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Research Article

Selegiline induced differentiation of rat bone marrow mesenchymal stem cells to dopaminergic neurons *in vitro*

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Abstract

Today, the use of mesenchymal stem cells (MSCs) for treating human diseases has attracted wide attention. The aim of this study is the expression of dopaminergic genes such as Nestin, patched Tumor Suppressor (PTCH), Sonic Hedgehog (SHH), Tyrosine Hydroxylase (TH) and Nuclear receptor-related factor 1 (NURR1) in MSCs after induction with selegiline. Rat bone marrow mesenchymal stem cells (rBMSCs) were extracted from femur and tibia bones and incubated with alpha Minimum Essential Medium (α-MEM) and 10% Fetal bovine serum (FBS). The stemness of cells at passage 4 was determined by the positive response to CD71 and CD90 markers and their differentiation into adipocytes and osteoblasts. The expression of SHH, PTCH, TH, NURR1 and Nestin genes in the cells after induction by 10-8 M selegiline for 48 hours was investigated by Reverse transcription polymerase chain reaction (RT-PCR) and Real Time-PCR methods. Isolated rBMSCs expressed CD71 and CD90 markers in culture conditions and could differentiate into adipocytes and osteoblasts. Induced cells showed neuronal morphology, positive response to Nestin and TH immunostaining. There was a significant increase of dopaminergic genes TH and NURR1 compared to the untreated cells. The results showed that selegiline with a dose of 10-8 M for 48 hours can lead to dopaminergic differentiation in rBMSCs.

Keywords

Selegiline, dopaminergic genes, *in vitro* culture

Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic cells discovered about 40 years ago from bone marrow by Friedenstein (Charbord et al. 2010). Many studies have shown that stem cells are in different tissues and successfully harvested from other organs such as the brain, liver, kidney, muscle, thymus, pancreas, skin, adipose tissue, bone marrow, lymph nodes, spleen, thymus, and umbilical cord. Also, MSCs are known as multipotent cells, which can differentiate into adipocytes, myocytes, osteocytes, and chondrocytes. Studies indicate that MSCs can differentiate into non-mesodermal cells such as intestinal epithelial cells, skin, hepatocytes, pneumocytes, and neurons (Lim et al. 2021). In 2006, the International Society of Cell Therapy proposed at least three characteristics for the human MSCs: 1- positive expression of CD105, CD90, and CD73 markers. 2- the negative expression of CD45,

CD34, CD14, CD11b, CD79α, CD19 markers, and HLA-DR surface molecules, and 3- the ability to adhere to the cell culture dish and to differentiate into osteocytes, chondrocytes, and adipocytes (Dominici et al. 2006). The stem cells can be divided into three main groups: embryonic, germline, and somatic (Hong et al. 2012). These pluripotent cells originate from the embryo's inner cell mass or blastocysts and have an unlimited life span related to telomerase activity. However, using ESCs in scientific studies and clinical trials is limited due to ethical considerations (Warmflash et al. 2012). Induced pluripotent stem cells (iPSCs) can be replaced with ESCs. iPSCs from a patient's somatic cells could be valuable for drug discovery and cell transplantation therapies. The differentiation ability of iPSCs is similar to ESCs. Still, these cells do not have the ethical or immunogenic limitations of ESCs, so for this reason, these cells may be used more clinically than ESCs (Worku 2021). Germinal stem cells originate from the embryo's primary germ layers; these cells can differentiate into progenitor cells to produce specific cells of the body tissues. Somatic or adult stem cells (SSCs) are precursor cells with less differentiation ability than embryonic stem cells. SSCs are in mature tissues such as hematopoietic, nervous, digestive, and mesenchymal tissues. The most common adult stem cells include: bone marrow stem cells, hematopoietic stem cells, and MSCs. BMSCs can differentiate into mesodermal cells such as cartilage, bone, fat, muscle, connective tissue, and tendons *in vitro*. In addition to the capacity for self-renewal and differentiation potential, these cells can suppress the immune system. Also, these cells have anti-tumor and migration properties. Stem cells express growth factors and cytokines that regulate an innate immune response. Stem cells can secrete factors such as MCP-1/CCL2 and physically interact with tumor cells. The evidence indicates that the types of chemokine and growth factor receptors expressed by MSCs may participate in the implantation of tumors. CXCR4/SDF1 factor plays a prominent role in migrating different stem cells (Hong et al. 2012; Prentice 2019).

Stem cells are used as autograft transplantation in neurological diseases such as stroke, spinal cord injuries, Parkinson's, Alzheimer's, Huntington's, and Autism. Differentiation of MSCs into neural-like cells has been reported both *in vitro* and in vivo after migration to the brain and spinal cord (Kousha et al. 2022; Pradhan et al. 2022). Recent studies indicate that MSCs can differentiate into different types of nerve cells after treatment with growth factors and chemical inducers (Iglesias-García et al. 2013). So, under proper conditions, these cells can differentiate into particular neurons, including dopaminergic neurons, and can be used to treat Parkinson's disease.

Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by a progressive and extensive loss of neurons in the substantia nigra pars compacta (SNpc) and their terminals in the striatum, which results in debilitating movement disorders. This devastating disease affects over 1 million individuals in the United States and is increasing in incidence worldwide. Available pharmacological and surgical therapies ameliorate clinical symptoms in the early stages of the disease, but they cannot stop or reverse the degeneration of dopaminergic neurons. Stem cell therapies have come to the forefront of the PD research field as promising regenerative therapies (Obeso et al. 2010; Parmar et al. 2020).

Selegiline is known to increase the survival of cultured nigral DAergic neurons, protecting them from oxidative stress. It has been reported that selegiline can protect hippocampal neurons from excitotoxic damage, most likely by induction of NGF protein (Semkova et al. 1996). The trophic effects of selegiline may play a significant role in treating neurodegenerative diseases (Vezina et al. 1992).

Chemical induction is simple and cost-effective compared to other methods. Selegiline is a selective, irreversible monoamine oxidase B (MAO-B) inhibitor at the conventional dose (10-8 M). MAO-B is an enzyme in the body that breaks down several chemicals in the brain, including dopamine, and slows the progression of PD (Ghorbanian et al. 2010; Haji Ghasem Kashani et al. 2013).

There have been reports that selegiline induced the differentiation of BMSCs into dopaminergic neurons (Beiki et al. 2022). Some researchers believe that chemical inducers have a toxic effect on cells and cause 50% of cell apoptosis after treatment for 24 hours. These cells expressed neuronal markers in the early hours after induction, but this phenomenon is reversible (Hokari et al. 2008). Many studies show that ascorbate, selegiline, vitamin E, and DMSO as essential antioxidants that protect against neurotoxic damage of dopaminergic neurons in the substantia nigra. Selegiline has been shown to prevent dopamine neuron death in Parkinson's disease (Hao et al. 1995; Kontkanen et al. 1999). This drug can improve the symptoms of Parkinson's disease by several mechanisms, which include: 1- it has trophic effects on dopaminergic neurons (Koutsiliere et al. 1994). Selegiline increased neuronal survival and the average neurite length and created branching in neurons and synapses (trophic effects) between neurons of dopaminergic neurons in rats treated with MPP+ (1-methyl-4-phenylpyridinium). 2- Selegiline, as a MAO-B inhibitor, prevents the production of peroxides resulting from dopamine oxidation and prevents disease progression. So, a neuroprotective role for this drug is considered (Mytilineou et al. 1997). 3- Selegiline reduces the apoptosis of neurons or stops it (Tatton et al. 1997). Selegiline at a concentration of 10-8 M had an anti-apoptotic effect. In addition, its concentrations of 10-7 to 10-9 M reduce oxidative damage of nerve cells in the culture condition (Suuronen et al. 2000). This drug can slow down the progress, weakness, and disability of the disease (Mytilineou et al. 1997). Selegiline reduces the neurodegeneration of PD by astrogliosis. Therefore, by releasing astroglial-derived neurotrophic factors, astrocytes cause the survival and protection of damaged neurons in PD (Hao et al. 1995). This study aimed to investigate the quantitative expression of dopaminergic genes in the cells treated by selegiline.

Materials and methods

Isolation and culture of rat bone marrow mesenchymal stem cells (rBMSCs)

In this experimental study, 15 adult male Wistar rats (weighing about 200–250 g) were purchased from the Pasteur Institute of Iran and housed at 20–24 °C under a 12-h light/dark cycle with free access to water and food for one week. The animals were sacrificed by chloroform, and the femur and tibia bones were separated. Under the hood, the epiphyses were cut, and bone marrow was flushed with alpha Minimum Essential Medium (α-MEM), 10% fetal bovine serum (FBS), and 1% penicillin/ streptomycin into the culture flask. After 72 hours of incubation, the cell culture medium was replaced with a fresh medium. The Cells were passaged at 70%–80% confluency (Locke et al. 2009). All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 23-80, revised 1996) and were authorized by the Islamic Azad University of Damghan's research ethical standards (IR.IAU.DAMGHAN.REC.1400.028).

Differentiation potential of rBMSCs

rBMSCs (passage 3) were cultured in 12-well plates and treated with adipogenic medium (StemPro Adipogenesis Differentiation Kit, A10070-01, Invitrogen) or osteogenic medium (StemPro Osteogenesis Differentiation Kit, A10072-01, Invitrogen). The medium was changed every three days. After 21 days, the cells were fixed with 4% formaldehyde for 1 hour at 4 °C, washed with 70% alcohol, and stained with oil-red for 10–15 min or alizarin red for 2 min. The cells were washed with PBS and observed by an inverted microscope (E600-Eclipse Nikon) equipped with a digital camera (DXM 1200 Camera Nikon Digital) (Locke et al. 2005).

