAB) and 0.003% hydrogen peroxide in 0.1M PB. The primary mesencephalic culture and brain sections was rinsed with 0.1M PB for DAB inhibition.

The immunostained cells were analyzed under bright-field microscopy.

6. Immunofluorescence double-labeling

The 4 μ m sections of embedded paraffin tissues and the 30 μ m-thick coronal brain sections were rinsed twice in PBS and incubated in 0.2% Triton X-100 for 30 min at RT. They were rinsed thrice with 0.5% BSA in 1 \times PBS for blocking. After blocking, they were incubated overnight at RT or 4 $^{\circ}$ C with primary antibodies; TH $^{+}$ NuMA, TH $^{+}$ syn, NuMA $^{+}$ syn, Ub $^{+}$ α -syn. After 24 hr, the brain sections were rinsed thrice in 0.5% BSA in 1 \times PBS (10 min each rinse) and incubated with secondary antibody; goat anti-mouse IgG (Alexa Fluor-488, green) and goat anti-rabbit IgG (Alexa Fluor-594, red) for 1 hr at RT. Brain tissues were then washed and mounted using Prolong Antifade Kit (Molecular Probes, USA). Stained tissues were viewed using an Olympus I \times 71 confocal laser scanning microscope (Olympus, Tokyo, Japan). To analyze the localization of different antigens in double-stained samples, immunofluorescence images were created from the same tissue section and merged using interactive software.

7. Gas chromatography-mass spectrometry (GC-MS, dopamine level assay)

GC-MS analyses both in scan and Selected Ion Monitoring (SIM) modes were performed with an Agilent 6890 gas chromatograph, interfaced to an Agilent 5973 mass-selective detector (70 eV, electron impact mode) and installed with an Ultra-2 (5% phenyl-95% methylpolysiloxane bonded phase; 25m×0.20mm i.d.,0.11 μm film thickness) cross-linked capillary column (Agilent Technologies, Atlanta, GA, USA). The temperatures of injector, interface and ion source were 260, 300 and 230 °C, respectively. Helium was used as carrier gas at a flow rate of 0.5 ml/min⁻¹ with constant flow mode. Samples were introduced in the split-injection mode (10:1) and the oven temperature was initially at 170°C for 2 min and programmed at 5°C/min to 205°C and at 2°C/min to 220°C then finally to 30°C/min to 300°C (3min). The mass range scanned was 50-800u at a rate of 0.42scan/s. All the GC-SIM-MS runs were performed in triplicate.

8. Western blot analysis

Brain tissues from the SN were dissected and homogenized in ice-cold lysis buffer containing (in mM) 20 Tris-HCl, pH 7.5, 1 EDTA, 5 MgCl₂, 1 dithiothreitol, 0.1 phenylmethylsulfonyl fluoride plus protease inhibitor cocktail (Sigma). Tissue homogenates was centrifuged for 20min at 14,000 × g at 4°C and the supernatant was transferred to a fresh tube. Their proteins were analyzed by Bio-Rad Protein Assay Kit (USA). Equal amounts of protein (50μg) were loaded in each lane with loading buffer containing 0.125M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 10% mercaptoethanol, and 0.002% bromophenol blue. Samples were boiled for 5min before gel loading. Protein analyzed on gel electrophoresis was

transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA) using an electrophoretic transfer system (Bio-Rad). The membranes were washed with Tris-buffered saline solution and 2.5 mM EDTA (TNE) and then blocked in TNE containing 5% skim milk for 1hr. Membranes were then incubated overnight at 4°C with specific primary antibodies of Ub and actin (1:2000; Santa Cruz). After washing, the membranes were incubated with secondary antibodies (1:2000; Amersham) for 1hr at RT, and wash again, and the blots were finally developed with the ECL Western blotting detection reagents (Amersham).

9. Rotarod test

For training, the animals were placed on the rotarod at a constant speed of 5 rpm for 10min during 3 consecutive days before the beginning of the experiment. For rotarod testing, the rats were placed on the rotarod and tested at different rotation speeds (for 10min each 5, 15, 25 rpm.). Each rat generally rested for 20-30 min between tests at each speed; this helps to reduce stress and fatigue. The test was performed on 2 consecutive days every week, and the result was averaged to obtain a stay time on the rod for each rat at a given rotation speed. The test commenced at the same time.

10. Stereological cell counts

The unbiased stereological estimation of the total number of the staining cells in the SN was made using the optical fractionator, as previously described in detail with some modifications (Kirik et al., 1998, 2000). This sampling technique is not affected by tissue volume changes and does not require reference volume determinations (West et al., 1991).

The sections used for counting covered the entire SN, from the rostral tip of the pars compacta back to the caudal end of the pars reticulata. This generally yielded 8-9 sections in a series. Sampling was performed using the Olympus C.A.S.T.-Grid system (Olympus Denmark A/S, Denmark), using an Olympus BX51 microscope, connected to the stage and feeding the computer with the distance information in the Z-axis. SN was delineated at 1.25× objective. A counting frame (60%, 35,650µm²) was placed randomly on the first counting area and systemically moved through all counting areas until the entire delineated area was sampled. Actual counting was performed using a 40× oil objective. Guard volume (4µm from the top 4-6µm 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 depth of 10µm) were counted. The total of staining cells was calculated according to the optical fractionator formula (West et al, 1991).

11. Statistical analysis

The Mann-Whitney test and Kruskal-Wallis analysis were used to compare the means in pairs of groups and multiple comparisons, respectively. P values less than 0.05 were considered statistically significant. Statistical analyses were performed using commercially available software (version 10.0; SPSS Inc., Chicago, IL, USA).

III. RESULTS

1. Characterization of hMSCs

The hMSCs plated on 12-mm round aclar plastic coverslips for characterization by immunocytochemistry. We were identified positive marker of CD 105 and negative marker of CD 34 of the hMSCs (Fig. 2).

2. Effect of hMSC on dopaminergic neurons in vitro

After 24 hr of treatment by proteasome inhibitor (MG-132, 10 μ M), dopaminergic neurons in primary mesencephalic culture were treated hMSCs for 24 hr. Immunocytochemical analyses revealed that hMSC treatment increased dopaminergic neuronal survival compared to dopaminergic neuronal death in MG-132 treatment (Fig. 3A). Quantitative analysis of TH⁺ cells showed that hMSC treatment significantly decreased dopaminergic neuronal loss induced by MG-132 ($p < 0.01$, Fig. 3B).

In addition, hMSCs treatment showed that there was a significant reduction of increased caspase 3 activity following MG-132 treatment (Fig. 3C).

3. Progressive decline in the number of TH-immunoreactive cells in MG-132-treated rats

Rats received systemic injection of MG-132 did not exhibit evidence of systemic toxicity and none died. MG-132 had no effect on significant weight changes compared with controls. At 2 and 4 weeks after initiation of treatment, mean body weights were, respectively, 291.8 ± 5.6 gm and 381 ± 10 gm for controls ($n = 5$) compared with 290.8 ± 5.1 and 386 ± 9.4 for MG-

132-treated rats (n=8).

