World Journal of Stem Cells

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4252/wjsc.v8.i3.106

World J Stem Cells 2016 March 26; 8(3): 106-117
ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Updates in the pathophysiological mechanisms of Parkinson's disease: Emerging role of bone marrow mesenchymal stem cells

Hanaa H Ahmed, Ahmed M Salem, Hazem M Atta, Emad F Eskandar, Abdel Razik H Farrag, Mohamed A Ghazy, Neveen A Salem, Hadeer A Aglan

Hanaa H Ahmed, Emad F Eskandar, Hadeer A Aglan, Hormones Department, Medical Research Division, National Research Centre, Giza 12622, Egypt

Ahmed M Salem, Mohamed A Ghazy, Biochemistry Department, Faculty of Science, Ain Shams University, Cairo 1156, Egypt

Hazem M Atta, Clinical Biochemistry Department, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Hazem M Atta, Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Kasralainy, Cairo 11562, Egypt

Abdel Razik H Farrag, Pathology Department, Medical Research Division, National Research Centre, Giza 12622, Egypt

Neveen A Salem, Narcotics, Ergogenic aids and Poisons Department, Medical Research Division, National Research Centre, Giza 12622, Egypt

Author contributions: Ahmed HH designed and coordinated the research as well as wrote the paper; Salem AM analyzed the data; Atta HM performed the isolation and preparation steps of bone marrow mesenchymal stem cells from rats; Eskandar EF participated in the designation of the research; Farrag AH performed the immunohistochemical examination and histopathological investigations; Ghazy MA performed the molecular investigations; Salem NA and Aglan HA participated in the induction of Parkinson's disease in rats and treatment as well as performed the biochemical measurements.

Institutional review board statement: The study was reviewed and approved by the National Research Centre Institutional Review Board.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the National Research Centre, protocol number: (09-200).

Conflict-of-interest statement: The authors declared no conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at hanaaomr@yahoo.com.

Correspondence to: Hanaa H Ahmed, PhD, Professor of Biochemistry, Hormones Department, Medical Research Division, National Research Centre, 33 El-Bohouth Street, Dokki, Giza 12622, Egypt. hanaaomr@yahoo.com
Telephone: +20-2-33335966
Fax: +20-2-33370931

Received: September 12, 2015
Peer-review started: September 16, 2015
First decision: November 7, 2015
Revised: February 1, 2016
Accepted: February 23, 2016
Article in press: February 24, 2016
Published online: March 26, 2016

Abstract

AIM: To explore the approaches exerted by mesenchymal stem cells (MSCs) to improve Parkinson's disease (PD) pathophysiology.

METHODS: MSCs were harvested from bone marrow

of femoral bones of male rats, grown and propagated in culture. Twenty four ovariectomized animals were classified into 3 groups: Group (1) was control, Groups (2) and (3) were subcutaneously administered with rotenone for 14 d after one month of ovariectomy for induction of PD. Then, Group (2) was left untreated, while Group (3) was treated with single intravenous dose of bone marrow derived MSCs (BM-MSCs). *SRY* gene was assessed by PCR in brain tissue of the female rats. Serum transforming growth factor beta-1 (TGF-β1), monocyte chemoattractant protein-1 (MCP-1) and brain derived neurotrophic factor (BDNF) levels were assayed by ELISA. Brain dopamine DA level was assayed fluorometrically, while brain tyrosine hydroxylase (TH) and nestin gene expression were detected by semi-quantitative real time PCR. Brain survivin expression was determined by immunohistochemical procedure. Histopathological investigation of brain tissues was also done.

RESULTS: BM-MSCs were able to home at the injured brains and elicited significant decrease in serum TGF-β1 (489.7 ± 13.0 *vs* 691.2 ± 8.0, $P < 0.05$) and MCP-1 (89.6 ± 2.0 *vs* 112.1 ± 1.9, $P < 0.05$) levels associated with significant increase in serum BDNF (3663 ± 17.8 *vs* 2905 ± 72.9, $P < 0.05$) and brain DA (874 ± 15.0 *vs* 599 ± 9.8, $P < 0.05$) levels as well as brain TH (1.18 ± 0.004 *vs* 0.54 ± 0.009, $P < 0.05$) and nestin (1.29 ± 0.005 *vs* 0.67 ± 0.006, $P < 0.05$) genes expression levels. In addition to, producing insignificant increase in the number of positive cells for survivin (293.2 ± 15.9 *vs* 271.5 ± 15.9, $P > 0.05$) expression. Finally, the brain sections showed intact histological structure of the striatum as a result of treatment with BM-MSCs.

CONCLUSION: The current study sheds light on the therapeutic potential of BM-MSCs against PD pathophysiology *via* multi-mechanistic actions.

Key words: Parkinson's disease; Pathophysiology; Bone marrow derived mesenchymal stem cells; Rotenone; Anti-inflammatory action; Ovariectomy; Anti-apoptotic effect; Neurogenic potential

Core tip: The current study was planned to clarify the mode of action of mesenchymal stem cells (MSCs) in targeting multiple systems implicated in the pathophysiology of Parkinson's disease (PD) in the rat model. For this purpose, the MSCs were isolated from bone marrow (BM) of rat femur bone and PD was induced in ovariectomized rats by rotenone administration for 14 d. Our results provided clear evidences for the therapeutic role of BM-derived MSCs against PD pathophysiology through their immunomodulatory properties, anti-inflammatory and anti-apoptotic effects as well as neurotrophic and neurogenic potentials.

Ahmed HH, Salem AM, Atta HM, Eskandar EF, Farrag AH, Ghazy MA, Salem NA, Aglan HA. Updates in the pathophysiological mechanisms of Parkinson's disease: Emerging role of bone marrow mesenchymal stem cells. *World J Stem Cells* 2016; 8(3): 106-117 Available from: URL: http://www.wjgnet.com/1948-0210/full/v8/i3/106.htm DOI: http://dx.doi.org/10.4252/wjsc.v8.i3.106

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, associated with extrapyramidal motor dysfunction[1] due to the progressive and specific loss of dopaminergic neurons in the substantia nigra pars compacta and declining levels of dopamine (DA) in the striatum[2]. It affects approximately seven million people globally[3]. The commonness of PD raises with age, as 1% of people over 60 years of age, 3.4% of those over 70, and 4% of those over 80 were affected by the disease[1]. Epidemiological studies and pathological investigations exhibit a mean period of onset of 70 in sporadic PD, which represents about 95% of patients[4,5]; but familial forms of the disease linked to transformation in a limited number of genes account for 4% and these patients suffer from early-onset disease before the age of 50[6].

Growing body of evidences have demonstrated that environmental factors play a critical role in the etiology of PD[7]. For example, the environmental toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was identified as one the causative agents of Parkinsonism[8]. Also, herbicides or pesticides usage increase the risk of PD[9,10]. As, the pesticide rotenone and the herbicide paraquat reproduce the PD phenotype in animals[11]. Additionally, it has been suggested that exposure to organic solvents, carbon monoxide and carbon disulfide[12] play roles in the etiology of PD. Epidemiological studies have proposed a potential link between pesticide exposure and increased risk of PD. For example, agrarian laborers, particularly individuals who work with pesticides, are at increased risk for suffering from PD[13].

At present, there is no therapy clinically accessible to postpone neurodegeneration, thusly modulation of the disease course is an imperative unmet clinical need. Along these lines, understanding of the pathophysiology and etiology of the disease at cellular and molecular levels to find new targets against which neuroprotective/disease-modifying therapy may be developed is the pivotal issue in the field of PD research[7].

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that have the capacity of self-renewal and differentiation into mesodermal lineage cells and other embryonic lineages, including adipocytes, osteocytes, chondrocytes, hepatocytes, neurons, muscle cells, epithelial cells, *etc*.[14]. Additionally, these cells have several advantages, such as easy availability as well as few ethical concerns and low immunogenicity. An expanding number of data has demonstrated that MSCs not only depend on their differentiation capacity to repair damaged tissue, but also rely on their ability to modify local environment, activate endogenous progenitor

cells, and secrete several factors[15]. The aforementioned properties make MSCs perfect candidate cell type for tissue engineering, regenerative medicine and autoimmune disease treatment[14].

The focus of our interest was to clarify the mode of action of bone marrow derived MSCs (BM-MSCs) in targeting multiple systems implicated in the pathophysiology of PD in the rat model.