Experimental groups

The cells at passage four were cultured in α-MEM containing 10% FBS as control. The selegiline group was induced cells with 10 -8 M selegiline for 24 h and transferred to serum-free medium for 48 h (Suuronen et al. 2000).

Identification of neural cells

In this study, the expression of neural markers such as βIII-tubulin, nestin, and Tyrosine Hydroxylase (TH) was investigated by immunocytochemistry. The term Sonic Hedgehog (SHH), patched Tumor Suppressor (PTCH), TH, NESTIN, and Nuclear receptor-related factor 1 (NURR1) genes was analyzed by RT-PCR. In addition, Real-time PCR was used to detect the expression levels of TH and NURR1 genes.

Immunocytochemistry

The differentiated cells were cultured on sterile coverslips coated with gelatin and fixed with 4% paraformaldehyde for half an hour at room temperature. After washing with PBS for 5 minutes, the samples were incubated with blocking serum. The samples were incubated overnight at 4 °C with monoclonal antibody to βIII-tubulin and HRP secondary antibody for 2 h. After incubation with DAB for 20 minutes, it was examined with a light microscope (Prentice 2019).

Gene expression detection methods

Extraction of total RNA

Total RNA was extracted from experimental groups using RNX-Plus (Sinaclon, Iran). The quality of the RNA was confirmed by agarose gel electrophoresis.

Reverse transcription polymerase chain reaction (RT-PCR)

cDNA synthesis was carried out using 0.5 μg of total RNA according to the manufacturer's protocol (k1622; Fermentase). Expressions of SHH, PTCH, Nestin, TH and NURR1 genes were measured by semiquantitative RT-PCR using a master cycler (Eppendorf, Germany). The PCR mix consisted of 5 μg of synthesized cDNA, 1× PCR buffer, 50 mM MgCl2, 10 mM dNTPs, 10 pmol forward and reverse primers, and 0.25 μl Taq DNA polymerase in a final volume of 25 μl. The PCR protocol comprised 2 min at 94 °C; 34 cycles of 30 sec at 94 °C, 30 sec at 55 °C, and 30 sec at 72 °C; and 5 min of a terminal extension at 72 °C. The primer sequences and product sizes were shown in Table 1. β2 microglobulin

Table 1. The primers used for RT-PCR.

Gene	Product size (bp)	Primer sequence	Accession number
SHH	173 bp	F: 5'-TTA AAT GCC TTG GCC ATC TC-3' R: 5'-CGA GCC AGC ATG CCA TAC TT-3'	NM_012604
PTCH	269 bp	F: 5'-CCT CCT TTA CGG TGG ACA AA-3' R:5'-ATC AAC TCC TGC CCA TG-3'	NM_012842
B2M	318 bp	F: 5'-CCG TGA TCT TTC TGG TGC TT-3' R: 5'-TTT TGG GCT TCA GAG TG-3'	NM_012512
NURR1	683 bp	F: 5′-TCC CGG AGG AAC TGC ACT TCG-3′ R: 5′-GTG TCT TCC TCT GCT CGA TCA-3′	U_72345
TH	276 bp	F: 5′-TGT CAC GTC CCC AAG GTT CAT -3′ R: 5′ –CGT GGG ACC AAT GTC TTC AGT G-3′	NM_012740
Nestin	431 bp	F: 5′- CAG- GCT –TCT- CTT- GGC- TTT-CTG′3- R: 5′- TGG- TGA- GGG-TTG -AGG -TTT- 3′	NM_012987

Table 2. The primers used for Real time RT-PCR.

Primer	Sequence	Product size
GAPDH-f	GCTGGGGCTCATTTGCAGG	258 bp
GAPDH-r	CGGAGGGGCCATCCACAGT	258 bp
TH-f	TGT CAC GTC CCC AAG GTT CAT	276 bp
TH-r	CGT GGG ACC AAT GTC TTC AGT G	276 bp
NURR1-f	TCC CGG AGG AAC TGC ACT TCG	683 bp
NURR1-r	TGG TGA GGG TTG AGG TTT	683 bp

(β2M) was used as the housekeeping gene (internal control). The PCR products were analyzed by 1.5% agarose gel electrophoresis, then visualized and photographed on a UV transilluminator (UVIdoc, EU). The intensity of gene bands was checked by the Image J software (Freeman et al. 1999).

Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Real-time PCR was done by Rotor-Gene 6000 PCR system, using RealQ Plus Master Mix Green (Amplicon, Denmark). The final volume of the reaction solution was 10μl, and the program was set as denaturation at 95 °C for 15 min, followed by 50 cycles at 95 °C for 15s and annealing/extension for 45s at 60 °C. Primer sequences were designed by AlleleID software version 7.5 (Premierbiosoft, USA), as shown in Table 2. The relative expression of target genes was calculated through the $2^{-\Delta\Delta Ct}$ formula.

Statistical analysis

Data analysis was performed using SPSS software version 16. After ensuring the normal distribution of data, the Kolmogorov test and the Independent-sample T-Test investigated a significant difference between the groups. $P<0.05$ was considered a significant level.

Results

rBMSCs morphology in culture condition

Colony formation and expansion during the second subculture were maximal when the cells were cultured in α-MEM containing 10% FBS (Fig. 1A, B). Cells with small rounded, fibroblast-like, and large flattened morphologies were observed in the 4th passage (Fig. 1C). Gradually, the fibroblast-like cells increased the heterogeneous cells decreased at high passage. The fibroblast-like cells (MSC) gradually increased, and the heterogeneous cells decreased in the culture.

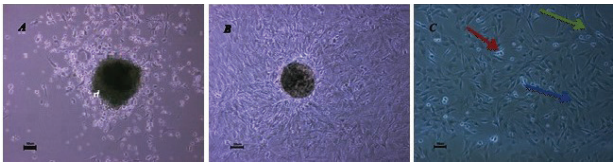

Figure 1. Phase-contrast images of rBMSCs at different passages. (**A**) The cultured cells formed colonies after the first subculture (200×); (**B**) The cells at passage 3 (200×); (**C**) Different morphologies of rBMSCs at passage 4: Round and proliferating cells (Red arrow), Fibroblast cells (Blue arrow), and Flat cells (Green arrow) (200×).

Rat BMSCs demonstrated a strong capacity for differentiation into adipogenic and osteogenic lineages. Control cells at passage 4 were shown in Fig. 2A, and Alizarin Red stained the cells of the control, and only the deposition of colored substances was observed in Fig. 2B. Osteogenic differentiation was confirmed by the production of calcium phosphate and mineralized extracellular matrix in

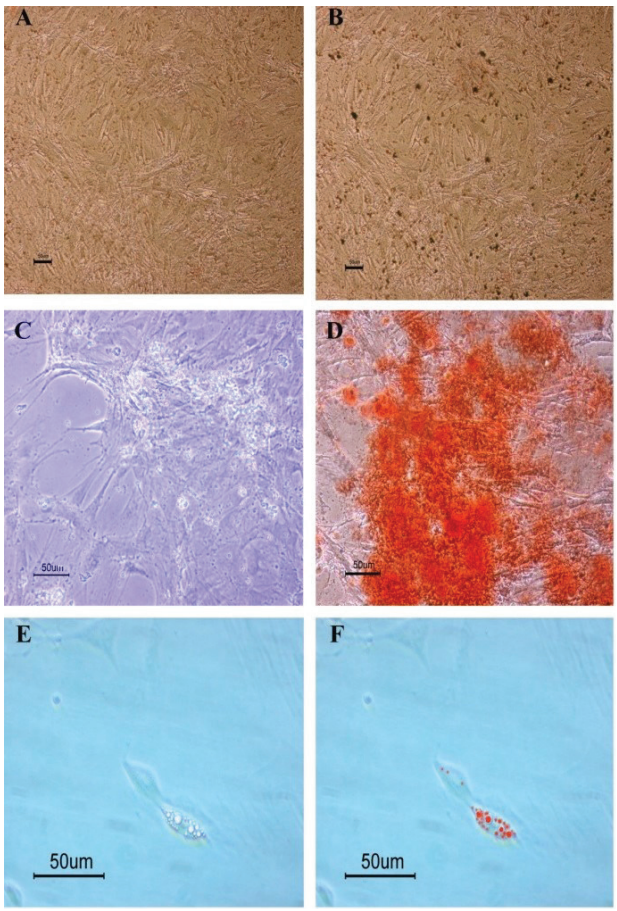

Figure 2. Rat BMSCs differentiation into osteoblasts or adipocytes. (**A**) Control (200×); (**B**) Alizarin Red stained the cells of the control group after 21 days (200×); (**C**) Differentiation of MSCs into osteoblasts and detection of calcium deposits (mineralization) after 21 days (400×); (**D**) Calcium deposits stained with Alizarin red (400×); (**E**) Differentiation of MSCs into adipocytes and production of lipid droplets after 21 days (400×); (**F**) Lipid droplets stained with oil red (400×).

induced cells stained with Alizarin Red (Fig. 2C, D). Adipogenic differentiation was confirmed by the appearance of small lipid vesicles formed after treatment and stained with oil red (Fig. 2E, F).

Selegiline-induced cells morphology

Within 4 hours after selegiline induction, the cells showed a triangular appearance with long processes. The cells showed a neuron-like morphology, at 24 h after induction (Fig. 3).