Immunohistochemical analysis showed that there was a progressive decline in the number of TH-immunoreactive cells in the SN of MG-132-treated animals (Fig. 4A). This TH⁺ neuronal loss was more prominent in the lateral portion of SN than medial region of SN. Quantification of the extent of neuronal loss assessed by stereological analysis revealed that TH⁺ positive cell loss was 23% ± 7.2% (p <0.05, n=5 per group) at 8 weeks, 70 ± 9.5 at 10 weeks (p <0.01, n=5 per group), and 92 ± 7.2 at 13 weeks (p <0.01, n=5 per group; Fig. 4B). In rats treated with MG-132, but not in controls, intracellular protein accumulation was identified, which was immunostained with α-synuclein and ubiquitin (Fig. 4C)

4. Effect of cell therapy with hMSC on animals treated with MG-132

3 weeks after last administration of MG-132, hMSCs were injected via tail vein 3 times for 3 weeks. Rats were killed at 10 weeks after the first hMSCs injection for immunohistochemistry, Western Blot, and HPLC. Immunohistochemical analysis showed that hMSCs treatment dramatically reduced a decline in the number of TH-immunoreactive cells in the SN of MG-132-treated rats (Fig. 5A). stereological analysis revealed that the number of TH⁺ positive cells was significantly higher in hMSC treatment group than in only MG-132-treated group (p<0.05), showing approximately 50% increase of survival in TH-immunoreactive cells in the SN (Fig. 5B). Consistent with the loss of dopaminergic neurons in the SNc, dopamine level in striatum was significantly decreased in MG-132-treated rat compared with controls. However, hMSCs treatment significantly increased dopamine level in the striatum of MG-132-treated rats, which was consistent with increased survival of TH-immunoreactive cells in the SN after hMSCs treatment (Fig. 5C).

Since inhibition of proteasomal enzymatic activity resulted in increased levels of ubiquitinated proteins due to the reduced clearance of proteins by the UPS (Rideout and Stefanis, 2002), we evaluated levels of high molecular weight poly-ubiquitinated proteins (200 and 20 kDa) in MG-132-treated animals. MG-132 treatment resulted in accumulation of poly-ubiquitinated proteins as determined by Western blot, however, hMSCs treatment markedly decreased accumulation of poly-ubiquitinated proteins in MG-132-treated rats (Fig. 5D).

To determine whether hMSCs modulate microglial activation, SN was immunostained with OX-42, a marker of microglia. In rats treated with MG-132, there was a marked increase in OX-42 immunoreactivity (a marker of microglia), however, there was little immunoreactivity with OX-42 in control rats. hMSC treatment in MG-132-treated rats revealed that increased OX-42 immunoreactivity induced by MG-132 was markedly decreased (Fig. 5E).

In rat treated with MG-132, there was a significant increase of caspase 3 activity compared with controls. However, hMSCs treatment in MG-132-treated rats significantly decreased caspase 3 activity (data not shown).

5. Histological analysis of transplanted hMSCs in animal model of PD induced by MG-132

To determine if transplanted hMSCs would survive, we identified existence of hMSCs in the SN of MG-132-treated rats using human specific NuMA staining. In controls and only MG-132-treated animals, there were no NuMA-positive cells (Fig. 6A). In contrast, NuMA-positive cells were only observed in animals treated with hMSCs. The number of NuMA-positive cells was 51957 ± 35 , which was corresponded to 1.7% of total injected hMSCs (Fig.

6B). To determine whether transplanted hMSC possessed phenotype of TH neurons, we co-stained NuMA-positive cells with antibodies to TH (Fig. 6C). The number of cell double-stained with NuMA and TH was 687 ± 35 , 35.7 % of NuMA-positive cells (Fig. 6D). Furthermore, we evaluated if the cell merged with NuMA and TH staining harbored immunoreactivity with human specific synaptophysin, a major membrane protein of synaptic vesicle using thin paraffin section (4 μ m). As shown in Fig. 6E, transplanted hMSC was triple-stained with NuMA, TH, and synaptophysin, indicating that transplanted hMSC harbored phenotype of TH as well as human specific synaptophysin.

6. Behavioral assessment

In controls, the mean stay time on the rod at a 25 rpm was increased to 100-150 sec after two weeks after initiation of rotarod test, however, MG-132-treated rat showed that the mean stay time at a 25 rpm was significantly reduced two weeks after MG-132 treatment ($p < 0.01$). hMSC treatment in MG-132-treated rats had a tendency of increase in mean stay time on the rod at a 25 rpm ($p < 0.1$). There was a significant difference of mean stay time on the rod at a 5 and 15 rpm among groups. (Fig. 7)

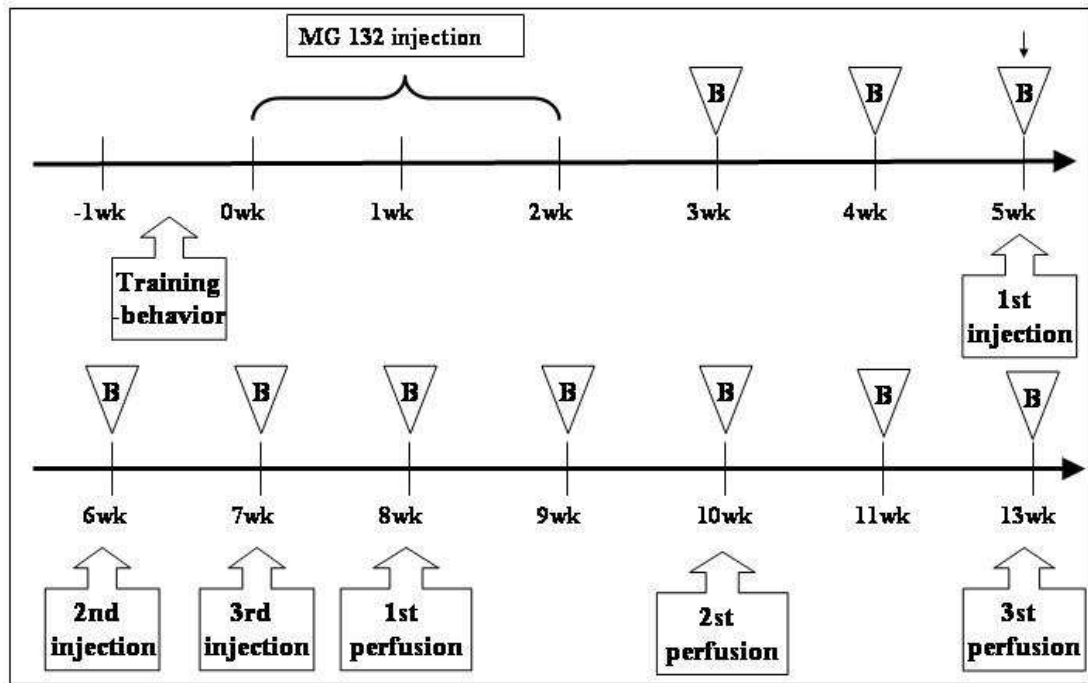


Fig. 1. Timeline and summary of treatment regimens and analyses. Before injection, adult Sprague-Dawley rats (SD rat) trained rotarod for 1week. MG132 (3mg/kg, SC) or vehicle (dimethyl sulfoxide;DMSO) as a control were injected six times into adult SD rats (approximately 200g at start) over a period of 2weeks (Mon, Wed, Fri, Mon, Wed, Fri). 2 weeks after injected MG132, rats injected human Mesenchymal stem cells once (1×10^6) at 1 week for 3weeks via tail vein. 8, 10, 12 weeks after MG-132, rats were killed for immunohistochemistry, western blot, RT-PCR. Behavior test at each week (B:behavior).

