Effect of intranasal stem cell administration on the nigrostriatal system in a mouse model of Parkinson's disease

MOHAMED SALAMA1,2, MAHMOUD SOBH1, MAHMOUD EMAM1, AHMED ABDALLA1, DINA SABRY1, MOHAMED EL-GAMAL1, AHMED LOTFY1, MAHMOUD EL-HUSSEINY3, MOHAMED SOBH1,3, ALI SHALASH4 and WAEL MY MOHAMED5,6

1Medical Experimental Research Center; 2Toxicology Department, Faculty of Medicine; 3Urology Nephrology Center, Mansoura University, Mansoura 35516; 4Neurology Department, Ain Shams Medical School, Ain Shams University, Cairo 11566; 5Department of Clinical Pharmacology, Menoufia Medical School, Menoufia University, Menoufia 32811, Egypt; 6Department of Basic Medical Science, Kulliyyah of Medicine, International Islamic University Malaysia, Kuantan, Pahang 53100, Malaysia

Received November 30, 2015; Accepted February 9, 2016

DOI: 10.3892/etm.2017.4073

Abstract. Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. It affects the locomotor system, leading to a final severe disability through degeneration of dopaminergic neurons. Despite several therapeutic approaches used, no treatment has been proven to be effective; however, cell therapy may be a promising therapeutic method. In addition, the use of the intranasal (IN) route has been advocated for delivering various therapies to the brain. In the present study, the IN route was used for administration of mesenchymal stem cells (MSCs) in a mouse model of PD, with the aim to evaluate IN delivery as an alternative route for cell-based therapy administration in PD. The PD model was developed in C57BL/6 mice using intraperitoneal rotenone administration for 60 consecutive days. MSCs were isolated from the mononuclear cell fraction of pooled bone marrow from C57BL/6 mice and incubated with micrometer-sized iron oxide (MPIO) particles. For IN administration, we used a 20 µl of 5x10^7 cell suspension. Neurobehavioral evaluation of the mice was performed, and after sacrifice, brain sections were stained with Prussian blue to detect the MPIO-labeled MSCs. In addition, immunohistochemical evaluation was conducted to detect tyrosine hydroxylase (TH) antibodies in the corpus striatum and dopaminergic neurons in the substantia nigra pars compacta (SNpc). The neurobehavioral assessment revealed progressive deterioration in the locomotor functions of the rotenone+MSC group, which was improved following MSC administration. Histopathological evaluation of brain sections in the rotenone+MSC group revealed successful delivery of MSCs, evidenced by positive Prussian blue staining. Furthermore, rotenone treatment led to significant decrease in dopaminergic neuron number in SNpc, as well as similar decrease in the corpus striatum fiber density. By contrast, in animals receiving IN administration of MSCs, the degeneration caused by rotenone treatment was significantly counteracted. In conclusion, the present study validated that IN delivery of MSCs may be a potential safe, easy and cheap alternative route for stem cell treatment in neurodegenerative disorders.

Introduction

Parkinson's disease (PD) is a degenerative disorder affecting the central nervous system (CNS), which results from the death of dopamine-generating cells in the substantia nigra of the midbrain. Thus, PD is characterized by depletion of dopaminergic cell bodies in substantia nigra that are subsequently lost in the nigrostriatal system. The nigrostriatal system is composed of the Substantia Nigra and Corpus striatum and is the affected dopaminergic system in cases of PD. The reported incidence rates of PD vary largely. The lowest PD incidence was reported to be 4.5/1,000,000 in Libya, while the highest incidence was reported in the USA at 20/1,000,000 (1). The early symptoms of PD include slowness of movement, rigidity, shaking and walking difficulties. Symptoms presented at later stages of the disease may include thinking and behavioral problems, with dementia and depression arising in the advanced stages (1). Other symptoms, including sensory, sleep and emotional problems, may also occur. The majority of PD cases occur after the age of 50 years, and the disorder is more prevalent among men rather than women. The pathological hallmark of the disease is accumulation of the protein α-synuclein into inclusions known as Lewy bodies in neurons (2). The intensity of the Lewy bodies is directly associated with the clinical symptoms of each individual. Mechanisms underlying PD may include mitochondrial dysfunction, oxidative stress, inflammation and defective...
protein handling. PD is the second most common neurodegenerative disorder after Alzheimer's disease (3). Upon clinical diagnosis of PD, the loss in dopaminergic neurons has already reached 80%, and thus neuroprotective therapies for PD are of little significance as the majority of dopaminergic neurons are lost. By contrast, the use of regenerative agents in PD patients appears to be more promising (4).

