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# Therapeutic potentials of human adipose-derived stem cells on the mouse model of Parkinson's disease

Hee Soon Choi [a,1], Hee Jin Kim [b,1], Jin-Hwan Oh [c], Hyeong-Geun Park [d], Jeong Chan Ra [d], Keun-A. Chang [c,e,**], Yoo-Hun Suh [e,f,g,*]

[a] Research Division, Department of Structure and Function of Neural Network, Korea Brain Research Institute, Daegu, Korea
[b] Research Division, Department of Neural Development and Disease, Korea Brain Research Institute, Daegu, Korea
[c] Neuroscience Research Institute, Gachon University, Incheon, Korea
[d] K-STEM CELL Institute, Seoul, Korea
[e] Department of Pharmacology, Gachon University, Incheon, Korea
[f] Korea Brain Research Institute, Daegu, Korea
[g] Department of Pharmacology, Seoul National University Medical College, Seoul, Korea

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ABSTRACT

The treatment of Parkinson's disease (PD) using stem cells has long been the focus of many researchers, but the ideal therapeutic strategy has not yet been developed. The consistency and high reliability of the experimental results confirmed by animal models are considered to be a critical factor in the stability of stem cell transplantation for PD. Therefore, the aim of this study was to investigate the preventive and therapeutic potential of human adipose-derived stem cells (hASC) for PD and was to identify the related factors to this therapeutic effect. The hASC were intravenously injected into the tail vein of a PD mouse model induced by 6-hydroxydopamine. Consequently, the behavioral performances were significantly improved at 3 weeks after the injection of hASC. Additionally, dopaminergic neurons were rescued, the number of structure-modified mitochondria was decreased, and mitochondrial complex I activity was restored in the brains of the hASC-injected PD mouse model. Overall, this study underscores that intravenously transplanted hASC may have therapeutic potential for PD by recovering mitochondrial functions.



## 1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease. The onset rate is estimated to be about 1%–2% of individuals aged >60 years and >3% of the number aged >75 years (Gasser, 2009; Pankratz and Foroud, 2007). Aging, genetic and environmental factors are jointly involved in the development of PD, though the exact mechanism was poorly understood. Especially, many epidemiological studies have reported that aging is the greatest risk factor for PD (Bennett et al., 1996; Morens et al., 1996; Tanner and Goldman, 1996). The signs and symptoms of PD are slow movement, rigidity, and tremor because of the loss of dopaminergic neurons in the substantia nigra (SN) that project to the striatum (Murrell et al., 2008). The progressive degeneration and loss of dopaminergic neurons in the SN are accompanied by the formation of intraneuronal inclusions, called "Lewy body," that are primarily composed of α-synuclein (Spillantini et al., 1997). However, no treatment has yet been developed because their interplay is still unclear. Lately, PD treatments have tried to compensate for the loss of striatal dopamine by administering its precursor L-DOPA and/or dopamine D2 receptor agonists (Collier et al., 2011; Dragicevic et al., 2014; Gazewood et al., 2013; Olanow and Schapira, 2013).

Recently, stem cell therapy for PD has been in the spotlight. The ideal transplantable cell should be easily accessible, have a high proliferation capacity in vitro, and have the ability to undergo differentiation into multiple cell lineages such as astrocytes, oligodendrocytes, and neuronal cells. In recent studies, human adipose-derived stem cell (hASC), a type of mesenchymal stem cell (MSC) isolated from adipose tissue, are well known for their pluripotent ability to differentiate into neuron-like cells

* Corresponding author at: Korea Brain Research Institute (KBRI), Daegu, 701-300, South Korea, Tel.: +82-53-980-8101; fax: +82-53-980-8239.
** Corresponding author at: Department of Pharmacology, Gachon University, Incheon, 406-799, South Korea, Tel.: +82-32-820-4332, fax: +82-32-820-4744
*E-mail addresses:* keuna705@gachon.ac.kr (K.-A. Chang), yhsuh@snu.ac.kr (Y.-H. Suh).
[1] Hee Soon Choi and Hee Jin Kim equally contributed to this work.


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(Chang et al., 2014; Kim et al., 2012). Autologous hASC has significant advantages, such as the lack of immune rejection responses, tumorigenesis, and ethical problems (Tomita et al., 2013; Zuk et al., 2002). Moreover, ASCs have already been used for some clinical applications (Traktuev et al., 2008). Unlike stem cells derived from other sources and induced pluripotent stem cells (Amariglio et al., 2009; Bjorklund et al., 2002; Brederlau et al., 2006; Duinsbergen et al., 2009), MSCs show a low probability of being tumorigenic, and a recent phase I study demonstrated the safety of MSC transplantation into PD patients (Glavaski-Joksimovic and Bohn, 2013; Venkataramana et al., 2010). Additionally, in our previous study, we found using an in vivo imaging technique that intravenously transplanted hASC could pass through the blood-brain barrier and migrate into the injuries of the brain (Chang et al., 2014; Kim et al., 2012). For these reasons, hASC may be the optimal stem cells for clinical therapies.

In many studies, it has been previously described that the structural and functional alterations of mitochondria were associated with neurodegenerative diseases including PD. The genes related to PD, such as α-synuclein, parkin, DJ-1, and PINK1, are directly or indirectly involved with mitochondrial functions (Shim et al., 2011). Mitochondria supply adenosine triphosphate to the cell through oxidative phosphorylation, synthesize key molecules, and buffer calcium gradients (Frazier et al., 2006). Therefore, mitochondrial health is closely associated with the metabolic component. Aging and energy-dependent disturbances involve mitochondrial defects (Wang et al., 2013b). In particular, impaired function of mitochondria leads to increased oxidative stress or reactive oxygen species, and both have a significant pathogenic role in the selective loss of dopaminergic neurons in human patients and in PD mouse models (Dexter et al., 1989; Moon et al., 2013; Sriram et al., 1997).