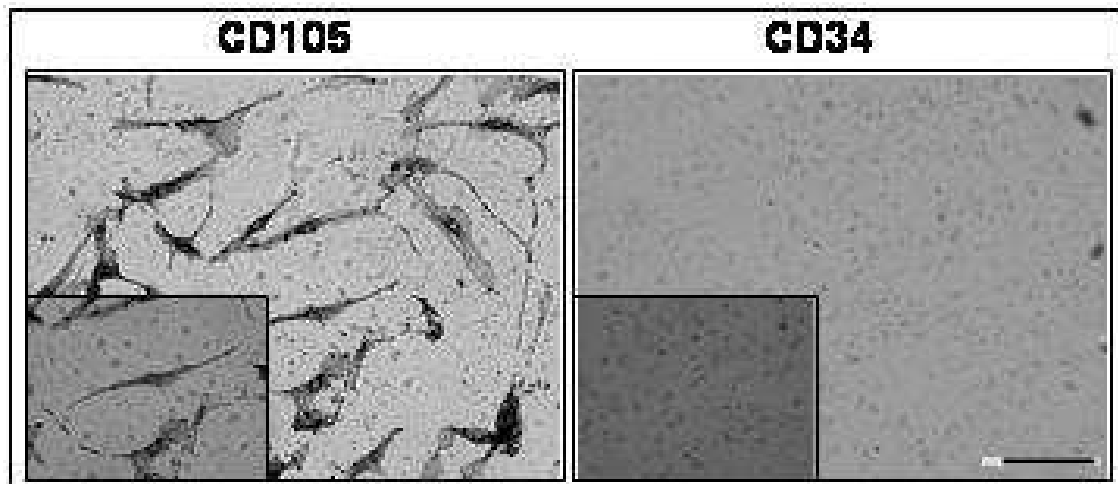


Fig. 2. Characterization of hMSCs. Isolation of hMSCs were identified positive marker of CD 105 and negative marker of CD 34 of the hMSCs. Big box is x200 and small box is x400. (scale bar – 100 μ M).

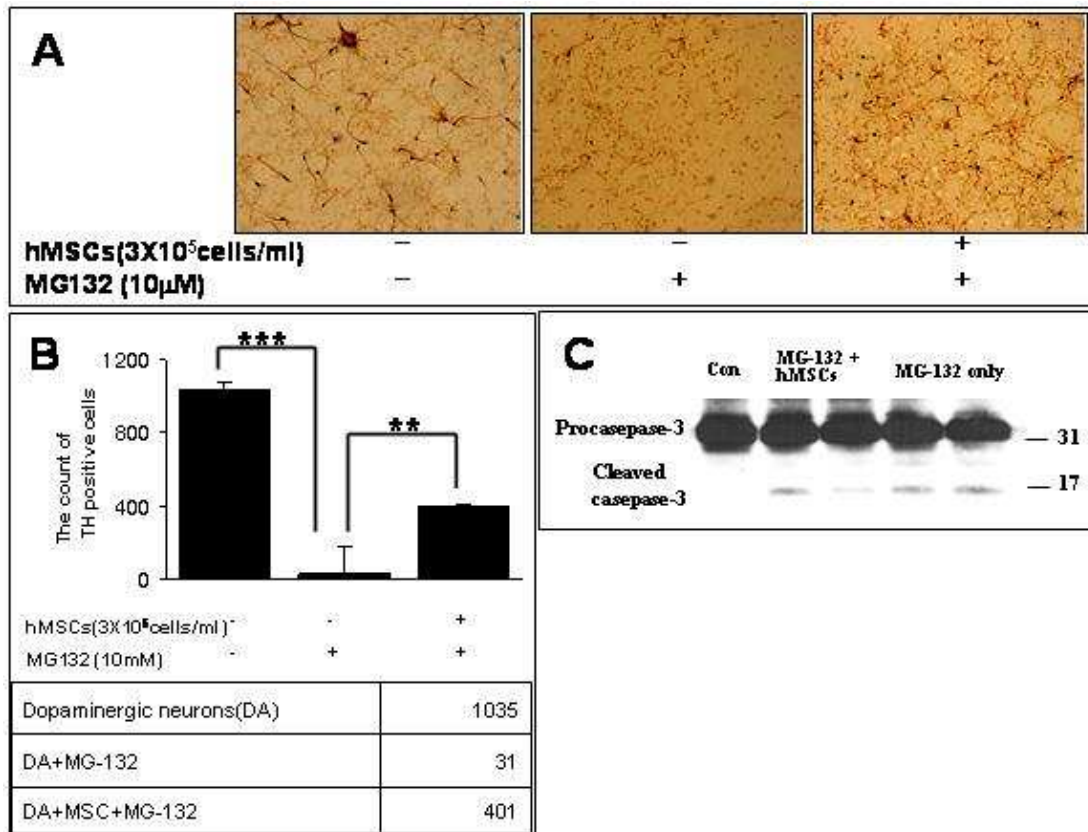


Fig. 3. Effect of hMSC on dopaminergic neurons in vitro. Primary culture cells of mesencephalic tissue were pretreated with MG132 (10 μ M) for 2h and then co-cultured hMSCs for 24hr. Immunocytochemistry with TH revealed that hMSCs co-culture group significantly decreased MG132-induced dopaminergic neurons death (A). The count of TH-positive cells in slip (B). The MSCs group showed reduction of increased caspase 3 activity following MG-132 treatment (Fig. 3C).