Cell-based therapy has been advocated for PD due to the high failure rate of other therapeutic strategies (5). Despite the improvement imparted by developing novel dopamine receptors agonists, these agents are considered weak in comparison with L-DOPA, thus resulting in their use as a complementary treatment rather than a substitute to the classical L-DOPA therapy (6). Cellular therapy, which was initially investigated using fetal tissues with limited long-term success, is currently the central component of regenerative treatment for PD. However, the identification of stem cells increased the possibility of more successful cellular therapy for neuroregeneration (7). However, to date, advances in stem cell research have failed to offer a successful regenerative therapy for PD patients due to various limiting factors. The most important problem for stem cell therapy in neurodegenerative diseases such as PD is the method of administration. The first mode of stem cell transplantation is through their direct introduction into the corpus striatum using stereotaxis, with the corpus being the preferred site for transplantation over the substantia nigra and subthalamic nucleus (8). However, this route is considered inapplicable in humans due to inconvenience, risk of several possible complications and high costs (9). By contrast, systemic administration of stem cells has not demonstrated sufficient encouraging results, which may be due to the difficulty of cells crossing the blood-brain barrier (BBB) (10).

The search for efficient delivery route for neurological diseases appears to be crucial. The majority of candidate drugs for CNS diseases that showed promising results on in vitro and in vivo studies failed to show similar efficacy in humans, leading to high attrition rates of novel CNS active drugs in clinical trials (11). The main reason for such failure is the presence of the BBB, which prevents the passage of the right concentration of the drug to the target tissue (12).

One approach for resolving this issue is targeted intranasal (IN) delivery, which is an applicable method used to circumvent BBB rather than attempting to cross it (13). The nasal passage is the only direct connection between the brain and the external environment. This connection occurs through the extension of axons from the olfactory bulb to the nose, allowing direct contact with the external environment. Another potential route is the nose to brain pathway, which is a controversial pathway suggesting the passage of medication through the deep structures in the nose that are innervated by cranial nerves (14). Based on this pathway, the IN route has been used for the delivery of a variety of agents for the treatment of different CNS conditions. For instance, drugs delivered using IN delivery system include growth factors, neuropeptides, genes and small molecules (9). Notably, previous animal studies showed the successful IN delivery of mesenchymal stem cells (MSCs) to the brain. In addition, animal models of Parkinson's disease have been successfully treated through IN administration of L-DOPA (15).

Based on these previous findings, the present study aimed to investigate the use of the IN route for administration of MSCs in a mouse model of PD.

Materials and methods

Rotenone mouse model and stem cells administration. A total of 30 B57BL/6 mice (age, 8 weeks; weight, 16-20 g) were provided by the Medical Experimental Research Center of Mansoura University (Mansoura, Egypt) and maintained in conditions of 21-23°C, with a humidity of 40-55% and a 12 h dark/light cycle. All animal experiments were performed according to the Guidelines for the Care and Use of Mammals in Neuroscience (2003), and were approved by the Ethical Committee for research at Mansoura University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

A PD model was developed in mice through the intraperitoneal administration of 3 mg/kg/day body weight rotenone (Sigma-Aldrich, St. Louis, MO, USA), for 60 consecutive days. The mice were divided into three groups (10 mice in each) as follows: Control group, which received daily intraperitoneal injection of 0.5% carboxymethyl cellulose (El-Nasr Chemicals Co., Cairo, Egypt); PD model group (rotenone group), receiving rotenone (3 mg/kg body weight) dissolved in 0.5% carboxymethyl cellulose intraperitoneally; and rotenone+MSC group, which received rotenone administration similarly to the PD model group, followed by IN bone marrow MSCs derived on day 60. All animals were sacrificed by perfusion through the aorta with 50 ml of 10 mM phosphate-buffered saline (PBS), followed by 150 ml of a cold fixative consisting of 4% paraformaldehyde, 0.35% glutaraldehyde and 0.2% picric acid in 100 mM phosphate buffer, under deep anesthesia with pentobarbital (100 mg/kg, intraperitoneally). Animals were sacrificed on day 70, thus after 10 days of MSCs treatment for the rotenone+MSC group.