MATERIALS AND METHODS

Preparation of BM-MSCs

BM was harvested by flushing the tibiae and femurs of 6-wk-old male Sprague Dawley rats with Dulbecco's modified Eagle's medium (DMEM; GIBCO/BRL, Grand Island, New York, United States, Cat. #42430-082) supplemented with 10% fetal bovine serum (FBS; GIBCO/BRL, Cat. #16000-044). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL, Cat. #10378-016). Cells were incubated at 37 ℃ in 5% humidified CO_2 for 12-14 d as primary culture or upon formation of large colonies. When large colonies developed (80%-90% confluence), cultures were washed twice with phosphate buffer saline (PBS; Gibco/BRL, Cat. #10010056) and the cells were trypsinized with 0.025% trypsin and 0.01% ethylenediaminetetraacetic acid (EDTA) (Gibco/BRL, Cat. #R-001-100) for 5 min at 37 ℃. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 mL falcon tube. The resulting cultures were referred to as first-passage cultures. MSCs in cultures were characterized by their adhesiveness and fusiform shape[16].

Experimental set up

Twenty four adult female Sprague-Dawley rats weighing 130-150 g were obtained from the Animal House Colony of the National Research Centre, Giza, Egypt and acclimated in a specific area where temperature (25 ℃ ± 1 ℃) and humidity (55%). Rats were controlled constantly with a 12 h light/dark cycles at National Research Centre Animal Facility Breeding Colony. Rats were individually housed with *ad libitum* access to standard laboratory diet consisted of casein 10%, salt mixture 4%, vitamin mixture 1%, corn oil 10%, cellulose 5% and completed to 100 g with corn starch and tap water. Rats were cared for according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research at National Research Centre, Giza, Egypt.

After the acclimatization period (2 wk), the female rats were ovariectomized surgically in Hormones Department, Medical Research Division at the National Research Centre. Then, after one month from ovariectomy the animals were classified into 3 different groups (8 rats/group). The first group (Ovariectomized control group) was untreated ovariectomized control group. While, the second and third groups were subcutaneously injected with rotenone (Sigma, United States, Cat. #R8875) in a dose of 12 mg/kg b. wt.[17] daily for 14 d for induction of PD. Thereafter, the second group (PD untreated group) was left untreated for 4 mo while, the third group (PD + BM-MSCs group) was infused intravenously with a single dose (3×10^6 cells/rat) of BM-MSCs[18]. For MSCs infusion, the PD induced rats were deeply anaesthetized *via* diethyl ether and MSCs were suspended in 100 μL PBS before transplantation and then slowly injected into the tail vein in 5 min with a 27G needle. The needle was kept in the tail vein for another 5 min to avoid regurgitation and then withdrawn.

At the end of the experimental period (4 mo), all animals were fasted for 12 h and the blood samples were collected from retro-orbital venous plexus under diethyl ether anaesthesia. The blood samples were left to clot and the sera were separated by cooling centrifugation (4 ℃) at 1800 × *g* for 10 min and then stored immediately at -20 ℃ in clean plastic Eppendorf until analyzed. Moreover, the whole brain of each rat was rapidly and carefully dissected. Then, each brain was sagittally divided into two portions. The first portion was immediately frozen in liquid nitrogen and stored at -80 ℃ prior to extraction for molecular study and DA level determination. While, the second portion was fixed in formalin buffer (10%) for histological investigation and immunohistochemical study.

Detection of male-derived MSCs in the brain of females

The genomic DNA was isolated from the brain tissues of female rats which were treated with BM-MSCs using phenol/chloroform extraction and ethanol precipitation method according to Sambrook *et al*[19] with minor modifications. The presence or absence of the sex determination region on the Y chromosome male (*SRY*) gene in recipient female rats was assessed by PCR. Primer sequences for *SRY* gene (forward 5′-CATCGAAGGGTTAAA-GTGCCA-3′, reverse 5′-ATAGTGTGTAGGTTGTTGTCC-3′, Invitrogen) were obtained from published sequences[20] and amplified to a product of 104 bp. The PCR conditions were as follows: Incubation at 94 ℃ for 4 min; 35 cycles of incubation at 94 ℃ for 50 s, 60 ℃ for 30 s, and 72 ℃ for 1 min; with a final incubation at 72 ℃ for 10 min. PCR products were separated using 2% agarose gel electrophoresis and stained with ethidium bromide.

Biochemical analyses

Serum transforming growth factor beta-1 (TGF-β1) level was assayed by enzyme linked immunosorbent assay (ELISA) using kit purchased from DRG Diagnostics Co., Germany (Cat. #EIA-1864), according to the method described by Kropf *et al*[21]. While, serum monocyte chemoattractant protein-1 (MCP-1) level was determined by ELISA method using kit purchased from Bender MedSystems GmbH, Europe (Cat. #BMS631INST), according to the method described by Baggiolini *et al*[22]. Moreover, serum brain derived neurotrophic factor (BDNF) level was evaluated by ELISA method using kit purchased

from Millipore Corporation, United States (Cat. #CYT306), according to the method described by Laske *et al*[23]. Finally, the quantitative determination of brain DA level was carried out according to the method described by Ciarlone[24] using a fluorometric method.

Detection of tyrosine hydroxylase and nestin genes expression level

Total RNA was isolated from brain tissues of female rats by the standard TRIzol® reagent extraction method (Invitrogen, Cat. #15596-026). Then, the complete Poly(A)$^+$ RNA was reverse transcribed into cDNA in a total volume of 20 μL using RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany, Cat. #K1631). An amount of total RNA (5 μg) was used with a reaction mixture, termed as master mix. The MM was consisted of 50 mmol/L $MgCl_2$, 5 × reverse transcription (RT) buffer (50 mmol/L KCl; 10 mmol/L Tris-HCl; pH 8.3; 10 mmol/L of each dNTP, 50 μmol/L oligo-deoxyribonucleotide primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 U M-MuLV reverse transcriptase. The RT reaction was carried out at 25 ℃ for 10 min, followed by 1 h at 42 ℃, and the reaction was stopped by heating for 5 min at 99 ℃. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through semi-quantitative real time PCR (sqRT-PCR). An iQ5-BIO-RAD Cycler (Cepheid, United States) was used to determine the rat cDNA copy number. PCR reactions were set up in 25 μL reaction mixtures containing 12.5 μL 1 × SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany, Cat. #RR820A), 0.5 μL 0.2 μmol/L forward primer, 0.5 μL 0.2 μmol/L reverse primer (Invitrogen), 6.5 μL distilled water, and 5 μL of cDNA template. Primer sequences were F: 5′-ACTGTGGAATTCGGGCTATG-3′, R: 5′-GACCTCAGGCTCCTCTGACA-3′ for tyrosine hydroxylase (TH)[25]; F: 5′-TGGAGCGGGAGTTAG-AGGCT-3′, R: 5′-ACCTCTAAGCGACACTCCCGA-3′ for nestin[26] and F: 5′-CTGTCTGGCGGCACCACCAT-3′, R: 5′-GCAACTAAGTCATAGTCCGC-3′ for β-actin[27]. The reaction program was allocated to 3 steps. First step was at 95.0 ℃ for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (1) denaturation at 95.0 ℃ for 15 s; (2) annealing at 58.0 ℃ for 30 s, 55.0 ℃ for 5 s and 60 ℃ for 30 s for TH, *nestin* and *β-actin* genes respectively; and (3) extension at 72.0 ℃ for 30 s. The third step consisted of 71 cycles started at 60.0 ℃ and then increased about 0.5 ℃ every 10 s up to 95.0 ℃ for melting curve analysis which was performed at the end of each sqRT-PCR to check the quality of the used primers. Each experiment included a distilled water control.

Immunohistochemical examination of brain survivin expression

Samples were taken from brain of rats of the different groups and fixed in 10% formalin buffer for 24 h. Washing was done in tap water then ascending grade of ethyl alcohol (30%, 50%, 70%, 90% and absolute) was used for dehydration. Specimens were cleared in xylene and embedded in paraffin (melting point 58 ℃-60 ℃) for 24 h. Sections were cut into 4 μ thick by sledge microtome then fixed on positive slides in a 65 ℃ oven for 1 h. Slides were placed in a coplin jar filled with 200 mL of triology working solution (Cell Marque, CA-United States, Cat. #920P-04) which combines the three pretreatment steps: Deparaffinization, rehydration and antigen unmasking. Then, the jar is securely positioned in the autoclave which was adjusted so that temperature reached 120 ℃ and maintained stable for 15 min after which pressure is released. Thereafter, the coplin jar is removed to allow slides to cool for 30 min. Sections were then washed and immersed in Tris-buffer saline to adjust the pH and these were repeated between each step of the immunohistochemical procedure. Quenching endogenous peroxidase activity was performed by immersing slides in 3% hydrogen peroxide for 10 min. Broad spectrum LAB-SA detection system (Invitrogen, Cat. #85-8943) was used to visualize any antigen-antibody reaction in the tissue. Background staining was blocked by putting 3 drops of 10% goat non immune serum blocker on each slide and incubating them in a humidity chamber for 10 min. Without washing, excess serum was drained and the working solution (1:100) of survivin mouse monoclonal (Thermo Scientific, United States, Cat. #RB-9245-P1) was prepared. Three drops of the working solution were applied and slides were incubated in the humidity chamber overnight at 4 ℃. Henceforward, biotinylated secondary antibody from ultravision detection system anti-polyvalent HRP/3,3′-diaminobenzidine (DAB) (Thermo Scientific, Cat. #TP-015-HD) was applied on each slide for 20 min followed by 20 min incubation with the streptavidin HRP enzyme conjugate (Thermo Scientific, Cat. #TP-015-HD). Then, DAB chromogen (Thermo Scientific, Cat. #TP-015-HD) was prepared and 3 drops were applied on each slide for 2 min. DAB was rinsed, after which counterstaining with Mayer hematoxylin and cover slipping were performed as the final steps before slides were examined under the light microscope (Olympus Cx21 with attached digital camera)[28]. Image analysis was performed using the image J, 1.41a NIH, United States analyzer.