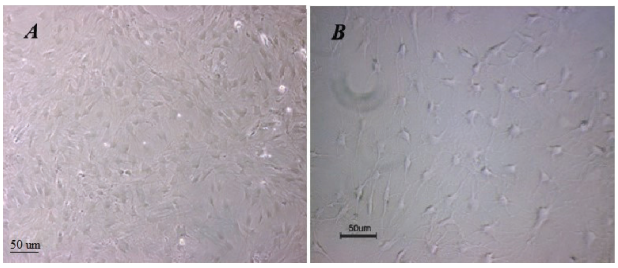

Figure 3. Induction of rBMSCs by selegiline. (**A**) The cells with long processes, at 4 h post-induction (200×); (**B**) rBMSCs morphological changes into neuron-like cells after 24 h of induction (200×).

Identification of induced neuron-like cells by nestin, TH and βIII-tubulin immunocytochemistry

About 70% of cells in the control group and more than 90% of 10 -8 selegiline-treated cells were nestin-positive (Fig. 4). About 45% and 62% of selegiline-treated cells expressed TH and β III-tubulin, respectively (Figs 5, 6).

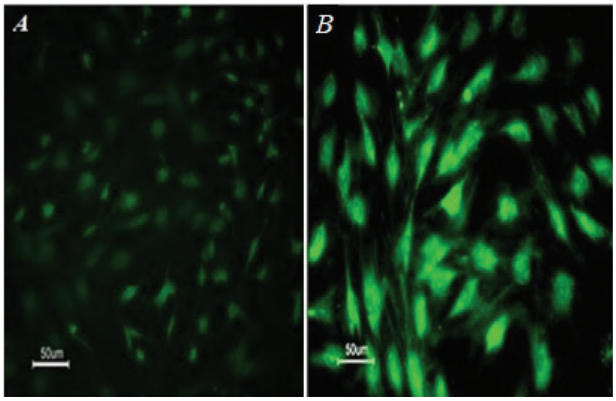

Figure 4. Immunocytochemical expression of Nestin. (**A**) Control: less than 70% of the cells were positive (400×); (**B**) Selegiline-treated group: more than 90% of the cells were positive (400×).

Analysis of genes expression

PCR results

There was a significant increase of NURR1 and TH genes expression in selegiline-induced cells compared to the control. There was no significant difference between experimental groups in SHH, PTCH, and Nestin expression (Figs 7, 8).

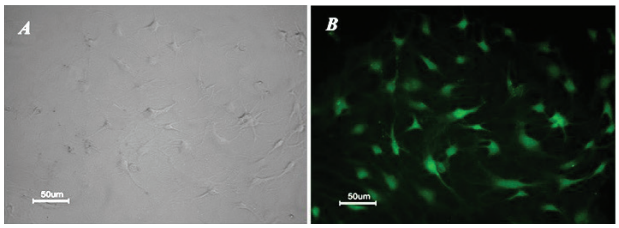

Figure 5. Immunocytochemical expression of TH. (**A**) Phase-contrast image of selegiline-treated cells (200×); (**B**) fluorescence microscopy image of TH positive cells (200×).

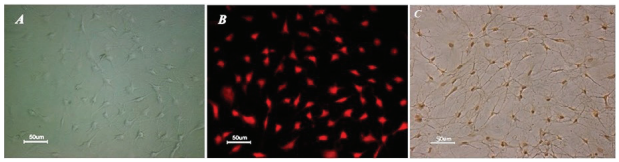

Figure 6. Immunocytochemical expression of βIII-tubulin. (**A**) Phase-contrast image of selegiline-treated cells (200×); (**B**) fluorescence microscopy image of βIII-tubulin positive cells (200×); (**C**) HRP image of βIII-tubulin positive cells (200×).

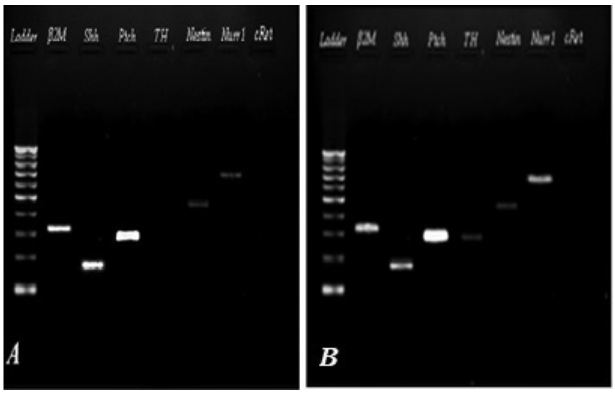

Figure 7. Agarose gel images of PCR product of dopaminergic genes in experimental groups. (**A**) Control; (**B**) Selegiline-induced cells.