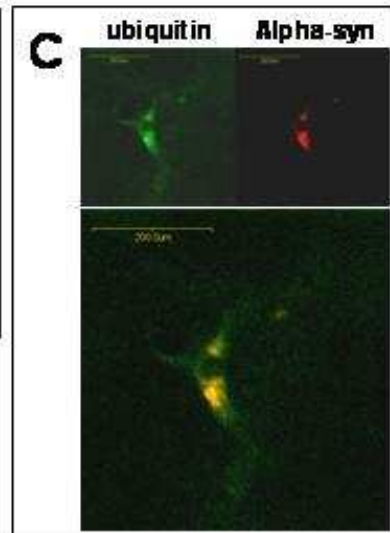
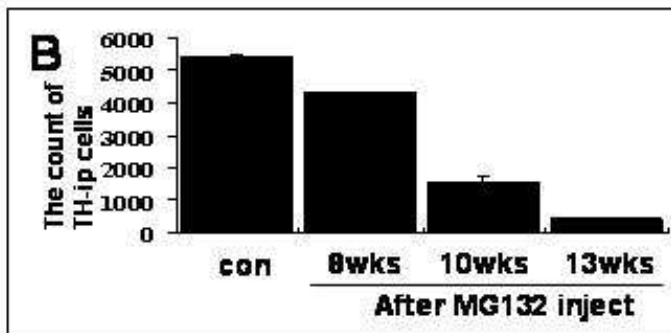
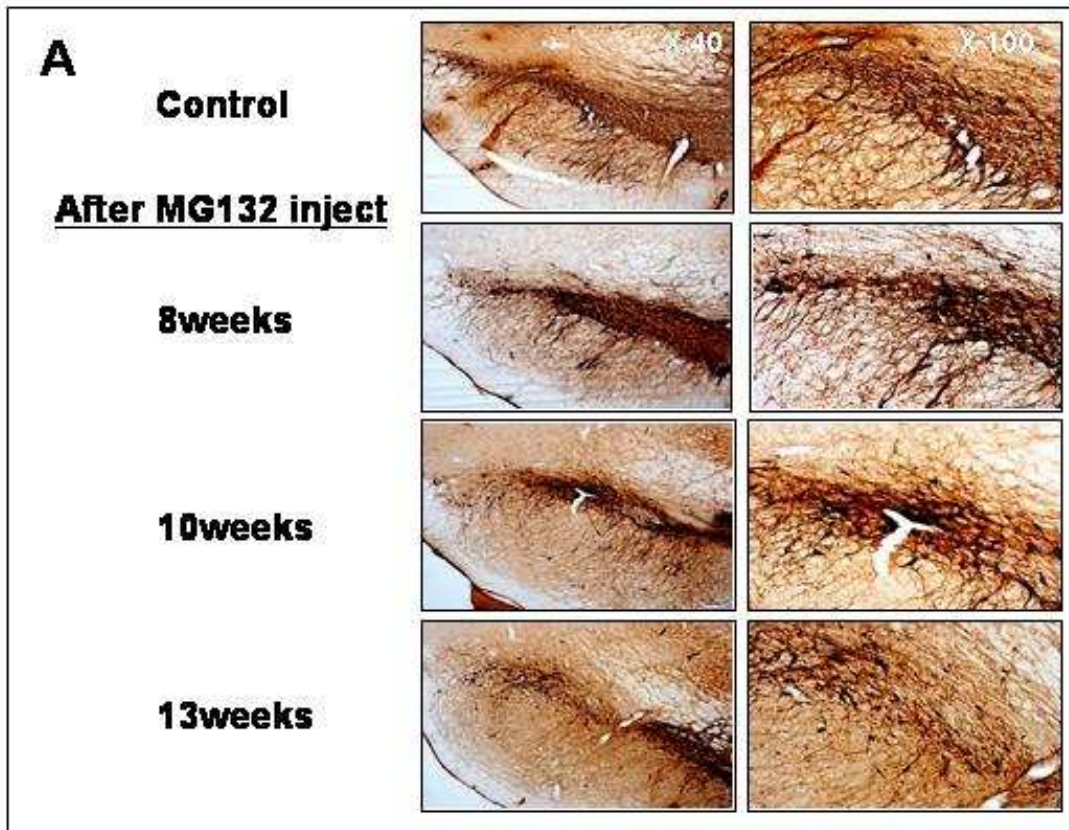


Fig. 4. Progressive decline in the number of TH-immunoreactive cells in MG-132-treated rats. SD rats were injected with MG132 (3mg/ml) for 2weeks and then identified progressive of dopaminergic neurons loss by immunohistochemistry (using TH) in SN (A). The count of TH-ip cells using C.A.S.T grid (B). The tissues identified inclusion by immunofluorescence (using ubiquitin-green, alpha-synuclein-red) (C).