Histopathological investigation of brain tissue of rats

Samples were taken from brain of rats in different groups and fixed in 10% formalin buffer for 24 h. Washing was done in tap water then ascending grade of ethyl alcohol (30%, 50%, 70%, 90% and absolute) was used for dehydration. Specimens were cleared in xylene and embedded in paraffin (melting point 58 ℃-60 ℃) for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 μ by sledge microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin (H and E) stain[29] for histopathological examination through the electric light microscope.

Statistical analysis

In the present study, all results were expressed as mean ± SE of the mean. Data were analyzed by one

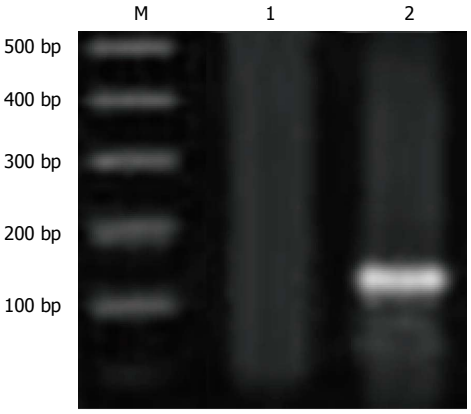

Figure 1 An agarose gel electrophoresis of DNA fragments showed *SRY* gene in recipient female rats for bone marrow derived mesenchymal stem cells in Parkinson's disease model. Lane (M) represents DNA ladder; Lane (1) represents ovariectomized control sample; Lane (2) represents sample from PD group treated with BM-MSCs. PD: Parkinson's disease; BM-MSCs: Bone marrow derived mesenchymal stem cells.

way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 14 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when *P* value was < 0.05.

Animal care and use statement

The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (25 ℃, 12 h/12 h light/dark, 55% humidity, *ad libitum* access to food and water) for 2 wk prior to experimentation. The animals were deeply anaesthetized *via* diethyl ether for intravenous infusion of MSCs. Also, blood samples were collected from retro-orbital venous plexus under diethyl ether anaesthesia.

RESULTS

BM-MSCs homing

To confirm that the intravenously transplanted MSCs derived from male bone marrow migrate and home to the female injured brain, DNA was isolated from the brain tissues of female rats and the presence or absence of the responsible region for sex determination on Y chromosome (*SRY* gene) was assessed by PCR. The agarose gel demonstrated that *SRY* gene was present in the brain tissues obtained from the group of rats treated with BM-MSCs. While, *SRY* gene was absent in the brain tissues obtained from the ovariectomized control rats (Figure 1).

Effect of treatment with BM-MSCs on inflammatory markers

Since, TGF-β1 has a pivotal role in the control of the transition between pro-inflammatory and anti-inflammatory response[30] and MCP-1 has a vital role in the migration of inflammatory cells across the blood-brain barrier as well as forms chemotactic gradients within the CNS to control the local inflammatory response[31]. Serum TGF-β1 and MCP-1 levels were determined by ELISA to evaluate the anti-inflammatory and immunomodulatory effects of the injected BM-MSCs in PD model.

Table 1 Effect of treatment with bone marrow derived mesenchymal stem cells on serum transforming growth factor beta-1 and monocyte chemoattractant protein-1 levels in Parkinson's disease model

	TGF-β1 (pg/mL)	MCP-1 (pg/mL)
Ovariectomized control	481.5 ± 7.5	88.1 ± 0.9
PD untreated	691.2 ± 8.0[a]	112.1 ± 1.9[a]
PD + BM-MSCs	489.7 ± 13.0[c]	89.6 ± 2.0[c]

Data are represented as mean ± SE of 8 rats/group. [a]Significant change at $P < 0.05$ in comparison with the ovariectomized control group; [c]Significant change at $P < 0.05$ in comparison with the untreated PD group. PD: Parkinson's disease; BM-MSCs: Bone marrow derived mesenchymal stem cells; TGF-β1: Transforming growth factor beta-1; MCP-1: Monocyte chemoattractant protein-1.

Our data revealed that rotenone administration causes significant ($P < 0.05$) elevation in serum TGF-β1 (43.6%) and MCP-1 (27.2%) levels *vs* the ovariectomized control group (Table 1). While, treatment with BM-MSCs elicits a significant ($P < 0.05$) reduction in both serum TGF-β1 and MCP-1 levels by 29.2% and 20.1% respectively relative to the group of rats left untreated.

Effect of treatment with BM-MSCs on neurotrophic and neurogenic markers

Brain derived neurotrophic factor plays an important role in supporting the survival of existing neurons and encouraging the growth as well as differentiation of new neurons and synapses[32]. Thusly, serum BDNF level was estimated by ELISA to evaluate the neurotrophic capacity of the injected BM-MSCs in PD model. In view of the data of the current work, rotenone administration experiences significant ($P < 0.05$) decline in serum BDNF level by 21.5% (Table 2) as compared to the ovariectomized control group. In contrast, treatment with BM-MSCs elevates serum BDNF level significantly ($P < 0.05$) by 26.1% (Table 2) relative to the group of rats left untreated.

Brain DA level was determined by a fluorometric method, while brain TH and nestin genes expression level was detected by sqRT-PCR to evaluate the neurogenic potential of the injected BM-MSCs in PD model. It is well known that DA is a neurotransmitter released by nerve cells to play crucial role in motor control, motivation, arousal, cognition and reward[33]. Furthermore, TH enzyme catalyzes the conversion of L-tyrosine to L-3,4-dihydroxy-phenylalanine[34]. While, nestin is one of the markers of neural precursors[35]. The data of our work revealed that rotenone administration leads to significant ($P < 0.05$) depletion of brain DA level (32.1%) and significant ($P < 0.05$) down-regulation in the expression level of brain TH and nestin genes by 54.6% and 48.5% respectively (Table 2) as compared to the ovariectomized control group.

Table 2 Effect of treatment with bone marrow derived mesenchymal stem cells on serum brain derived neurotrophic factor and brain dopamine levels as well as brain tyrosine hydroxylase and nestin genes expression level in Parkinson's disease model

	BDNF (pg/mL)	DA (μg/g tissue)	Relative expression of TH gene (TH/β-actin)	Relative expression of nestin gene (nestin/β-actin)
Ovariectomized control	3700 ± 26.4	882 ± 20.3	1.19 ± 0.004	1.30 ± 0.004
PD untreated	2905 ± 72.9[a]	599 ± 9.8[a]	0.54 ± 0.009[a]	0.67 ± 0.006[a]
PD + BM-MSCs	3663 ± 17.8[c]	874 ± 15.0[c]	1.18 ± 0.004[c]	1.29 ± 0.005[c]

Data are represented as mean ± SE of 8 rats/group. [a]Significant change at $P < 0.05$ in comparison with the ovariectomized control group; [c]Significant change at $P < 0.05$ in comparison with the untreated PD group. PD: Parkinson's disease; BM-MSCs: Bone marrow derived mesenchymal stem cells; BDNF: Brain derived neurotrophic factor; DA: Dopamine; TH: Tyrosine hydroxylase.

Table 3 Effect of treatment with bone marrow derived mesenchymal stem cells on brain survivin expression in Parkinson's disease model

	Survivin (cell number)
Ovariectomized control	288 ± 16.5
PD untreated	271.5 ± 13.9
PD + BM-MSCs	293.2 ± 15.9

Data are represented as mean ± SE of 8 rats/group. PD: Parkinson's disease; BM-MSCs: Bone marrow derived mesenchymal stem cells.

However, treatment with BM-MSCs produces significant ($P < 0.05$) elevation in brain DA level by 45.9% and significant ($P < 0.05$) up-regulation in brain TH and nestin genes expression level by 122.2% and 92.5% respectively (Table 2) *vs* the group of rats left untreated.