Stem cells isolation. MSCs were isolated from the mononuclear cell fraction of pooled bone marrow from healthy C57BL/6 mice. Mice were sacrificed by cervical dislocation and their femurs and tibiae were carefully cleaned from the skin and cut at the ankle bone. The muscle and connective tissue were scraped, and the bones were placed in 10% ethyl alcohol for sterilization and left for a few seconds. Next, the ends of the tibia and femur were cut by sharp scissors and a 27-gauge needle was inserted, after which the sample was flushed with Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and collected in a 15-ml tube. The cell suspension was then filtered through a 70-µm filter mesh. Bone marrow cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution (Thermo Fisher Scientific, Inc.) in a 25-cm² tissue culture flask and incubated at 37°C and 5% CO₂. Subsequent to adhesion to the plastic wall of the dish and culture in DMEM, the stem cells were tested for plasticity through in vitro differentiation, including investigation of adipogenesis, chondrogenesis and osteogenesis (16). Isolated MSCs were then incubated overnight with micrometer-sized iron oxide (MPIO) particles (Bangs Laboratories, Inc., Fishers, IN, USA). For IN application, a 20 µl cell suspension...
Mice were adapted to the open field rotenone+MSC. For assessment of dopaminergic stem cells tracking and anti-TH antibody (dilution, 1:1,000; the slides were incubated with primary mouse monoclonal serum) for 10 min. Subsequently, aldehyde was blocked using 30% hydrogen peroxide in methanol for specimens then cut by a microtome at 4‑5 micron on glass slides. Next, the brain specimens were processed into paraffin blocks, and specimens in 100 mM PBS containing 0.1% sodium azide at 4˚C. Following several washes with PBS, the samples were incubated with the required biotinylated secondary antibody (1:10,000; cat no. A4416; Sigma-Aldrich) overnight at 4˚C. After perfusion, the brain was subjected to image analysis. In order to bilaterally evaluate the TH-positive fiber innervation in the striatum, mean optical density measurements were performed using the Image J software (http://imagej.nih.gov/ij/) as previously described (19); and i) Striatal TH-fiber density measurement was performed using Image J software (version 1.33-1.34; National Institutes of Health, Bethesda, MD, USA). In addition, images of coronal sections were captured at seven rostral-caudal levels, in order to cover the entire striatal complex. The striatum was included from the lateral ventricle to the external capsule and a horizontal line connecting the ventral end of the ventricle via the anterior commissure to the external capsule. The data are expressed as percentage of the controls and represent the average of the seven levels (20). Furthermore, assessment of dopaminergic neurons in the substantia nigra pars compacta (SNpc) was determined by counting the number of TH-positive cells in the SNpc of both hemispheres, in every fourth section throughout the entire nucleus. The anatomical levels considered in the anteroposterior (AP) extension were between -5.20 and -5.80 mm with respect to bregma. Results are expressed as the percentage of TH-positive cells in the lesioned SNpc with respect to the control (21).