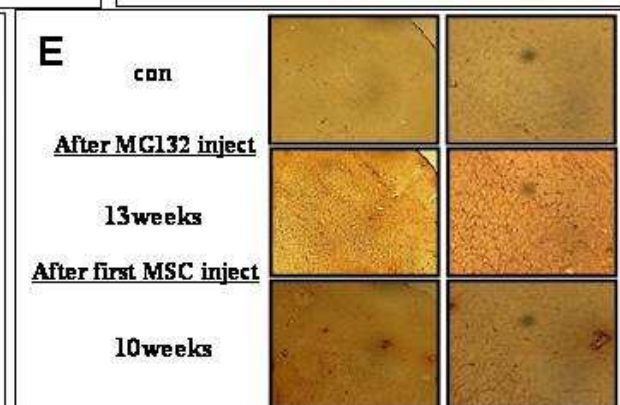
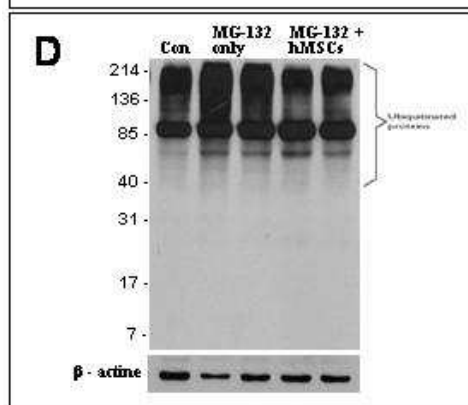
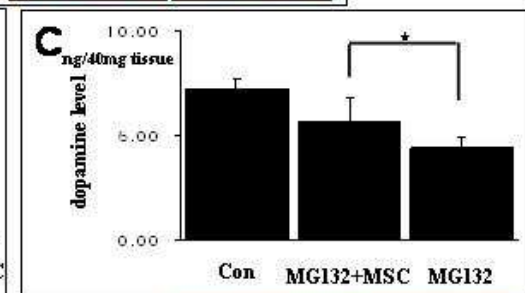
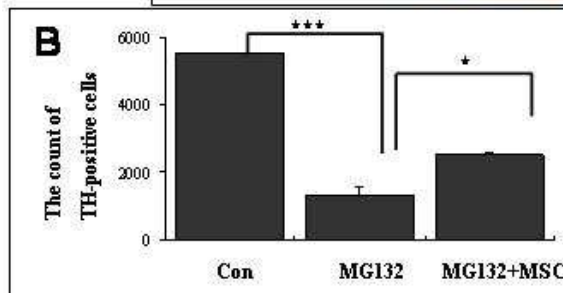
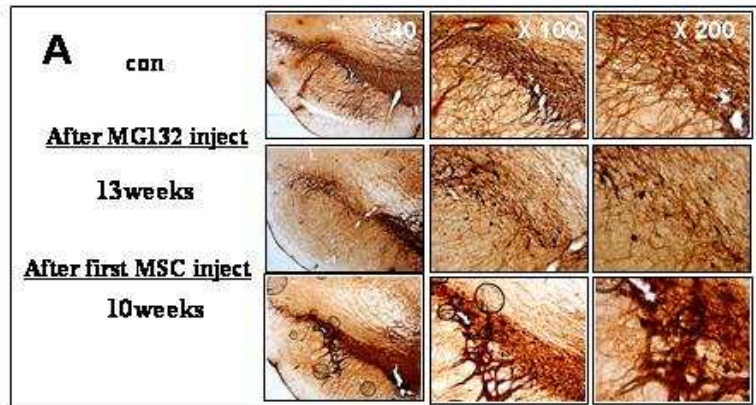
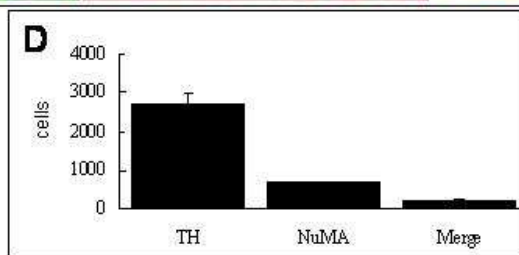
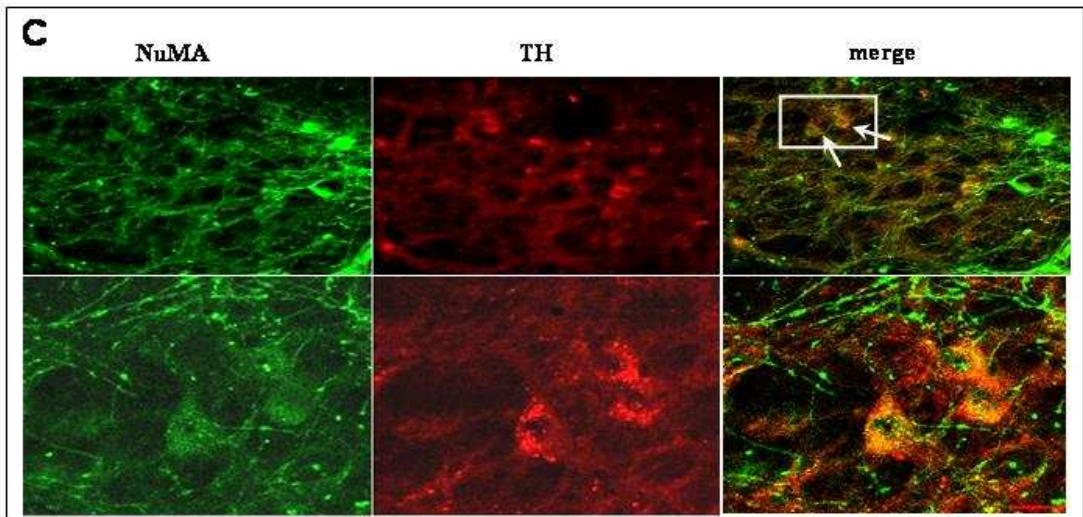
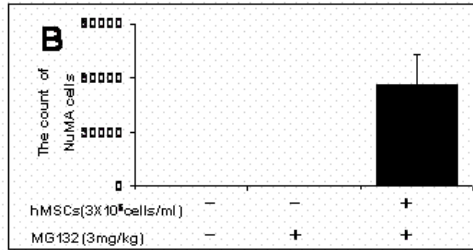
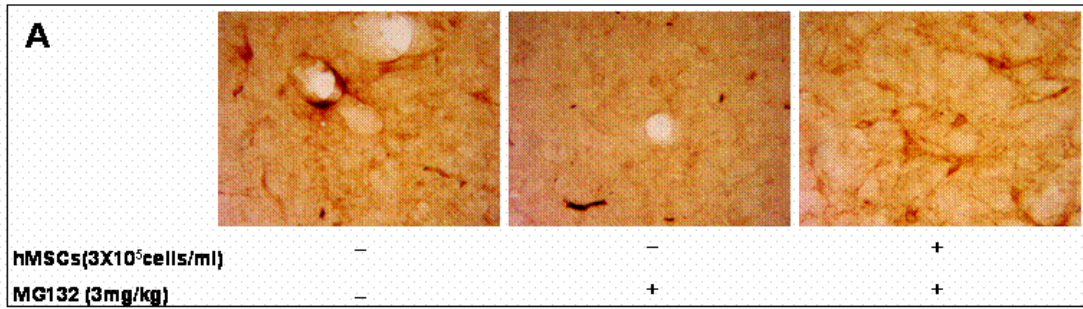


Fig. 5. Effect of cell therapy with hMSC on animals treated with MG-132. Rats were killed at 10 weeks after the first hMSCs injection or 13 weeks after the first MG132 injection for immunohistochemistry. The hMSCs treatment dramatically reduced a decline in the number of TH-immunoreactive cells in the SN of MG-132-treated rats (A). Stereological analysis revealed that the number of TH⁺ positive cells was significantly higher in hMSC treatment group than in only MG-132-treated group ($p < 0.05$) (B), Dopamine level in striatum was significantly decreased in MG-132-treated rat compared with controls. However, hMSCs treatment significantly increased dopamine level in the striatum of MG-132-treated rats (C), hMSCs treatment markedly decreased accumulation of poly-ubiquitinated proteins in MG-132-treated rats (D). hMSC treatment in MG-132-treated rats revealed that increased OX-42 immunoreactivity induced by MG-132 was markedly decreased (E).