Effect of treatment with BM-MSCs on anti-apoptotic marker

The anti-apoptotic action of the single intravenous dose of BM-MSCs in PD model was evaluated through the detection of brain survivin expression using immuno-histochemical technique. As, survivin belongs to a family of endogenous cellular inhibitors of caspases that directly repress apoptotic cell death through interactions with pro-apoptotic caspases[36]. In view of the current data, rotenone administration causes insignificant ($P > 0.05$) decrease in the number of positive cells for survivin expression by 5.7% (Table 3 and Figure 2B) relative to the ovariectomized control group. While, treatment with BM-MSCs produces insignificant ($P > 0.05$) increase in the number of positive cells for survivin expression by 8.0% (Table 3 and Figure 2C) in comparison with the group of rats left untreated.

Effect of treatment with BM-MSCs on brain structure

The brain section photomicrograph of ovariectomized control rat shows congestion in the blood vessels in striatum area (Figure 3A). While, brain section photomicrographs of untreated rotenone administered rat show congestion in the blood vessels and capillaries (Figure 3B) in the striatum as well as hyalinization and plaques formation in the matrix of the striatum indicating the occurrence of neurodegeneration (Figure 3C). Finally, the brain section photomicrograph of rotenone administered rat treated with BM-MSCs shows intact histological structure of the striatum (Figure 3D).

DISCUSSION

MSCs have been considered as an effective tool for regenerative cell therapy. These cells could be isolated from both healthy and patient tissues and expanded *in vitro* on a therapeutic scale without posing significant ethical or procedural problems[37]. Furthermore, it has been proposed that stem cells may replace lost cells by differentiating into functional neural tissue; provide source of trophic support for the diseased nervous system or alter the immune system to prevent further neurodegeneration[38]. Therefore, the current study was planned to elucidate the mechanisms by which BM-MSCs could attenuate PD pathophysiology in the experimental model.

In consistent with Yoon *et al*[39] who found that intravenously transplanted BM-MSCs could migrate and home into the brain, the data presented in this work demonstrated that the intravenously transplanted MSCs were able to migrate to the site of injury (brain). The homing property afforded by MSCs was likely attributable to their broader expression of homing molecules[40]. Furthermore, it has been reported that, chemokines released from tissue or endothelial cells may contribute to the activation of adhesion ligands, transendothelial migration, chemotaxis, and/or subsequent retention in surrounding tissue[41].

In view of the data of the current work, rotenone administration for 14 d in ovariectomized rats elevated the level of serum TGF-β1 and MCP-1 significantly. This finding is greatly supported by those of Rota *et al*[42] and Reale *et al*[43] who stated that both TGF-β1 and MCP-1 levels are increased in several chronic neurodegenerative pathologies such as PD. It has been reported that the inflammatory response due to Parkinsonism is characterized by activation of microglia in the brain. The proposed explanation in regards to the reason of degeneration in dopaminergic neurons is that PD is caused by activation of microglial cells as a result of increased levels of cytokines[44]. Activated microglia release a wide array of pro-inflammatory and cytotoxic factors as well as eicosanoids and nitric oxide[45], which work in concert to develop neurodegeneration[46]. Moreover, Gao *et al*[47] reported that the dopaminergic neurodegeneration enhanced by rotenone might be attributed primarily to

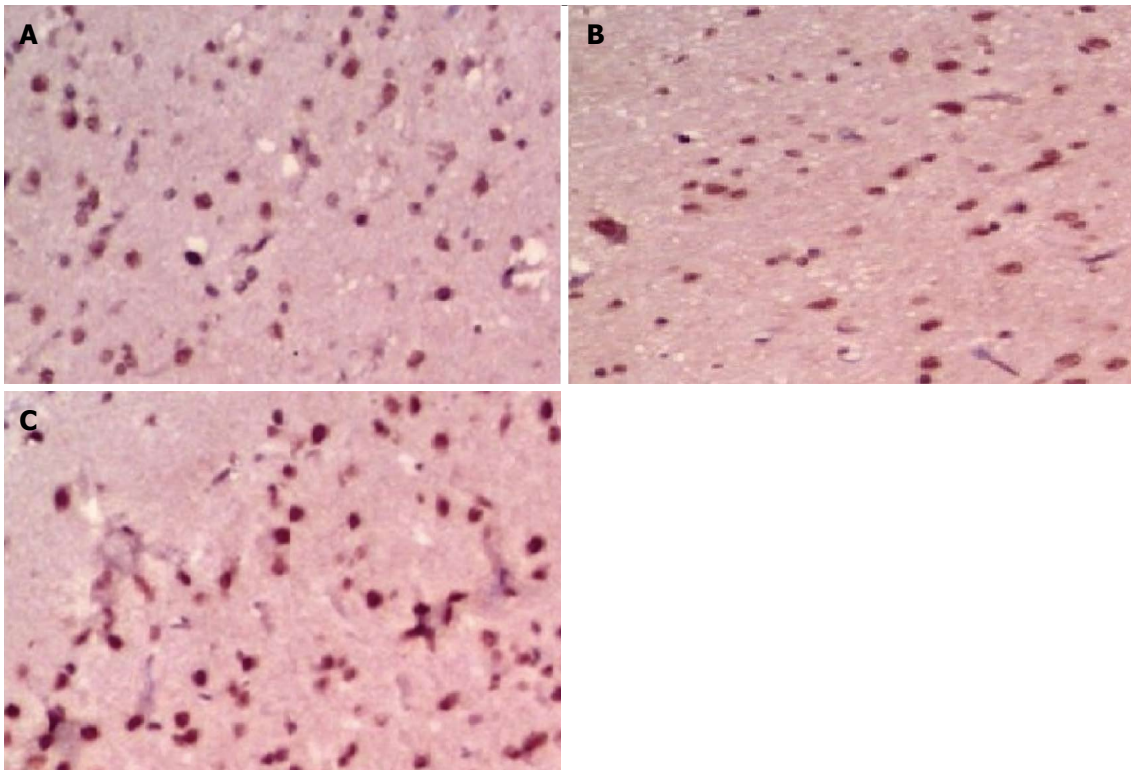

Figure 2 Immunohistochemical examination of survivin expression in Parkinson's disease model groups. A: Ovariectomized control; B: PD untreated; C: PD + BM-MSCs. PD: Parkinson's disease; BM-MSCs: Bone marrow derived mesenchymal stem cells.

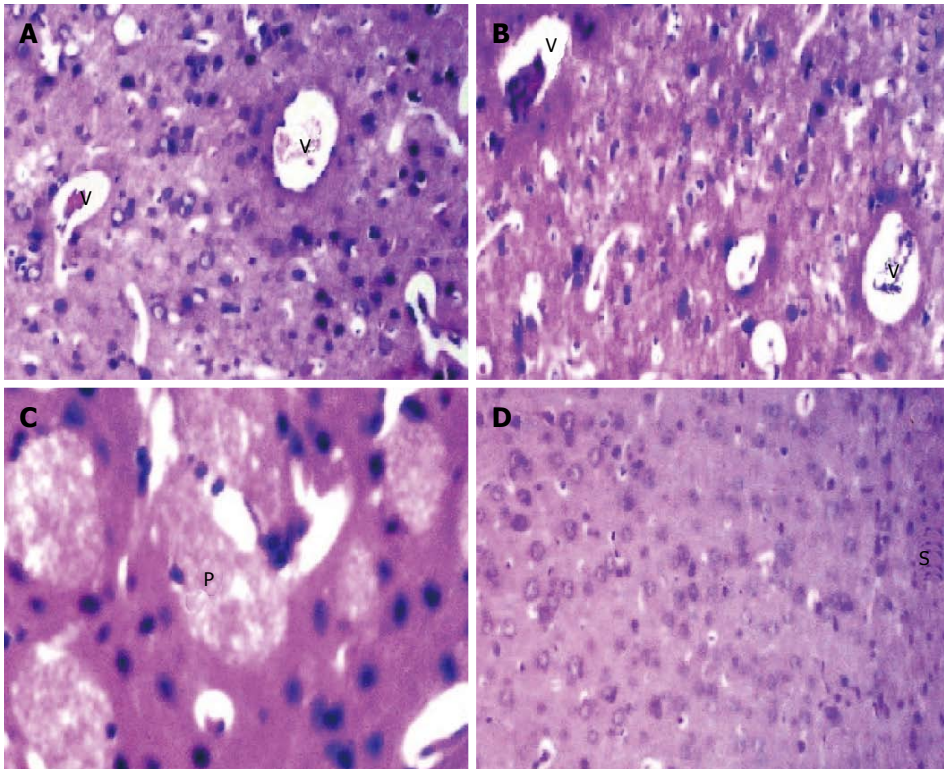

Figure 3 Photomicrograph of brain section of: A: Ovariectomized control group shows congestion in blood vessels of striatum (v) (H and E × 80); B: untreated Parkinson's disease (PD) group shows congestion in blood vessels and capillaries of striatum (v) (H and E × 80); C: Untreated PD: Parkinson's disease group shows hyalinization with plaques formation in the matrix of striatum (H and E × 160); and D: PD group treated with bone marrow derived mesenchymal stem cells shows intact histological structure of the striatum (H and E × 80).

the activation of microglia and consequently their release of superoxide free radicals that play an important role in

the inflammation mediated oxidative damage to neurons. This effect might be ascribed to the known susceptibility of dopaminergic neurons to oxidative stress as a result of reduced antioxidant capacity, high content of iron and DA, and possible defect in mitochondrial function[48]. The release of cytokines from the brain into the peripheral blood supply through the blood brain barrier[49] could explain the observed increase in serum TGF-β1 and MCP-1 levels.