Statistical analysis. All data are presented as the mean ± standard deviation. Two groups of data were analyzed by Student's t-test. Three groups of data were analyzed by analysis of variance with a Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Behavioral evaluation. Behavioral assessment revealed progressive deterioration in the locomotor functions of the rotenone group (PD model group) compared with the control group. However, IN administration of MSCs (rotenone+MSC group) was found to improve the locomotor performance of the animals when compared with the rotenone-only treated animals. In general, increased salivation and hyperventilation were observed following injections, but with no accompanied convulsions or mortality in any group.
The ease and efficacy of IN administration, besides the rapid delivery of therapeutic agents, resulted in several trials investigating the use of this route in various CNS disorders, particularly in neurodegenerative diseases. The...
IN administration of therapeutic agents was attempted with numerous vehicles and tracking methodologies (29,30). In the majority of studies, IN administration (alone or assisted with drug delivery agents) successfully reached the intracranial region and led to significant improvement in the disease (31). In addition, investigation of the role of IN stem cell delivery in PD has been attempted in several animal models, with promising results. Although long-term follow-up of transplanted stem cells revealed disappearance of cells after a certain period of time, this is predicted in neurodegenerative diseases, which mandates repetitive doses, thus further supporting the use of the easy IN route.

In spite of the positive results obtained from previous studies addressing the use of IN stem cells in PD, translation into clinical practice has yet to be achieved. This is due to the lack of an ideal animal model of PD that can recapitulate the pathology that occurs in human cases (32). Therefore, it is important to study new therapeutic agents for PD on different animal models. Previously, the IN route was assessed in 6-OHDA rat model (21) and in transgenic mice (22). The main issue with animal models of PD is the absence of an ideal model that can recapitulate all PD pathological findings (23). Therefore, it appears that investigating new therapeutic approaches on different animal models may be more effective (24).

Although the 6-OHDA and transgenic models are important tools to study PD in animals, they present major limitations that may reduce the credibility of their use for therapeutic agent testing (33). The 6-OHDA model involves the local injection of the agent into the nigrostriatal system, leading to immediate and severe degeneration in this area. With the exception of damage caused in the dopaminergic system, this model is not associated with other characteristics of PD. It lacks the cascade of events or pathogenic pathways that lead to PD in human cases, including lack of the neuro-inflammatory nature of PD which is important when studying cell transplantation. Furthermore, it does not represent the progressive nature of the disease, and since PD is a unilateral disease, the model does not accurately represent the locomotor disturbances occurring in PD. By contrast, transgenic models of PD can serve as successful models for the rare familial type of the disease; however, they lack the sequence
of events that lead to the development of typical PD. In idio-
pathic PD, the role of environmental exposure is major, and
thus this is a limiting point of transgenic models. An alter-
native model that carries numerous of the PD characteristics
is the rotenone-induced model. Rotenone can induce a PD
model in animals that has a chronic and progressive nature.
The disease process is accompanied by various features of
PD pathogenesis, particularly the neuroinflammatory effects
and BBB influence, which are critical points in evaluating cell
therapy.

Based on the aforementioned observations and limita-
tions, the present study used the IN route for delivering MSCs
in a rotenone-induced PD model in mice. To evaluate the
therapeutic efficiency of this route, animals were evaluated
behaviorally using a variety of neurobehavioral tests (such as
the open field and parallel rod tests) and histopathological eva-
uation of brain sections through immunostaining against TH,
which is the main marker of dopaminergic cells. Behavioral
assessment assists in the study of the symptom-relieving
effects of therapy, as behavioral tests can be translated into
clinical performance in human cases. In addition, histopatho-
logical evaluation helps to study the improvement of disease
pathology following treatment. In the present study, the trans-
planted stem cells were tracked to ensure their successful
delivery intracranially and that they reached the site of the
lesion. This step is of paramount importance to verify that
any therapeutic effects of MSCs are caused by their direct
regenerative effects and not due to a systemic body reaction.

In the current study, IN delivery of MSCs administered to
a rotenone animal model were found to result in improvements
in all affected neurobehavioral tests, which indicates the
efficient therapeutic effect of this treatment. The successful
passage of IN stem cells, as observed by stem cell tracking
in the mouse brain tissue, shows that the therapeutic effects
observed on the behavioral level can be attributed to the
physical presence of MSCs inside the target brain tissues. The
therapeutic efficiency of IN delivery of MSCs was then veri-
fied by immunostaining with TH antibodies, showing reduced
degenerative effects compared with the rotenone-only treated
type.

The results reported in the present study complement
previous research findings that denote the success of IN
delivery of stem cells in animal PD models. The use of a
rotenone PD model appears to be of great importance, as this
model carries certain important features of PD, such as envi-
ronmental contribution and the chronic progressive pattern of
the disease (25).