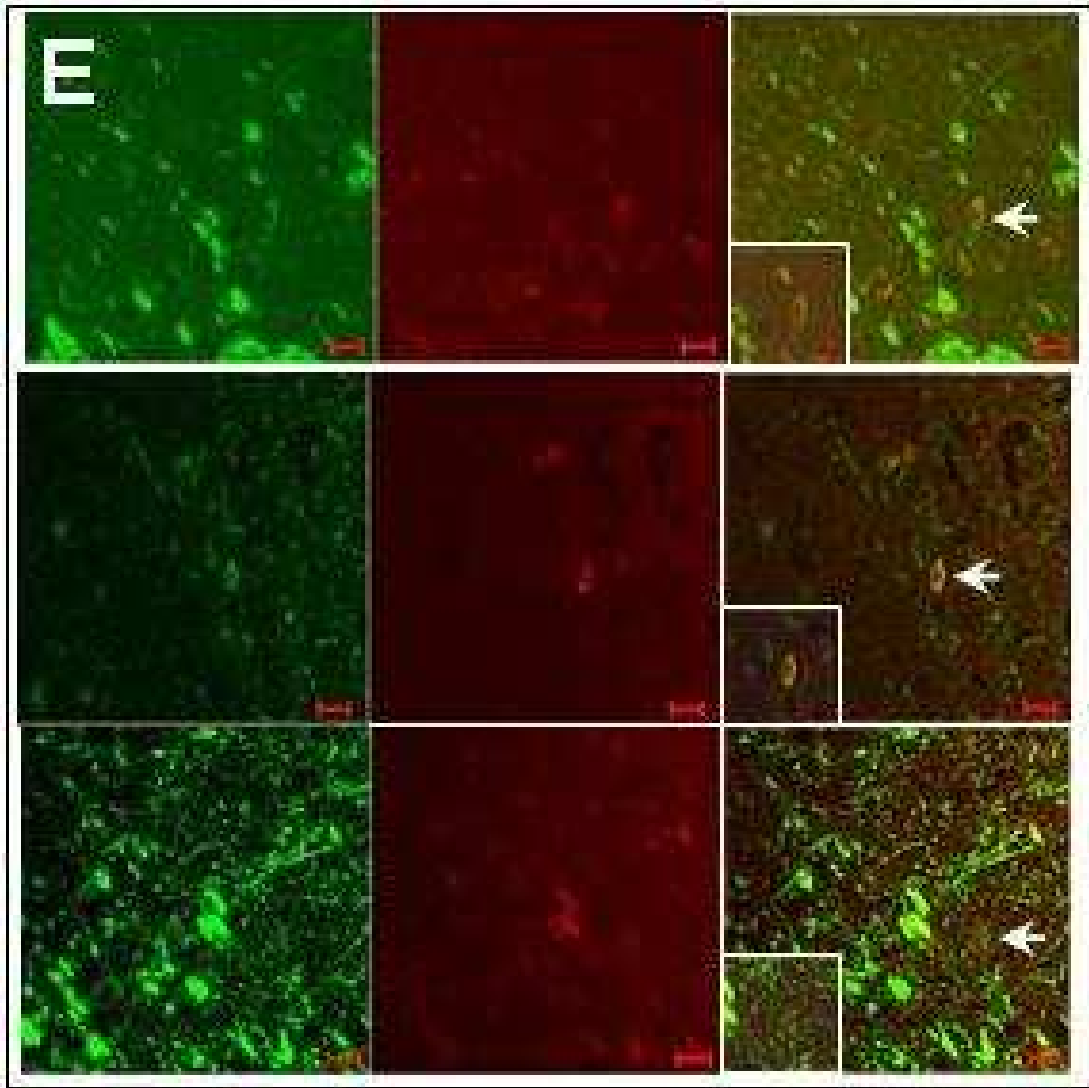


Fig. 6. Histological analysis of transplanted hMSCs in animal model of PD induced by MG-132. Rats were killed at 10 weeks after the first hMSCs injection for immunohistochemistry. The existence of hMSCs in the SN of MG-132-treated rats was identified by human specific NuMA staining (A), The count of NuMA-ip cells in the SN of MG132-treated rats (B), The differentiation of hMSCs in the SN of MG-132-treated rats was identified by Immunofluorescence double-labeling (using TH, NuMA) (C), the count of TH+NuMA merge cells in the SN of MG-132-treated rats (D). The transplanted hMSC was triple-stained with NuMA, TH, and synaptophysin, indicating that transplanted hMSC harbored phenotype of TH as well as human specific synaptophysin (E).

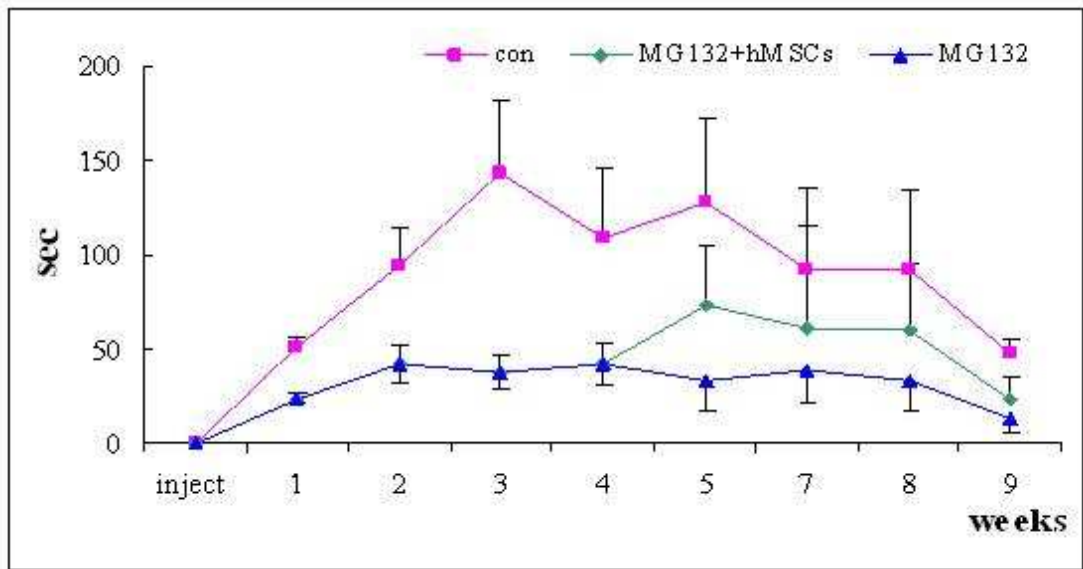


Fig. 7. Behavioral assessment of transplanted hMSCs in animal model of PD induced by MG-132 **Characterization of hMSCs.** MG-132-treated rat showed that the mean stay time at a 25 rpm was significantly reduced two weeks after MG-132 treatment. The hMSC treatment in MG-132-treated rats had a tendency of increase in mean stay time on the rod at a 25 rpm.

IV. DISCUSSION

Although PD is the only chronic neurodegenerative disease for which there are effective for symptomatic treatment of dopaminergic regimens, these therapies do not change the progressive nature of PD and moreover, some axial symptoms of gait freezing and postural instability which are more disability than tremor and rigidity do not respond to dopaminergic therapies. In addition, as PD progress, dopaminergic therapies results in disabling drug-induced motor complications such as wearing off and dyskinesia and a variety of non-motor symptoms such as dementia, autonomic dysfunction, and mood disorders also emerge, which is comparatively disabling as much as motor symptoms. Therefore, neuroprotective strategy that favorably influences the disease process or underlying pathogenesis to produce enduring benefits for PD patients is required.

There were numerous candidates for neuroprotective agents which are tested in animal model of PD.

However, most drug candidates have been tested in acute or subacute neurotoxin-induced animal model and these compounds are administrated before the development of PD model by neurotoxin. To test neuroprotective agents in animal model of PD similar to clinical settings, animal model of PD should have the nature of chronic progressive nature and administration of candidate drugs should start after neuronal loss of SN has started. Like progressive PD model studied by McNaught and colleagues using proteasome inhibitors, chronic administration of MG-132 in this study also showed progressive decline in the number of TH-immunoreactive cells, from approximately 23% TH-positive cell loss at 8 weeks to 92% TH-positive cell loss at 13 weeks, and this decline in TH-positive neurons in

SN accompanied the decreased dopamine level in the striatum as well as mean stay time on the rod compared with controls. In addition, the treatment of hMSCs in this study started 4 weeks after initial administration of MG-132 in animal and 24 hr after MG-132 treatment in primary mesencephalic culture.