The results of the current study manifested that treatment with BM-MSCs lessen the level of serum TGF-β1 and MCP-1 significantly. This finding is in great accordance with our previous work on adipose tissue derived MSC[50] that proved its anti-inflammatory and immunomodulatory activities which are implicated in mitigating neuroinflammation characterizing PD. Accordingly, the observed role of BM-MSCs in depleting serum TGF-β1 and MCP-1 levels could be allied to the ability of BM-MSCs to modulate microglia/macrophage activation including inflammatory responses as documented by Németh *et al*[51] and Choi *et al*[52].

Growing body of evidence indicates that there is a link between pro-inflammatory cytokines and neurotrophic factors in the CNS[53]. It has been postulated that there is a balance between cytokine and neurotrophin in the brain and disruption of this balance cause injurious changes in the CNS[54]. Moreover, Borchelt[55] observed that astrocytes stimulated by mediators released from microglia down-regulate neurotrophic factors expression and release additional inflammatory mediators that in turn activate microglia. Parallel to these evidences, our results indicated that rotenone administration elicited significant decrease in serum BDNF level. This finding could be allied to the diminished level of brain BDNF due to inflammation. As, Klein *et al*[56] reported that BDNF level in the blood correlates with alteration in the level of BDNF in the brain.

In view of the current data, treatment with BM-MSCs experienced significant increase in serum BDNF level. This preferable effect could be related to the ability of MSCs to secrete BDNF as observed by Lattanzi *et al*[57] and Han *et al*[58]. Blandini *et al*[59] documented that MSCs have the ability to differentiate into glial cells that release diverse neurotrophic factors to provide protection against neurotoxin after their grafting into Parkinsonian rat brains. Additionally, there is an evidence that MSCs may modulate the expression of neurotrophic factors according to the environment in which they exist[60,61].

The data presented in this work revealed that rotenone administration led to significant down-regulation in brain *TH* gene expression level in concomitant with significant decline in brain DA level. This observation could be ascribed to the dopaminergic degeneration[62] due to elevated sensitivity of dopaminergic neurons to oxidative damage[47] as well as inhibition of complex Ⅰ activity and decrement of the mitochondrial membrane potential as a result of rotenone administration[47,63].

Our previous findings indicated the neurotrophic and neuroprotective potentials of adipose tissue derived MSC against neurodegenerative insult of PD[50]. Similarly, the data of the present work demonstrated that treatment with BM-MSCs elicited significant increase in brain DA level as well as brain TH gene expression level. This finding comes in line with the study of Shetty *et al*[64] who demonstrated that BM-MSCs can be transdifferentiated efficiently into functional dopaminergic neurons capable of secreting DA and alleviating behavioral deficiencies. Moreover, the results of Bouchez *et al*[25] study showed that grafting of BM-MSCs caused an increase in the immunostaining of TH in striatum associated with elevation in the number of TH^+ neurons in the substantia nigra pars compacta. Also, Blondheim *et al*[65] and Offen *et al*[66] stated that the transplantation of BM-MSCs into the animal model induced with 6-hydroxydopamine resulting in an increase in the level of TH in the striatal region thus improving motor behavior in a mouse model of PD. Since, TH is the rate-limiting enzyme in DA synthesis, the increase in the level of TH would increase the production of DA. Additionally, the observed increase in brain DA content and TH expression level as a result of treatment with BM-MSCs could be explained by the ability of MSCs to secrete a wide array of cytokines and growth factors, including BDNF[57] which exert neurotrophic and neuroprotective effects on DA neurons[67]. Furthermore, Trzaska *et al*[68] reported that BDNF has a crucial role in the functional maturation of MSC-derived DA progenitors.

In line with previous studies reported by Höglinger *et al*[69] and Abdipranoto *et al*[70], the current study manifested that rotenone administration caused significant down-regulation in brain nestin gene expression level. This finding could be imputed to the depletion in DA level due to degeneration of dopaminergic neurons as documented by Crews *et al*[71]. In contrast, treatment with BM-MSCs induced significant up-regulation in nestin gene expression level. Bouchez *et al*[25] found that rat MSCs express neuronal proteins such as nestin at the RNA and protein levels. Moreover, the study of Ye *et al*[72] indicated the presence of nestin positive cells in brain tissue of PD rat after transplantation of undifferentiated BM-MSCs. The suggested mechanism by which BM-MSCs treat PD rat model could be related to that transplanted BM-MSCs might become nestin-positive stem cells that differentiate into astrocytes or other non-dopaminergic neurons and participate in the reconstruction of dopaminergic neurons circuits[72].

The data of this work revealed that rotenone administration produced slight decrease in the number of positive cells for survivin expression. This finding harmonizes with that of Zhang *et al*[73] who reported that degenerating neurons lacked survivin expression. Jiang *et al*[74] results showed that survivin is critically required for the survival of developing CNS neurons. Moreover, Zhang *et al*[75] suggested that there is a connection between the expression of survivin and adult neurogenesis. Thus, the observed decrement in survivin expression might be attributed to the decreased neurogenesis due to DA depletion[71]. Another possible mechanism by which rotenone could decrease survivin expression might be related to its effect on p53

which was shown to be over expressed by rotenone[76]. Under normal conditions, p53 protein levels are low and regulated by IκB kinase (IKK) and prominently by mouse double minute 2 (Mdm2), an ubiquitin ligase responsible for p53 degradation. Cellular stress reduces the interaction between p53 and Mdm2 leading to accumulation of the former[77]. Wu *et al*[76] reported that the degeneration of dopaminergic neurons by rotenone was accompanied by an increase in p53 protein level which in turn induces p21 expression. Then, the increased level of p21 suppresses the expression of cycline dependent kinases leading to accumulation of hypophosphorylated retinoblastoma that interact with E2F (a transcriptional activator) to repress survivin expression[78].

In the light of our results, treatment with BM-MSCs caused insignificant increase in the number of positive cells for survivin expression. This increment is in agreement with Okazaki *et al*[79] and it could be imputed to the ability of MSCs to enhance neurogenesis and inhibit apoptosis through their secreted BDNF as documented by Ye *et al*[72]. Moreover, Kim *et al*[80] reported that grafted MSCs attenuate dopaminergic neuronal loss through their anti-apoptotic effects. Also, the increase in survivin expression by MSCs treatment might be related to their inhibitory action on P53 through the inactivation of ERK1/2[81].

In view of the histopathological investigations of brain tissues section of the current work, rotenone administration resulted in congestion in the blood vessels and capillaries of striatum. Also, there were hyalinization and plaques formation in the matrix of striatum indicating the occurrence of neurodegeneration. Sai *et al*[82] demonstrated that rotenone causes dopaminergic neurons degeneration *in vivo* and substantia nigra pars compacta and striatum are the main targets of rotenone in the rat brain. These findings could be allied to the inhibition of neuronal mitochondrial complex I activity[47] and consequently oxidative damage[83] as a result of rotenone administration.

Brain tissue sections examination indicated that single infusion with BM-MSCs resulted in intact histological structure of the striatum. This finding coincides with Dezawa *et al*[84] who reported that nerve system recovery after BM-MSCs transplantation could be related to their secretion of neurotrophic factors that restore the function of nervous system, promotion of local angiogenesis and vascular reconstruction and neuronal regeneration through promotion of autologous neuronal regeneration and differentiation of transplanted cells into neural cells.

In conclusion, the current study provided experimental evidences for the ability of BM-MSCs to mitigate PD pathophysiology through multi-mechanistic approaches (immunomodulatory, anti-inflammatory and anti-apoptotic effects as well as neurotrophic and neurogenic potentials). These promising results pave the way for the clinical trial application of MSCs in the treatment of neurodegenerative diseases particularly PD.