Traditionally, the experimental models for PD have been created in rodents and primates via the delivery of neurotoxins, such as 6-hydroxydopamine (6-OHDA) (Stott and Barker, 2014). It has been reported that 6-OHDA is a dopamine analog that specifically induces dopaminergic neuronal cell death via either uncoupling mitochondrial oxidative phosphorylation, resulting in energy deprivation or alternatively through its ability to produce hydrogen peroxide, hydroxyl, and superoxide radicals (Soto-Otero et al., 2008; Wang et al., 2013b). 6-OHDA is also known to inhibit complexes I and IV of the mitochondrial respiratory chain in the SN (Ben-Shachar et al., 1995; Glinka and Youdim, 1995; Glinka et al., 1996; Mizuno et al., 1989).

In the present study, we focused on the applicability of hASC by a convenient and safe intravenous injection and the relevance of hASC to mitochondrial functions in a PD mouse model induced by 6-OHDA for the first time. We investigated whether hASC injection could rescue the behavioral deficits in the PD mouse model using the apomorphine-induced rotation test and the rotarod performance test. To confirm a neuropathological difference, we evaluated the change in the numbers of tyrosine hydroxylase (TH)-positive neurons in hASC-injected PD mice compared with sham control PD mice. To assess whether injected hASC rescued alterations in the dopamine level in the striatum, we performed positron emission tomography (PET) imaging analysis with [$^{11}$C]raclopride, an antagonist of DA-D2R, in the PD mouse model injected with hASC or saline. Additionally, we focused on changes in the mitochondrial structure and complex I activity in the experimental PD model.

## 2. Experimental procedures

### 2.1. Materials

hASCs (RNL-Bio_SM081201 P3), male C57BL6 mice, desipramine, apomorphine, 6-OHDA (Sigma Aldrich Korea, Kyounggi-do, Korea), 25% glutaraldehyde (Electron Microscopy Sciences, PA, USA), Dako EnVision+ system–horseradish peroxidase–labeled polymer (Dako Korea, Seoul, Korea), DAB+ substrate chromogen (Dako Korea), anti-TH antibody (Santa Cruz Biotechnology, CA, USA), and the complex I enzyme activity microplate assay kit (Abcam, Cambridge, UK) were used in the experiments.

### 2.2. Method

#### 2.2.1. PD mouse model preparation and behavioral testing

Seven-week-old male C57BL/6N mice weighing 20–25 g were housed in a specific pathogen-free room, automatically maintained on a 12-hour light-dark cycle at 25 °C and proper humidity, and were given food and water ad libitum. Desipramine (25 mg/kg, Sigma, St. Louis, MO, USA) was injected intraperitoneally (ip) to block norepinephrine reuptake 1 hour before 6-OHDA injection. The animals were anesthetized with Rompun and Zoletil (1 μg/g, ip) and injected with 10 μg of 6-OHDA (4 μg/μL containing 0.2 mg/mL L-ascorbic acid) or the same volume of saline for the PD model or control, respectively. 6-OHDA or saline was injected unilaterally into the SN (A/P = −3.2, M/L = −1.5, D/V = −4.6) with a flat skull position (coordinates in mm, with anterior-posterior and lateral coordinates measured from bregma, and ventral from dura) using a Kopf stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The injections were made at a rate of 0.5 μL/min using a 25-gauge Hamilton syringe (Hamilton Company, NV, USA). The in vivo experimental protocol that followed is shown in Fig. 1A. After the surgery, each mouse was kept in an individual cage.

Two weeks after the 6-OHDA injection, motor coordination, and fatigue resistance were tested using the accelerating rotarod and apomorphine-induced rotation tests for the measurement of baseline behavior (n = 10 per group). The control mouse and PD mouse induced by saline or 6-OHDA, respectively, was selected by the baseline behavior testing and distributed to each group. The injection of hASC ($1 \times 10^6$ cells) was conducted twice at an interval of 2 weeks, and the behavioral performances were measured at 3 weeks after the final injection of hASC via the rotation and accelerating rotarod tests. For the rotation test, mice were habituated in the basket for 10 minutes. After subcutaneous injection with apomorphine (0.05 mg/kg), the behavior of the mouse was tracked and the number of right and left rotations and the net number of rotations for 30 minutes were analyzed by an EthoVision video tracking system. For the rotarod test, mice were conditioned at a speed of 8 rpm for 5 minutes and at a speed of 12 rpm for 5 minutes at an interval of 1 hour. One day after the training, the motor performance was tested at an accelerated speed from 2 rpm to 20 rpm for 10 minutes. The time schedule of the experimental procedure is shown in Fig. 1.

#### 2.2.2. Micro-positron emission tomography

The PET study with [$^{11}$C]raclopride was performed 4–5 weeks after the 2nd adipose-derived stem cells treatment (n = 4 per group). Anesthesia was induced and maintained with passive 1.5% isoflurane at an oxygen level of 1.5 L/min with vacuum. Each mouse was positioned on a bed with its brain centered in the gantry. The PET scan was performed using a Focus 120 Micro PET system (Concorde Microsystems, Knoxville, USA). Dynamic scans were performed for 90 minutes immediately after a [$^{11}$C]raclopride injection (18.50–24.64 MBq, 200 μL) via the tail vein. The acquisition of data was reconstructed with a 2D-filtered back-projection algorithm (microPET Manager, Siemens Medical Solutions, Knoxville, USA). The dynamic frame was composed of 32 frames: 1 × 60 seconds, 6 × 20 seconds, 2 × 30 seconds, 5 ×