Present study demonstrated that hMSC treatment significantly decreased neurodegeneration of dopaminergic neuron resulted from MG-132 treatment, showing approximately 50% increase of survival in TH-immunoreactive cells in the SN. Increased dopamine level in the striatum and a tendency of reduction in behavioral deterioration after hMSCs treatment were well corresponded to the increase in survival of dopaminergic neurons following MSCs treatments in MG-132-treated animals. The mechanism of neuroprotective effects in hMSCs seems to be more complex and pleiotropic, which may be mediated by trophic mechanisms. First, this study demonstrated that hMSCs treatment markedly decreased accumulation of poly-ubiquitinated proteins and the activity of caspase 3 activity in vitro as well as in vivo following MG-132 treatment. Second, hMSC treatment had an effect of anti-inflammatory effect, showing significantly reduced microglial activation in MG-132-treated animals. Although initially triggering events in the dopaminergic neuronal death in PD are still unknown, apoptosis and alteration of proteasome activity play a pivotal role in the pathogenesis of PD. The presence of DNA fragmentation and chromatin clumping in the dopaminergic neurons coupled with upregulation of signals associated with apoptosis in PD patients supports that dopaminergic neurons would be die by apoptosis in PD (annals). Recent genetic, postmortem, and experimental studies suggest that proteasomal dysfunction might play an important role in the accumulation of toxic proteins and consequently, neurodegeneration in the SN, mediating by imbalance between the degradation and clearance

of abnormal proteins. Evidence has been presented in several studies of in vitro or in vivo disease models that inhibition of the inflammatory response can prevent degeneration of nigrostriatal dopaminergic neurons. For example, sodium salicylate, COX-2 inhibitor, or minocycline have been shown to significantly reduce striatal dopaminergic depletion and dopaminergic neuronal loss induced by MPTP or LPS-models (Aubin et al. 1998; Du et al. 2001; He et al. 2001; Sairam et al. 2003; Teismann et al. 2003). Furthermore, recent epidemiological study has demonstrated that regular use of nonsteroidal anti-inflammatory drugs (NSAID) is associated with a 45% lower risk of PD compared to a nonuser (Chen et al. 2003), supporting the neuroprotective effects of NSAID in the development or progression of PD. It has been known that the MSCs could be mobilized from the marrow and produce a variety of trophic factors that inhibit apoptosis and inflammation (J Cell Biochem. 2006 Aug 1;98(5):1076-84). MSCs have a characteristic of migration towards damaged tissues in animal model of ischemia as well as PD model by 6-hydroxydopamine. In this study, the number of survival hMSCs in SN (a key structure damaged from MG-132 treatment) 5 weeks after last hMSCs administration was approximately 1.7% of total injected hMSCs. We speculate that these migrated cells may contribute to the production of numerous trophic factors and inhibit microenvironmental cascade of the neurodegenerative process in nigral dopaminergic neurons.

Recent studies indicate that mouse, rat, and human MSCs can be induced to differentiate into neuron-like cells. Moreover, Blondheim and colleagues demonstrated that MSCs had an expression of several specific neuronal markers and transcriptional factors, of which a large proportion of the genes was participating in the neuro-dopaminergic system, and suggested that the expression of neural gene as well as gene associated with dopaminergic system is a

widespread phenomenon of MSCs. However, there were few reports regarding that MSCs can differentiate into TH-immunoreactive neurons in vivo study and their results were contradictory. Li and colleagues and Blondheim and colleagues reported that using 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine and 6-hydroxydopamine -PD model, respectively, MSCs injected intrastrially express the phenotype of dopaminergic neurons. In a recent study by Ye and colleague, they did not find BrdU and TH-positive cell in the striatum and suggested that functional recovery in MSCs-treated rat may not be associated with differentiation of MSCs into TH-positive neurons. Our data demonstrated that approximately 35.7% of survival hMSCs in SN had an expression of TH-positive immunoreactivity and furthermore, TH-positive cells were immunostained with human specific synaptophysin, suggesting that TH-positive cells may have a dopaminergic function. Taken with trophic effects of anti-apoptosis, anti-inflammation, and decreased poly-ubiquitinated proteins, differentiation of hMSCs into functional TH-positive neurons may in part contribute to functional recovery and decreased neurodegeneration in MG-132 treated animals.

Besides molecular and cellular benefits of hMSCs, cell therapy with hMSCs has an advantage in clinical application. hMSCs can be easily harvested from self bone marrow, culture in vitro, and administrated to patients through various roots including intravenous, intraarterial, intrathecal, or intralesional infusion. In contrast with therapy with embryonic stem cell, there is no immunological rejection, and cell therapy with hMSCs is free from ethical problems. Importantly, regarding the safety in hMSCs application, our group recently documented that cell therapy with hMSCs in patients with ischemic stroke is feasible and quite safe. This is the first report of hMSCs application in similar clinical setting of PD with

the viewpoint of neuroprotective strategies, and we believe it is an approach with direct clinical application.

V. CONCLUSION

In conclusion, we have shown that hMSC treatment have a protective effect of dopaminergic neuronal cell death induced by MG-132 in vitro and in vivo. Complex mechanisms mediated by trophic effect of hMSCs and differentiation of hMSCs into functional TH-positive neurons may work in the neuroprotective process of hMSCs. In addition, neuroprotective strategies of hMSCs in this study may be clinically applicable.

REFERENCES

1. Adler CH: Nonmotor Complications in Parkinson's Disease. *Mov Disord* 20 Suppl 11, S23-29, 2005.
2. Aubin N, Curet O, Deffois A, Carter C: Aspirin and Salicylate Protect Against MPTP-Induced Dopamine Depletion in Mice. *Journal of neurochemistry* 71, 1635-1642, 1998.
3. Bang OY, Lee JS, Lee PH, Lee G: Autologous Mesenchymal Stem Cell Transplantation in Stroke Patients. *Annals of neurology* 57, 874-882, 2005.
4. Blondheim NR, Levy YS, Ben-Zur T, Burshtein A, Cherlow T, Kan I, Barzilai R, Bahat-Stromza M, Barhum Y, Bulvik S et al: Human Mesenchymal Stem Cells Express Neural Genes. *Stem cells and development* 15, 141-164, 2006.
5. Caplan AI, Dennis JE: Mesenchymal Stem Cells as Trophic Mediators. *Journal of cellular biochemistry* 98, 1076-1084, 2006.
6. Chen H, Zhang SM, Hernan MA, Schwarzschild MA, Willett WC, Colditz GA, Speizer FE, Ascherio A: Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Archives of neurology* 60, 1059-1064, 2003.
7. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M: Intravenous Bone Marrow Stromal Cell Therapy Reduces Apoptosis and Promotes Endogenous Cell Proliferation After Stroke in Female Rat. *Journal of neuroscience research* 73, 778-786, 2003.
8. Cho BP, Song DY, Sugama S, Shin DH, Shimizu Y, Kim SS et al: Pathological dynamics of activated microglia following medial forebrain bundle transaction. *Glia* 53:92-102, 2006.