COMMENTS

Background

Parkinson's disease (PD) is one of the neurodegenerative diseases, accompanied by extrapyramidal motor dysfunction due to the progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta and declining levels of dopamine in the striatum. So, it is very important to stop or halt neurodegeneration. However, to date, there is no therapy clinically available that delays the neurodegenerative process itself, therefore modification of the disease course is an important unmet clinical need. Transplantation of mesenchymal stem cells (MSCs) for treating neurodegenerative disorders has received growing attention recently because these cells are readily available, easily expanded in culture, and when transplanted survive for relatively long periods of time.

Research frontiers

MSCs are a heterogeneous subset of stromal stem cells that have the ability of self-renewal and multipotency. In the area of neurodegenerative disorders treatment, the current research hotspot is how to modify the disease course by specifically target the pathophysiologic cascade, hoping to delay the onset of the disease and slow its progression.

Innovations and breakthroughs

Modern research has focused on discovering effective disease-modifying therapies, which specifically target the pathophysiologic cascade, hoping to delay the onset of the disease and slow its progression. The study provided a non invasive approach for mitigating PD pathophysiology *via* bone marrow derived MSCs (BM-MSCs) transplantation which has immunomodulatory, anti-inflammatory and anti-apoptotic effects as well as neurotrophic and neurogenic potentials.

Applications

The study results shed light on the therapeutic potential of BM-MSCs against PD pathophysiology *via* multi-mechanistic actions.

Terminology

PD is the second most common neurodegenerative disease, accompanied by extrapyramidal motor dysfunction which resulting from the progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta and declining levels of dopamine in the striatum. MSCs are a heterogeneous subset of stromal stem cells that have the ability of self-renewal and multipotency, which could differentiate into cells of the mesodermal lineages and other embryonic lineages, including adipocytes, osteocytes, chondrocytes, hepatocytes, neurons, muscle cells, epithelial cells, *etc*.

Peer-review

This article is well written, clearly demonstrating the therapeutic effect of BM-MSCs for the treatment of PD. Authors also presented the molecular basis for the amelioration of PD pathology by showing decrements and increments in inflammatory mediators and neurotrophic factors in the serum, respectively. The overall data presented in this manuscript are sound.

REFERENCES

1 **Olanow CW**, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology* 2009; **72**: S1-136 [PMID: 19470958 DOI: 10.1212/WNL.0b013e3181a1d44c]

2 **Xu L**, Chen WF, Wong MS. Ginsenoside Rg1 protects dopaminergic neurons in a rat model of Parkinson's disease through the IGF-I receptor signalling pathway. *Br J Pharmacol* 2009; **158**: 738-748 [PMID: 19703168 DOI: 10.1111/j.1476-5381.2009.00361.x]

3 **Yao SC**, Hart AD, Terzella MJ. An evidence-based osteopathic approach to Parkinson disease. *Osteopathic Family Physician* 2013; **5**: 96-101 [DOI: 10.1016/j.osfp.2013.01.003]

4 **Tanner CM**. Is the cause of Parkinson's disease environmental or hereditary? Evidence from twin studies. *Adv Neurol* 2003; **91**: 133-142 [PMID: 12442672 DOI: 10.1016/j.bbadis.2013.09.007]

5 **Farrer MJ**. Genetics of Parkinson disease: paradigm shifts and future prospects. *Nat Rev Genet* 2006; **7**: 306-318 [PMID: 16543934 DOI: 10.1038/nrg1831]

6 **Mizuno Y**, Hattori N, Kitada T, Matsumine H, Mori H, Shimura H, Kubo S, Kobayashi H, Asakawa S, Minoshima S, Shimizu N.

Familial Parkinson's disease. Alpha-synuclein and parkin. *Adv Neurol* 2001; **86**: 13-21 [PMID: 11553970]
7 **Kim YS**, Kim YK, Hwang O, Kim DJ. Pathology of neurodegenerative diseases, Brain damage - Bridging between basic research and clinics. In: Dr. Alina Gonzalez-Quevedo, editor. USA: InTech., 2012: 99-138 [DOI: 10.5772/38028]
8 **Langston JW**, Ballard PA. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *N Engl J Med* 1983; **309**: 310 [PMID: 6602944 DOI: 10.1056/NEJM198308043090511]
9 **Kamel F**, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J Epidemiol* 2007; **165**: 364-374 [PMID: 17116648 DOI: 10.1093/aje/kwk024]
10 **Tanner CM**, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW. Occupation and risk of parkinsonism: a multicenter case-control study. *Arch Neurol* 2009; **66**: 1106-1113 [PMID: 19752299 DOI: 10.1001/archneurol.2009.195]
11 **Betarbet R**, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000; **3**: 1301-1306 [PMID: 11100151 DOI: 10.1038/81834]
12 **Corrigan FM**, Murray L, Wyatt CL, Shore RF. Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. *Exp Neurol* 1998; **150**: 339-342 [PMID: 9527905 DOI: 10.1006/exnr.1998.6776]
13 **Gorell JM**, Peterson EL, Rybicki BA, Johnson CC. Multiple risk factors for Parkinson's disease. *J Neurol Sci* 2004; **217**: 169-174 [PMID: 14706220]
14 **Si YL**, Zhao YL, Hao HJ, Fu XB, Han WD. MSCs: Biological characteristics, clinical applications and their outstanding concerns. *Ageing Res Rev* 2011; **10**: 93-103 [PMID: 20727988 DOI: 10.1016/j.arr.2010.08.005]
15 **Tögel F**, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol* 2007; **292**: F1626-F1635 [PMID: 17213465 DOI: 10.1152/ajprenal.00339.2006]
16 **Alhadlaq A**, Mao JJ. Mesenchymal stem cells: isolation and therapeutics. *Stem Cells Dev* 2004; **13**: 436-448 [PMID: 15345137 DOI: 10.1089/scd.2004.13.436]
17 **Antkiewicz-Michaluk L**, Karolewicz B, Romańska I, Michaluk J, Bojarski AJ, Vetulani J. 1-methyl-1,2,3,4-tetrahydroisoquinoline protects against rotenone-induced mortality and biochemical changes in rat brain. *Eur J Pharmacol* 2003; **466**: 263-269 [PMID: 12694809 DOI: 10.1016/S0014-2999(03)01565-6]
18 **Zhao DC**, Lei JX, Chen R, Yu WH, Zhang XM, Li SN, Xiang P. Bone marrow-derived mesenchymal stem cells protect against experimental liver fibrosis in rats. *World J Gastroenterol* 2005; **11**: 3431-3440 [PMID: 15948250 DOI: 10.3748/wjg.v11.i22.3431]
19 **Sambrook L**, Fritsch EF, Manitatis T. Molecular cloning: a laboratory manual. NY: Cold Spring Harbor Press, Cold Spring Harbor, 1989 [DOI: 10.1016/0092-8674(90)90210-6]
20 **Wu GD**, Tuan TL, Bowdish ME, Jin YS, Starnes VA, Cramer DV, Barr ML. Evidence for recipient derived fibroblast recruitment and activation during the development of chronic cardiac allograft rejection. *Transplantation* 2003; **76**: 609-614 [PMID: 12923453 DOI: 10.1097/01.TP.0000066362.37931.6D]
21 **Kropf J**, Schurek JO, Wollner A, Gressner AM. Immunological measurement of transforming growth factor-beta 1 (TGF-beta1) in blood; assay development and comparison. *Clin Chem* 1997; **43**: 1965-1974 [PMID: 9342020]
22 **Baggiolini M**, Dewald B, Moser B. Human chemokines: an update. *Annu Rev Immunol* 1997; **15**: 675-705 [PMID: 9143704 DOI: 10.1146/annurev.immunol.15.1.675]
23 **Laske C**, Stransky E, Eschweiler GW, Klein R, Wittorf A, Leyhe T, Richartz E, Köhler N, Bartels M, Buchkremer G, Schott K. Increased BDNF serum concentration in fibromyalgia with or without depression or antidepressants. *J Psychiatr Res* 2007; **41**: 600-605 [PMID: 16600301 DOI: 10.1016/j.jpsychires.2006.02.007]
24 **Ciarlone EA**. Determination of catecholamines spectrophotoflurometrically. *Am J Physiol* 1978; **125**: 731-737
25 **Bouchez G**, Sensebé L, Vourc'h P, Garreau L, Bodard S, Rico A, Guilloteau D, Charbord P, Besnard JC, Chalon S. Partial recovery of dopaminergic pathway after graft of adult mesenchymal stem cells in a rat model of Parkinson's disease. *Neurochem Int* 2008; **52**: 1332-1342 [PMID: 18372079 DOI: 10.1016/j.neuint.2008.02.003]
26 **Huang C**, Tang C, Feigin A, Lesser M, Ma Y, Pourfar M, Dhawan V, Eidelberg D. Changes in network activity with the progression of Parkinson's disease. *Brain* 2007; **130**: 1834-1846 [PMID: 17470495 DOI: 10.1093/brain/awm086]
27 **Gaytan F**, Barreiro ML, Caminos JE, Chopin LK, Herington AC, Morales C, Pinilla L, Paniagua R, Nistal M, Casanueva FF, Aguilar E, Diéguez C, Tena-Sempere M. Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. *J Clin Endocrinol Metab* 2004; **89**: 400-409 [PMID: 14715878 DOI: 10.1210/jc.2003-031375]
28 **Bancroft JD**, Gamble M. Theory and practice of histological techniques. 6th Ed. USA: Churchill Livingstone-Elsevier, 2008: 433-469. Available from: URL: http://trove.nla.gov.au/version/208775733
29 **Banchroft JD**, Stevens A, Turner DR. Theory and practice of histological techniques. 4th ed. Philadelphia, PA, USA: Churchill Livingstone, 1996: 25-90. Available from: URL: http://trove.nla.gov.au/version/45634524
30 **Lawrence DA**. Transforming growth factor-beta: a general review. *Eur Cytokine Netw* 1996; **7**: 363-374 [PMID: 8954178 DOI: 10.1016/j.lfs.2013.10.004]
31 **Iarlori C**, Gambi D, Gambi F, Lucci I, Feliciani C, Salvatore M, Reale M. Expression and production of two selected beta-chemokines in peripheral blood mononuclear cells from patients with Alzheimer's disease. *Exp Gerontol* 2005; **40**: 605-611 [PMID: 15935590 DOI: 10.1016/j.exger.2005.04.007]
32 **Huang EJ**, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 2001; **24**: 677-736 [PMID: 11520916 DOI: 10.1146/annurev.neuro.24.1.677]
33 **Schultz W**. Multiple dopamine functions at different time courses. *Annu Rev Neurosci* 2007; **30**: 259-288 [PMID: 17600522 DOI: 10.1146/annurev.neuro.28.061604.135722]
34 **Nagatsu T**. Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays Biochem* 1995; **30**: 15-35 [PMID: 8822146 DOI: 10.1016/j.neuroscience.2014.04.063]
35 **Li Y**, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. *J Cereb Blood Flow Metab* 2000; **20**: 1311-1319 [PMID: 10994853 DOI: 10.1097/TP.0000000000000535]
36 **Salvesen GS**, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002; **3**: 401-410 [PMID: 12042762 DOI: 10.1038/nrm830]
37 **Tanna T**, Sachan V. Mesenchymal stem cells: potential in treatment of neurodegenerative diseases. *Curr Stem Cell Res Ther* 2014; **9**: 513-521 [PMID: 25248677]
38 **Wilkins A**, Kemp K, Ginty M, Hares K, Mallam E, Scolding N. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. *Stem Cell Res* 2009; **3**: 63-70 [PMID: 19411199 DOI: 10.1016/j.scr.2009.02.006]
39 **Yoon JK**, Park BN, Shim WY, Shin JY, Lee G, Ahn YH. In vivo tracking of 111In-labeled bone marrow mesenchymal stem cells in acute brain trauma model. *Nucl Med Biol* 2010; **37**: 381-388 [PMID: 20346878 DOI: 10.1016/j.nucmedbio.2009.12.001]
40 **Payne NL**, Sun G, McDonald C, Layton D, Moussa L, Emerson-Webber A, Veron N, Siatskas C, Herszfeld D, Price J, Bernard CC. Distinct immunomodulatory and migratory mechanisms underpin the therapeutic potential of human mesenchymal stem cells in autoimmune demyelination. *Cell Transplant* 2013; **22**: 1409-1425