In conclusion, the present study identified the positive
effects of IN delivery of MSCs in a progressive mouse model
of PD. Thus, this treatment may have a possible similar effect
in clinical practice, suggesting potential application in human
cases of PD. According to the present results along with those
of previous studies, IN delivery of stem cells appears to be a
potential safe, easy and cheap route for stem cell treatment in
neurodegenerative disorders. Although this study offered
proof of the potential therapeutic benefit of IN route for
delivering MSCs as a treatment for PD, further investigation
is required prior to clinical application, such as comparison
between different drug delivery vehicles, evaluation of
nanosubstance addition, and investigation of the ideal type of
stem cells and timing of transplantation.

Acknowledgements

The present study was supported by the Medical Experimental
Research Center of Mansoura University (grant no. 2014-01).

References

1. Chen RC, Chang SF, Su CL, Chen TH, Yen MF, Wu HM, Chen ZY and Liuo HH: Prevalence, incidence, and mortality of
PD: A door-to-door survey in Ilan county, Taiwan. Neurology 57:

2. Aarsland D, Londos E and Ballard C: Parkinson’s disease
dementia and dementia with Lewy bodies: Different aspects of


4. Sengupta U, Guerrero-Munoz MJ, Castillo-Carranza DL,
Lagnana-Reeves CA, Gerson JE, Paulucci-Holthauzen AA, Krishnamurthy S, Farhed M, Jackson GR and Kayed R:
Pathological interface between oligomeric alpha-synuclein and

5. Shalman JM, De Jager PL and Feany MB: Parkinson’s disease:

6. Toulouse A and Sullivan AM: Progress in Parkinson’s

7. Street V and Stacy M: Dopamine agonists. In: Handbook of

8. Laguna Goya R, Tyers P and Barker RA: The search for a
curative cell therapy in Parkinson’s disease. J Neurol Sci 265:

9. Inden M, Kim D, Qi M, Kitamura Y, Yanagisawa D, Nishimura K,
Tsuchiya D, Takata K, Hayashi K, Taniguchi T, et al: Transplantation of mouse embryonic stem cell-derived neurons into the striatum, subthalamic nucleus and substantia nigra, and
behavioral recovery in hemiparkinsonian rats. Neurosci Lett 387:
151-156, 2005.

10. Hanson LR and Frey WH II: Intranasal delivery bypasses
the blood-brain barrier to target therapeutic agents to the central

11. Chao YY, He BP and Tay SS: Mesenchymal stem cell
transplantation attenuates blood brain barrier damage and neuro-
inflammation and protects dopaminergic neurons against MPTP
toxicity in the substantia nigra in a model of Parkinson’s disease.

12. Ruijgrok MJ and de Lange EC: Emerging insights for transla-
tional pharmacokinetic and pharmacokinetic pharmacodynamic
studies: Towards prediction of nose-to-brain transport in humans.

13. de Lange EC: The mastermind approach to CNS drug therapy:
Translational prediction of human brain distribution, target site
kinetics, and therapeutic effects. Fluids Barriers CNS 10: 12,
2013.

approach to delivering treatment for brain diseases: An anatomic,
physiologic, and delivery technology Overview. Ther Deliv 5:
709-733, 2014.

15. Lochhead JJ and Thorne RG: Intranasal delivery of biologics to
the central nervous system. Adv Drug Deliv Rev 64: 614-628,
2012.

16. Gambaryan PY, Kondrashova IG, Severin ES, Guseva AA and
Kamensky AA: Increasing the efficiency of Parkinson’s disease
treatment using a poly (lactic-co-glycolic acid) (PLGA) based

17. Ha EF, He BP, Dheen ST and Tay SS: Interactions of chemokines
and chemokine receptors mediate the migration of mesenchymal
stem cells to the impaired site in the brain after hypoglossal

18. Sarkar, S, Thomas B, Muralikrishnan D and Mohanakumar KP:
Effects of serotoninergic drugs on tremor induced by physyo-