9. Chung ES, Joe EH, Ryu JK, Kim J, Lee YB, Cho KG et al: GT1b ganglioside induces death of dopaminergic neurons in rat mesencephalic cultures. *Neuroreport* 12: 611-614, 2003.
10. Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL et al: Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* 98, 14669-14674, 2001.
11. Emborg ME: Evaluation of animal models of Parkinson's disease for neuroprotective strategies. *Journal of neuroscience methods* 139, 121-143, 2004.
12. Han BS, Hong HS, Choi WS, Markelinos GJ, Oh YJ: Caspase-dependent and – independent cell death pathways in primary cultures of mesencephalic dopaminergic neurons after neurotoxin treatment. *J Neurosci* 23: 5068-5078, 2003.
13. Hattori N, Mizuno Y: Pathogenetic mechanisms of parkin in Parkinson's disease. *Lancet* 364, 722-724, 2004.
14. He Y, Appel S, Le W: Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain research* 909, 187-193, 2001.
15. Healy DG, Abou-Sleiman PM, Wood NW: PINK, PANK, or PARK? A clinicians' guide to familial parkinsonism. *Lancet neurology* 3, 652-662, 2004.
16. Hellmann MA, Panet H, Barhum Y, Melamed E, Offen D: Increased survival and migration of engrafted mesenchymal bone marrow stem cells in 6-hydroxydopamine-lesioned rodents. *Neuroscience letters* 395, 124-128, 2006.

17. Jin HK, Carter JE, Huntley GW, Schuchman EH: Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. *J Clin Invest* 109, 1183-1191, 2002.
18. Kirik D, Rosenbald C, Björklund 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.
19. Li Y, Chen J, Wang L, Zhang L, Lu M, Chopp M: Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neurosci Lett* 316, 67-70, 2001.
20. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H et al: Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 103, 697-705, 1999.
21. Mareschi K, Novara M, Rustichelli D, Ferrero I, Guido D, Carbone E, Medico E, Madon E, Vercelli A, Fagioli F: Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K_p channel types. *Experimental hematology* 34, 1563-1572, 2006.
22. Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, Olivieri C, Pastore I, Marasso R, Madon E: Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. *Amyotroph Lateral Scler Other Motor Neuron Disord* 4, 158-161, 2000.
23. McNaught KS, Perl DP, Brownell AL, Olanow CW: Systemic Exposure to Proteasome Inhibitors Causes a Progressive Model of Parkinson's Disease. *Annals of neurology* 56, 149-162, 2004.

24. Moore DJ, West AB, Dawson VL, Dawson TM: Molecular Pathophysiology of Parkinson's Disease. *Annual review of neuroscience* 28, 57-87, 2005.
25. Morris HR: Genetics of Parkinson's disease. *Annals of medicine* 37, 86-96, 2005.
26. Olanow CW, McNaught KS: Ubiquitin-Proteasome System and Parkinson's Disease. *Mov Disord* 21, 1806-1823, 2006.
27. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage Potential of Adult Human Mesenchymal stem cells. *Science* 284, 143-147, 1999.
28. Reaney SH, Johnston LC, Langston WJ, Di Monte DA: Comparison of the neurotoxic effects of proteasomal inhibitors in primary mesencephalic cultures. *Exp Neurol* 202, 434-440, 2006.
29. Sairam K, Saravanan KS, Banerjee R., Mohanakumar KP: Non-steroidal anti-inflammatory drug sodium salicylate, but not diclofenac or celecoxib, protects against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats. *Brain research* 966, 245-252, 2003.
30. Schrag A, Schott JM: Epidemiological, clinical, and genetic characteristics of early-onset parkinsonism. *Lancet neurology* 5, 355-363, 2006.
31. Tatton WG, Chalmers-Redman R, Brown D, Tatton N: Apoptosis in Parkinson's Disease: Signals for Neuronal Degradation. *Annals of neurology* 53 Suppl 3, S61-70; discussion S70-62, 2003.
32. Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S, Vila M, Jackson-Lewis V, Przedborski S: Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America* 100, 5473-5478, 2003.

33. West MJ, Slomianka L, Gundersen HJ: 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.
34. Woodbury D, Schwarz EJ, Prockop DJ, Black IB: Adult Rat and Human Bone Marrow Stromal Cells Differentiate Into Neurons. *J Neurosci Res* 61, 364-370, 2000.
35. Yang MS, Park EJ, Sohn S, Kwon HJ, Shin WH, Pyo HK et al: Interleukin-13 and -4 induce death of activated microglia. *Glia* 38: 273-280, 2002.
36. Ye M, Wang XJ, Zhang YH, Lu GQ, Liang L, Xu JY, Sheng-Di C: Therapeutic effects of differentiated bone marrow stromal cell transplantation on rat models of Parkinson's disease. *Parkinsonism Relat Disord*, 2006.

SD rat 의 PD model 에서 Human Mesenchymal Stem Cell 의 효과

박 현 정

아주대학교 대학원

의학과

(지도교수 : 이 필 휴)

파킨슨 씨 병은 흑색질 안에 도파민 세포의 감소가 원인이 되는 진행성 신경 퇴화병이다. 현재 우리의 실험에선, 단백질 분해 억제제인 MG 132 를 이용하여 파킨슨 씨 병의 진행성 신경퇴화 랫트 동물 모델과 세포 모델을 만든 후 human mesenchymal stem cells (hMSCs) 치료제의 효과를 확인하였다. 중뇌 신경 세포의 면역화학염색법을 이용하여 MG-132 만을 첨가한 그룹과 hMSCs 를 함께 첨가한 그룹을 비교하였을 때 hMSCs 를 첨가한 그룹의 세포 사멸이 감소한 것을 확인하였다. 또한 MG-132 만을 첨가한 그룹에서는 caspase-3 의 활성이 증가하였는데 hMSCs 를 첨가한 그룹에서는 caspase-3 의 활성이 감소하였다.

MG-132 만를 투여한 동물 모델들의 신경 세포는 8 주 때 $23\% \pm 7.2\%$, 10 주 때 70 ± 9.5 , 13 주 때 92 ± 7.2 로 진행성 있게 감소하였다. hMSCs 를 반복적으로 정맥에 투여 후에 흑색질의 도파민 세포의 감소는 MG 132 만 투여한 그룹에 비해 50% 정도 줄어들었고 운동성은 증가하였다. hMSCs 를 투여한 그룹에서 염증반응의 활성화 감소를 확인하기 위해 OX-42 항체를 이용한 면역화학염색결과 피질의 마이크로글리아의 활성화가 매우 감소되는 것을 관찰할 수 있었다. 또한 면역염색법을 통해 흑색질 안에 hMSCs 마커인 NuMA positive 세포가 hMSCs 총량의 1.7%가 확인됨으로써 hMSCs 의 존재를 확인하였고, 도파민 세포 마커인 tyrosine hydroxylase 와 NuMA 의 merge 되는 세포가 NuMA-positive 세포 총량의 35.7%가 확인됨으로써 hMSCs 는 흑색질에서 도파민 세포로 분화 된다는 것을 보여주었다. 추가로 이 세포들의 기능적인 면도 TH, NuMA, synaptophysin 의 merge 를 보여주는 면역염색실험과 행동분석실험을 통해 확인하였다.

결론적으로, hMSCs 의 trophic effect 와 기능성이 있는 도파민 세포로 분화되는 것은 MG-132 로 인한 도파민 세포의 사멸을 보호하는 역할일 것이고, 추가로, 우리가 밝힌 hMSCs 의 이 세포 보호 작용은 임상 적용 치료제로 가능할 것을 시사한다.

핵심어 : 파킨슨 씨 병, MG-132, 단백질 분해 억제제, human mesenchymal stem cell