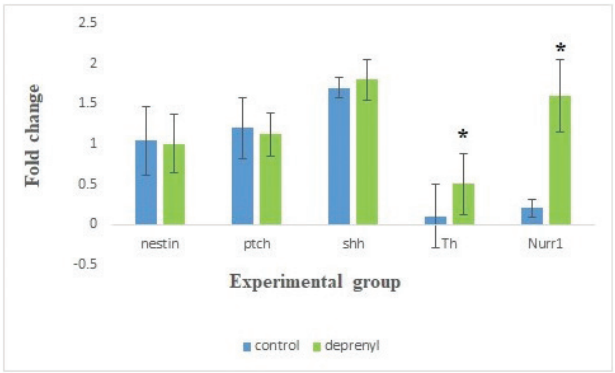

Figure 8. Comparison of SHH, PTCH, TH, NESTIN, and NURR1 gene expression levels in experimental groups. Data are represented as mean ± SD, n = 3. * Significant difference versus control.

Real Time RT-PCR results

The expression of the TH and NURR1 genes in the cells treated with selegiline was significantly increased compared to the control (Fig. 9).

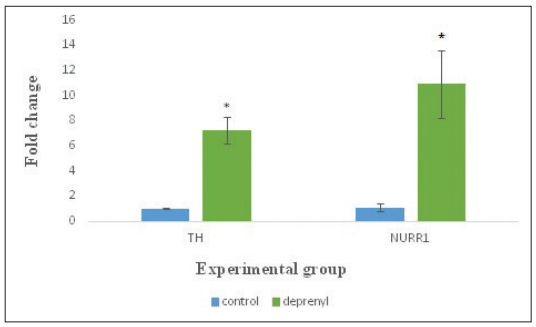

Figure 9. Comparison of the expression level of TH and NURR1 genes in experimental. Data are represented as mean ± SD, n = 3. * Significant difference versus control.

Discussion

Many reports indicate that MSCs, due to their ability to differentiate into dopaminergic neurons, are a great therapeutic cell source to treat Parkinson's disease. Chen et al. have reported that MSCs can proliferate and differentiate into neurons and maintain survival in the endogenous neurogenic niche of the Alzheimer's disease model. They also observed that transplantation of MSCs in rodents' Alzheimer's models led to neurogenesis and improved spatial learning (Andrzejewska et al. 2021; Chen et al. 2021). Liu et al. reported that MSCs can stimulate astrocyte proliferation and maintain neurons' survival (Liu 2022). This study aimed to investigate the effect of selegiline on the neural differentiation of rBMSCs. MSCs derived from bone marrow are a heterogeneous population of cells that expand quickly *in vitro*. These cells expressed CD71 and CD90 (Friedenstein et al. 1970). There are also reported that rBMSCs differentiate into non-mesodermal cells, including neurons, under appropriate conditions (Xiang et al. 2008). In this study, a homogenous population of cells was obtained after the third passage. The cells in passage 3 were spindle-shaped but became flat upon cell aging. Positive response (more than 95%) to CD71 and CD90 markers showed stemness.

In addition, a positive response to nestin antigen confirmed the neural progenitor cells of selegiline- induced group. But its expression level differed in experimental groups, so it was expressed about 70% in the control group and more than 90% in the selegiline-treated group. The cells of the selegiline-treated group responded positively to TH and βIII -tubulin by immunocytochemical staining. The analysis of RT-PCR data showed no significant difference in dopaminergic genes such as SHH, PTCH, and nestin between the two experimental groups. Still, a significant increase of TH and NURR1 genes in the selegiline- treated cells was observed compared to the control. In addition, the Real Time-PCR data confirmed the mentioned results. Some researchers believed that the expression of neural factors occurs during the first hours after induction, and this feature was reversibly lost (Hokari et al. 2008). However, no such result was observed in this study. A dose 10-8 M of selegiline has recently been shown to exert anti-apoptotic and neuroprotective effects. Produced dopaminergic neurons expressing TH and NURR1 genes. The selegiline-induced cells showed a significant increase in these genes, as compared to the control. These results are under Barzilay and colleagues, who generated dopaminergic neurons from MSCs by 12 different induction protocols. They showed that a two-step protocol can produce dopaminergic neurons from bone marrow MSCs. In the first stage, the cells were cultured in the serum-free medium containing EGF and FGF-2 for 48 to 72 hours, then transferred to the induction medium containing cAMP, IBMX, and rich in various neurotrophic factors for another 48 to 72 hours (Barzilay et al. 2008). The results showed that a basic level of TH is expressed in all induction protocols. A significant increase of TH mRNA was found, using BDNF alone or combined with GDNF, TGF-β3. They showed that using various neurotrophic factors, about 30% of MSCs express a detectable level of TH. Dopamine secretion was also observed in the mentioned study (Ahmed et al. 1995; Herzog et al. 2003). In 2021, Kashani et al. reported that the exposure of hASCs cells to 10-7 M selegiline for 24 hours led to the induction of neural-like cells, as well as a significant increase in the expression of NTF3 and BDNF genes compared to cells cultured in serum-free medium. This treatment did not affect the expression of NGF and GDNF genes (Amiri et al. 2021).