[PMID: 23057962 DOI: 10.3727/096368912X657620]
41 **Belema-Bedada F**, Uchida S, Martire A, Kostin S, Braun T. Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. *Cell Stem Cell* 2008; **2**: 566-575 [PMID: 18522849 DOI: 10.1016/j.stem.2008.03.003]
42 **Rota E**, Bellone G, Rocca P, Bergamasco B, Emanuelli G, Ferrero P. Increased intrathecal TGF-beta1, but not IL-12, IFN-gamma and IL-10 levels in Alzheimer's disease patients. *Neurol Sci* 2006; **27**: 33-39 [PMID: 16688597]
43 **Reale M**, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun* 2009; **23**: 55-63 [PMID: 18678243 DOI: 10.1016/j.bbi.2008.07.003]
44 **Teismann P**, Tieu K, Cohen O, Choi DK, Wu DC, Marks D, Vila M, Jackson-Lewis V, Przedborski S. Pathogenic role of glial cells in Parkinson's disease. *Mov Disord* 2003; **18**: 121-129 [PMID: 12539204 DOI: 10.1002/mds.10332]
45 **McGuire SO**, Ling ZD, Lipton JW, Sortwell CE, Collier TJ, Carvey PM. Tumor necrosis factor alpha is toxic to embryonic mesencephalic dopamine neurons. *Exp Neurol* 2001; **169**: 219-230 [PMID: 11358437 DOI: 10.1006/exnr.2001.7688]
46 **Liu B**, Gao HM, Wang JY, Jeohn GH, Cooper CL, Hong JS. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann N Y Acad Sci* 2002; **962**: 318-331 [PMID: 12076984 DOI: 10.1111/j.1749-6632.2002.tb04077.x]
47 **Gao HM**, Hong JS, Zhang W, Liu B. Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 2002; **22**: 782-790 [PMID: 11826108]
48 **Jenner P**, Olanow CW. Understanding cell death in Parkinson's disease. *Ann Neurol* 1998; **44**: S72-S84 [PMID: 9749577 DOI: 10.1002/ana.410440712]
49 **Banks WA**, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation* 2002; **10**: 319-327 [PMID: 12907838 DOI: 10.1159/000071472]
50 **Ahmed H**, Salem A, Atta H, Ghazy M, Aglan H. Do adipose tissue-derived mesenchymal stem cells ameliorate Parkinson's disease in rat model? *Hum Exp Toxicol* 2014; **33**: 1217-1231 [PMID: 24567299 DOI: 10.1177/0960327114524238]
51 **Németh K**, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009; **15**: 42-49 [PMID: 19098906 DOI: 10.1038/nm.1905]
52 **Choi H**, Lee RH, Bazhanov N, Oh JY, Prockop DJ. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages. *Blood* 2011; **118**: 330-338 [PMID: 21551236 DOI: 10.1182/blood-2010-12-327353]
53 **Johnson VJ**, Sharma RP. Aluminum disrupts the pro-inflammatory cytokine/neurotrophin balance in primary brain rotation-mediated aggregate cultures: possible role in neurodegeneration. *Neurotoxicology* 2003; **24**: 261-268 [PMID: 12606298 DOI: 10.1016/S0161-813X(02)00194-8]
54 **Aloe L**, Fiore M, Probert L, Turrini P, Tirassa P. Overexpression of tumour necrosis factor alpha in the brain of transgenic mice differentially alters nerve growth factor levels and choline acetyltransferase activity. *Cytokine* 1999; **11**: 45-54 [PMID: 10080878]
55 **Borchelt DR**. Amyotrophic lateral sclerosis--are microglia killing motor neurons? *N Engl J Med* 2006; **355**: 1611-1613 [PMID: 17035656 DOI: 10.1056/NEJMcibr064358]
56 **Klein AB**, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol* 2011; **14**: 347-353 [PMID: 20604989 DOI: 10.1017/S1461145710000738]
57 **Lattanzi W**, Geloso MC, Saulnier N, Giannetti S, Puglisi MA, Corvino V, Gasbarrini A, Michetti F. Neurotrophic features of human adipose tissue-derived stromal cells: in vitro and in vivo studies. *J Biomed Biotechnol* 2011; **2011**: 468705 [PMID: 22219658 DOI: 10.1155/2011/468705]
58 **Han C**, Zhang L, Song L, Liu Y, Zou W, Piao H, Liu J. Human adipose-derived mesenchymal stem cells: a better cell source for nervous system regeneration. *Chin Med J* (Engl) 2014; **127**: 329-337 [PMID: 24438624 DOI: 10.3760/cma.j.issn.0366-6999.20120064]
59 **Blandini F**, Cova L, Armentero MT, Zennaro E, Levandis G, Bossolasco P, Calzarossa C, Mellone M, Giuseppe B, Deliliers GL, Polli E, Nappi G, Silani V. Transplantation of undifferentiated human mesenchymal stem cells protects against 6-hydroxydopamine neurotoxicity in the rat. *Cell Transplant* 2010; **19**: 203-217 [PMID: 19906332 DOI: 10.3727/096368909X479839]
60 **Boucherie C**, Caumont AS, Maloteaux JM, Hermans E. In vitro evidence for impaired neuroprotective capacities of adult mesenchymal stem cells derived from a rat model of familial amyotrophic lateral sclerosis (hSOD1(G93A)). *Exp Neurol* 2008; **212**: 557-561 [PMID: 18539273 DOI: 10.1016/j.expneurol.2008.04.030]
61 **Crisostomo PR**, Wang Y, Markel TA, Wang M, Lahm T, Meldrum DR. Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. *Am J Physiol Cell Physiol* 2008; **294**: C675-C682 [PMID: 18234850 DOI: 10.1152/ajpcell.00437.2007]
62 **Bousquet M**, St-Amour I, Vandal M, Julien P, Cicchetti F, Calon F. High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. *Neurobiol Dis* 2012; **45**: 529-538 [PMID: 21971528 DOI: 10.1016/j.nbd.2011.09.009]
63 **Greenamyre JT**, MacKenzie G, Peng TI, Stephans SE. Mitochondrial dysfunction in Parkinson's disease. *Biochem Soc Symp* 1999; **66**: 85-97 [PMID: 10989660]
64 **Shetty P**, Ravindran G, Sarang S, Thakur AM, Rao HS, Viswanathan C. Clinical grade mesenchymal stem cells transdifferentiated under xenofree conditions alleviates motor deficiencies in a rat model of Parkinson's disease. *Cell Biol Int* 2009; **33**: 830-838 [PMID: 19465139 DOI: 10.1016/j.cellbi.2009.05.002]
65 **Blondheim NR**, Levy YS, Ben-Zur T, Burshtein A, Cherlow T, Kan I, Barzilai R, Bahat-Stromza M, Barhum Y, Bulvik S, Melamed E, Offen D. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. *Stem Cells Dev* 2006; **15**: 141-164 [PMID: 16646662 DOI: 10.1089/scd.2006.15.141]
66 **Offen D**, Barhum Y, Levy YS, Burshtein A, Panet H, Cherlow T, Melamed E. Intrastriatal transplantation of mouse bone marrow-derived stem cells improves motor behavior in a mouse model of Parkinson's disease. *J Neural Transm Suppl* 2007; **72**: 133-143 [PMID: 17982886 DOI: 10.1007/978-3-211-73574-9_16]
67 **Jin GZ**, Cho SJ, Choi EG, Lee YS, Yu XF, Choi KS, Yee ST, Jeon JT, Kim MO, Kong IK. Rat mesenchymal stem cells increase tyrosine hydroxylase expression and dopamine content in ventral mesencephalic cells in vitro. *Cell Biol Int* 2008; **32**: 1433-1438 [PMID: 18778785 DOI: 10.1016/j.cellbi.2008.08.014]
68 **Trzaska KA**, King CC, Li KY, Kuzhikandathil EV, Nowycky MC, Ye JH, Rameshwar P. Brain-derived neurotrophic factor facilitates maturation of mesenchymal stem cell-derived dopamine progenitors to functional neurons. *J Neurochem* 2009; **110**: 1058-1069 [PMID: 19493166 DOI: 10.1111/j.1471-4159.2009.06201.x]
69 **Höglinger GU**, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat Neurosci* 2004; **7**: 726-735 [PMID: 15195095 DOI: 10.1038/nn1265]
70 **Abdipranoto A**, Wu S, Stayte S, Vissel B. The role of neurogenesis in neurodegenerative diseases and its implications for therapeutic development. *CNS Neurol Disord Drug Targets* 2008; **7**: 187-210 [PMID: 18537646 DOI: 10.2174/187152708784083858]
71 **Crews L**, Mizuno H, Desplats P, Rockenstein E, Adame A, Patrick C, Winner B, Winkler J, Masliah E. Alpha-synuclein alters Notch-1 expression and neurogenesis in mouse embryonic stem cells and in the hippocampus of transgenic mice. *J Neurosci* 2008; **28**: 4250-4260 [PMID: 18417705 DOI: 10.1523/JNEUROSCI.0066-08.2008]
72 **Ye M**, Wang XJ, Zhang YH, Lu GQ, Liang L, Xu JY. Therapeutic