Conclusion

This research showed that selegiline-induced cells expressed TH antigen and relatively some neurotrophic factors that play a role in different stages of survival and differentiation of dopaminergic neurons. Selegiline at a dose of 10-8 M causes the expression of specific genes of dopaminergic neurons, such as TH and NURR1.

References

Aggarwal PM (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105(4): 1815–1822. https://doi.org/10.1182/blood-2004-04-1559

Ahmed SH, Reynolds BA, Weiss S (1995) BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronel precursors. The Journal of Neuroscience 15(8): 5765–5778. https://doi.org/10.1523/JNEUROSCI.15-08-05765.1995

Amiri A, HG Kashani M, Ghorbanian MT (2021) Expression of neurotrophic factor genes by human adipose stem cells post-induction by deprenyl. Anatomy & Cell Biology 54(1): 74–82. https://doi.org/10.5115/acb.19.229

Andrzejewska A, Dabrowska S, Lukomska B, Janowski M (2021) Mesenchymal stem cells for neurological disorders. Advanced Science 8(7): 2002944. https://doi.org/10.1002/advs.202002944

Barzilay R, Kan I, Ben-Zur T, Bulvik S, Melamed E, Offen D (2008) Induction of human mesenchymal stem cells into dopamine-producing cells with different differentiation protocols. Stem Cells and Development 17(3): 547–554. https://doi.org/10.1089/scd.2007.0172

Beiki R, Khaghani M, Esmaeili F, Dehghanian F (2022) Synergistic effects of combined Nurr1 overexpression and natural inducers on the more efficient production of dopaminergic neuron-like cells from stem cells. Frontiers in Cellular Neuroscience 15: 803272. https://doi.org/10.3389/fncel.2021.803272

Charbord P (2010) Bone marrow mesenchymal stem cells: historical overview and concepts. Human gene therapy 21(9): 1045–1056. https://doi.org/10.1089/hum.2010.115

Chen YA, Lu CH, Ke CC, Chiu S-J, Jeng F-S, Chang C-W, Yang B-H, Liu R-S (2021) Mesenchymal stem cell-derived exosomes ameliorate Alzheimer's disease pathology and improve cognitive deficits. Biomedicines 9(6): 594. https://doi.org/10.3390/biomedicines9060594

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4): 315–317. https://doi.org/10.1080/14653240600855905

Freeman WM, Walker SJ, Vrana KE (1999) Quantitative RT-PCR: pitfalls and potential. BioTechniques 26(1): 112–122. https://doi.org/10.2144/99261rv01

Friedenstein CR, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell and Tissue Kinetics 3(4): 393–403. https://doi.org/10.1111/j.1365-2184.1970.tb00347.x

Ghorbanian MT, Mesbah-Namin SA, Fathollahi Y (2010) Selegiline is an efficient and potent inducer for bone marrow stromal cell differentiation into neuronal phenotype. Neurological Research 32(2): 185–193. https://doi.org/10.1179/174313209X409016

Haji Ghasem Kashani M, Ghorbanian MT, Hosseinpour L (2013) Transplantation of deprenyl-induced tyrosine hydroxylase-positive cells improves 6-OHDA-lesion rat model of parkinson's disease: behavioral and immunohistochemical evaluation. Cell Journal 15(1): 55–64. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3660025/pdf/Cell-J-15-55.pdf

Hao R, Ebadi M, Pfeiffer R (1995) Selegiline protects dopaminergic neurons in culture from toxic factor(s) present in the cerebrospinal fluid of patients with Parkinson's disease. Neuroscience Letters 200: 77–80. https://doi.org/10.1016/0304-3940(95)12113-I

Herzog EL, Chai L, Krause DS (2003) Plasticity of marrow-derived stem cells. Blood 102(10): 3483–3493. https://doi.org/10.1182/blood-2003-05-1664

Hokari KS, Shichinohe H, Yano S, Hida K, Iwasaki Y (2008) Bone marrow stromal cells protect and repair damaged neurons through multiple mechanisms. Journal of Neuroscience Research 86(5): 1024–1035. https://doi.org/10.1002/jnr.21572

Hong HS, Kim YH, Son Y (2012) Perspectives on mesenchymal stem cells: tissue repair, immune modulation, and tumor homing. Archives of Pharmacal Research 35(2): 201–211. https://doi.org/10.1007/s12272-012-0201-0

Iglesias-García O, Pelacho B, Prósper F (2013) Induced pluripotent stem cells as a new strategy for cardiac regeneration and disease modeling. Journal of Molecular and Cellular Cardiology 62: 43–50. https://doi.org/10.1016/j.yjmcc.2013.04.022

Kontkanen O, Castren E (1999) Trophic effects of selegiline on cultured brain dopaminergic neurons. Brain Research 829(1–2): 190–192. https://doi.org/10.1016/S0006-8993(99)01363-3

Kousha A, Haji Ghasem Kashani M, Vaezi GH, Hojati V (2022) Expression of neurotrophins in adipose-derived stem cells during *in vitro* culture and posttransplantation in parkinsonian rat model. Iranian South Medical Journal 25(3): 227–239. https://doi.org/10.52547/ismj.25.3.227

Koutsiliere E, O'Callaghan JF, Chen TS, Riederer P, Rausch W-D (1994) Selegiline enhances survival and neurite outgrowth of MPP-treated dopaminergic neuron. European Journal of Pharmacology 269: R3–4. https://doi.org/10.1016/0922-4106(94)90048-5

Lim SK, Khoo BY (2021) An overview of mesenchymal stem cells and their potential therapeutic benefits in cancer therapy. Oncology Letters 22(5): 785. https://doi.org/10.3892/ol.2021.13046

Liu C (2022) The Role of Mesenchymal Stem Cells in Regulating Astrocytes-Related Synapse Dysfunction in Early Alzheimer's Disease. Frontiers in Neuroscience 16: 927256. https://doi.org/10.3389/fnins.2022.927256

Locke M, Windsor J, Dunbar PR (2009) Human adipose-derived stem cells: isolation, characterization and applications in surgery. ANZ Journal of Surgery 79(4): 235–244. https://doi.org/10.1111/j.1445-2197.2009.04852.x

Mytilineou RP, Leonardi E (1997) L-Deprenyl protects mesencephalic dopamine neurons from glutamate receptor-mediated toxicity *in vitro*. Journal of Neurochemistry 68(1): 33–39. https://doi.org/10.1046/j.1471-4159.1997.68010033.x

Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AHV, Halliday G (2010) Missing pieces in the Parkinson's disease puzzle. Nature Medicine 16 (5): 653–661. https://doi.org/10.1038/nm.2165

Parmar M, Grealish S, Henchcliffe C (2020) The future of stem cell therapies for parkinson disease. Nature Reviews Neuroscience 21(2): 103–115. https://doi.org/10.1038/s41583-019-0257-7

Pradhan AU, Uwishema O, Onyeaka H, Adanur I, Dost B (2022) A review of stem cell therapy: An emerging treatment for dementia in Alzheimer's and Parkinson's disease. Brain and Behavior 12(9): e2740. https://doi.org/10.1002/brb3.2740

Prentice DA (2019) Adult Stem cells. Circulation Research 124(6): 837–839. https://doi.org/10.1161/CIRCRESAHA.118.313664

Semkova L, Wolz P, Schilling M, Krieglstein J (1996) Selegiline enhances NGF synthesis and protects central nervous system neurons from excitotoxic and ischemic damage. European Journal of Pharmacology 315(1): 19–30. https://doi.org/10.1016/S0014-2999(96)00593-6

Suuronen T, Kolehmainen P, Salminen A (2000) Protective effect of L-deprenyl against apoptosis induced by okadaic acid in cultured neuronal cells. Biochemical Pharmacology 59(12): 1589–1595. https://doi.org/10.1016/S0006-2952(00)00282-3

Tatton WG, Chalmers-Redman RM, Ju WY, Wadia J, Tatton NA (1997) Aptosis in neurodegenerative disorders: potential for therapy by modifying gene transcription. Journal of Neural Transmission 49: 245–268. https://doi.org/10.1007/978-3-7091-6844-8_25

Vezina P, Mohr E, Grimes D (1992) Deprenyl in Parkinson's disease: mechanisms, neuroprotective effect, indications and adverse effects. Canadian Journal of Neurological Sciences 19(1): 142–146. https://doi.org/10.1017/S0317167100041524

Warmflash A, Arduini BL, Brivanlou AH (2012) The molecular circuitry underlying pluripotency in embryonic stem cells. Wiley. Interdiscip. WIREs Systems Biology and Medicine 4(5): 443–456. https://doi.org/10.1002/wsbm.1182

Worku MG (2021) Pluripotent and multipotent stem cells and current therapeutic applications. Stem Cells and Cloning 14(7): 3–7. https://doi.org/10.2147/SCCAA.S304887

Xiang LX, Chen Y, Dong XJ (2008) Mesenchymal stem cells: A promising candidate in regenerative medicine. The International Journal of Biochemistry & Cell Biology 40(5): 815–820. https://doi.org/10.1016/j.biocel.2008.01.007