effects of differentiated bone marrow stromal cell transplantation on rat models of Parkinson's disease. *Parkinsonism Relat Disord* 2007; **13**: 44-49 [PMID: 17005432 DOI: 10.1016/j.parkreldis.2006.07.013]

73 **Zhang QG**, Wang R, Khan M, Mahesh V, Brann DW. Role of Dickkopf-1, an antagonist of the Wnt/beta-catenin signaling pathway, in estrogen-induced neuroprotection and attenuation of tau phosphorylation. *J Neurosci* 2008; **28**: 8430-8441 [PMID: 18716201 DOI: 10.1523/JNEUROSCI.2752-08.2008]

74 **Jiang Y**, de Bruin A, Caldas H, Fangusaro J, Hayes J, Conway EM, Robinson ML, Altura RA. Essential role for survivin in early brain development. *J Neurosci* 2005; **25**: 6962-6970 [PMID: 16049172 DOI: 10.1523/JNEUROSCI.1446-05.2005]

75 **Zhang L**, Yan R, Zhang Q, Wang H, Kang X, Li J, Yang S, Zhang J, Liu Z, Yang X. Survivin, a key component of the Wnt/β-catenin signaling pathway, contributes to traumatic brain injury-induced adult neurogenesis in the mouse dentate gyrus. *Int J Mol Med* 2013; **32**: 867-875 [PMID: 23900556 DOI: 10.3892/ijmm.2013.1456]

76 **Wu F**, Wang Z, Gu JH, Ge JB, Liang ZQ, Qin ZH. p38(MAPK)/p53-Mediated Bax induction contributes to neurons degeneration in rotenone-induced cellular and rat models of Parkinson's disease. *Neurochem Int* 2013; **63**: 133-140 [PMID: 23714208 DOI: 10.1016/j.neuint.2013.05.006]

77 **Momand J**, Wu HH, Dasgupta G. MDM2--master regulator of the p53 tumor suppressor protein. *Gene* 2000; **242**: 15-29 [PMID: 10721693 DOI: 10.1016/S0378-1119(99)00487-4]

78 **Jiang Y**, Saavedra HI, Holloway MP, Leone G, Altura RA. Aberrant regulation of survivin by the RB/E2F family of proteins. *J Biol Chem* 2004; **279**: 40511-40520 [PMID: 15271987 DOI: 10.1074/jbc.M404496200]

79 **Okazaki T**, Magaki T, Takeda M, Kajiwara Y, Hanaya R, Sugiyama K, Arita K, Nishimura M, Kato Y, Kurisu K. Intravenous administration of bone marrow stromal cells increases survivin and Bcl-2 protein expression and improves sensorimotor function following ischemia in rats. *Neurosci Lett* 2008; **430**: 109-114 [PMID: 18053646 DOI: 10.1016/j.neulet.2007.10.046]

80 **Kim HJ**, Lee JH, Kim SH. Therapeutic effects of human mesenchymal stem cells on traumatic brain injury in rats: secretion of neurotrophic factors and inhibition of apoptosis. *J Neurotrauma* 2010; **27**: 131-138 [PMID: 19508155 DOI: 10.1089/neu.2008-0818]

81 **Liu L**, Cao JX, Sun B, Li HL, Xia Y, Wu Z, Tang CL, Hu J. Mesenchymal stem cells inhibition of chronic ethanol-induced oxidative damage via upregulation of phosphatidylinositol-3-kinase/Akt and modulation of extracellular signal-regulated kinase 1/2 activation in PC12 cells and neurons. *Neuroscience* 2010; **167**: 1115-1124 [PMID: 20153405 DOI: 10.1016/j.neuroscience.2010.01.057]

82 **Sai Y**, Chen J, Wu Q, Liu H, Zhao J, Dong Z. Phosphorylated-ERK 1/2 and neuronal degeneration induced by rotenone in the hippocampus neurons. *Environ Toxicol Pharmacol* 2009; **27**: 366-372 [PMID: 21783966 DOI: 10.1016/j.etap.2008.12.004]

83 **Jenner P**. Parkinson's disease, pesticides and mitochondrial dysfunction. *Trends Neurosci* 2001; **24**: 245-247 [PMID: 11311359 DOI: 10.1016/S0166-2236(00)01789-6]

84 **Dezawa M**, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, Mimura T, Kitada M, Suzuki Y, Ide C. Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. *J Clin Invest* 2004; **113**: 1701-1710 [PMID: 15199405 DOI: 10.1172/JCI200420935]

P- Reviewer: Gharaee-Kermani M, Saeki K **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com