Therapeutic effects of melatonin-treated bone marrow mesenchymal stem cells (BMSC) in a rat model of Alzheimer's disease

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Melatonin

A B S T R A C T

The therapy based on mesenchymal stem cells (MSCs) has received growing attraction for Alzheimer's disease (AD). However, a great challenge in this regard is the low survival rate of MSCs following transplantation. This study seeks to improve the therapy based on Bone Marrow MSCs (BM-MSCs) through melatonin (MT) pre-treatment, which is 'a known antioxidant' in an animal model of AD. In this paper, we separated BMSCs from the rat tibia and femur bones and then pretreated cells were with 5μM of MT for 24 h. The sample consisted of 40 male Wistar rats randomly assigned to the control, sham, MT-pretreated BMSCs and amyloid-beta (Aβ) peptide BMSCs groups. Two months after the cell transplantation, a number of tests including novel object recognition, Morris water maze, passive avoidance test, and open field test were undertaken. 69 days after the cell therapy, the rats were sacrificed. We removed brain tissues histopathological analysis and carried out immunohistochemistry for Beta tubulin, GFAP and iba1 proteins. The results suggested that both MT-BMSCs and BMSCs moved to brain tissues following the intravenous transplantation. However, MT-BMSCs had a significant effect on boosting learning, cognition and memory in comparison with BMSCs (P < 0.05). Furthermore, there was a significant rise in GFAP and Beta tubulin and substantial fall in microglial cells in the BMSCs in comparison with MT-BMSCs. Stem cell therapy has been proposed as an effective strategy for neurodegenerative diseases, but its therapeutic features are restricted. It has been shown that the pretreatment of MSCs with melatonin partly would boost cells efficiency and thereby alleviate AD complications such as memory and cognition.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized with a progressive impairment of cognitive function and the loss of synapsis and neurons involved in learning, memory and behavior (Querfurth and LaFerla, 2010; Shahidi et al., 2017b) (Asadbegi et al., 2017). The pathogenesis of AD has not accurately discovered yet, but there is no doubt that amyloid-β (Aβ) accumulation is a crucial part of this disease (Nell et al., 2017), as it plays a key role in the AD-related neurodegenerative process (Asadbegi et al., 2017). Aβ is a peptide containing 37–43 amino acids, which is produced from amyloid precursor protein (Bozner et al. (1997)). Currently, there is no effective treatment for AD and existing drugs only treat symptoms and slow the progression of cognitive decline (Babaei et al., 2012; Eftekharzadeh et al., 2015; Nasiri et al., 2019).

Nerve growth factor (NGF) is a member of neurotropic factors that support the survival and function of neurons, and promote neurite growth. NGF can boost choline acetyltransferase (ChAT) activity and cholinergic cell survival, reverse spatial memory impairments, and induce the long-term memory (Zhang et al., 2016). Mesenchymal Stem Cells (MSCs) can produce neurotrophic factors and neuroprotective cytokines (Alizadeh et al., 2019a, c; Nasiri et al., 2019). The use of bone marrow stromal cells (BMSC) offers a new strategy for the treatment of neurological diseases (Alizadeh et al., 2013; Zhang et al., 2016). BMSCs have a chemotactic proterty as they can migrate into injured sites and reside there and have capacity to differentiate into local cells of injured sites. They exert therapeutic properties through paracrine effects and secrete chemokines, cytokines, and growth factors that help in...
tissue regeneration. (Fu et al., 2019). Given that there are various microenvironments participating in in vitro cell propagation or injured tissue following injection, BMSCs need to have the capacity to shield themselves against potentially deleterious effects (Der Sarkissian et al., 2017). Thus it is necessary to promote the survival of BMSCs before BMSC-based therapies (Jeong et al., 2017).

Melatonin (N-acetyl-5-methoxytryptamine) is produced from mitochondria of different cells including oocytes, pinealocytes, neurons, endothelial cells (Tan and Reiter, 2019), which is involved in various physiological functions, including antioxidant (Mortezaei et al. (2020); Mortezaei et al., 2016, 2015; Rudintskaia et al., 2015) and radical scavengers that mitigate the harmful effects of free radicals (Acuna Castroviejo et al., 2011; Mortezaei et al., 2016). It has been shown that melatonin enhances MSCs survival and melatonin may be the regulation of precursor cell commitment and differentiation (Rafat et al., 2018a, b). The molecular signals in the extracellular context influence MSC differentiation. As a powerful free radical scavenger, melatonin provides crucial protection mechanisms for MSC against damaged tissues (Luchetti et al., 2010).

Melatonint- pretreatment can enhance the homing of MSCs following transplantation (Mortezaei et al., 2016). In addition, current evidence proved that melatonin preconditioning improves dramatically the proliferative, prosurvival, paracrine secretion of MSCs following transplantation. (Zhao et al., 2020). Preconditioning MSCs with MT has reinforced therapeutic results in animal models of myocardial infarction, cerebral ischemia, renal ischemia, and liver fibrosis (Sabeti et al., 2019). BMSC-transplantation in rats through tail vein injection increase neurogenesis (Yang et al., 2015b). The mesenchymal stem cell transplantation via tail vein alleviated inflammatory response and improved blood brain barrier condition (Chi et al., 2019). There is a paucity of studies on MSCs with melatonin for AD. This paper explores the impact of transplanting bone marrow mesenchymal stem cells (BMSC) with melatonin pretreatment in rat model of AD.

2. Materials and methods

2.1. Isolation of BMSCs

We sacrificed the old male Wistar rats (about 6 weeks old) by diethyl ether. To collect the tibia and femur bones of rats, they were flushed the bone with α- Minimum Essential Medium Eagle (α-MEM) (Sigma, USA) that contained 1% PenStrep (Gibco, Germany) in a sterile condition. We centrifuged the cells at 1000 × g for 5 min and then dispersed them in α-MEM with 10% fetal bovine serum (Sigma, USA) that contained 100 μg/mL streptomycin and 100U/mL penicillin (Golipoor et al., 2010).

2.2. Cell culture and expansion

The isolated cells were plated at a density of 10 × 10^5 cells in T75 tissue culture flasks and incubated at 37 °C and 5% CO2. Cell subculture was carried out by trypsin/ ethylenediaminetetraacetic acid (Sigma, USA) when 90% confluency of flasks was reached.

2.3. Flow cytometric analysis

We harvested ADSCs from 3 to 5 passages using trypsinization. They were then stained by CD44- FITC, CD90- PerCP CY5.5, CD45- FITC, CD34- PE antibodies for 20 min. In the next step, they were washed twice in PBS and subjected to analysis using a Attune™ NxT Flow Cytometer from Thermo Fisher Scientific (Alizadeh et al., 2017, 2019d; Simorgh et al., 2019).

2.4. Melatonin preconditioning of ADSCs

The isolated cells were pretreated with 5μM melatonin (Sigma, USA) for 24 h.

2.5. RT-PCR

2 days after preconditioning with melatonin, we extracted total RNA from cells employing RNA Extraction Solution (RX™, Cinnagen, Iran), which was stored at −70 °C for further analysis. cDNA synthesis was conducted with 5 μg of the total RNA taken from Fermentas kit (Fermentas, Canada) in accordance with the instructions provided by the manufacturer. The primers of genes were identified in this study are listed in Table 1. We used 2.5 μL of PCR product consisted of 0.2 pM of each primer, 0.5 mM MgCl2, 0.3 mM dNTP, 1× PCR bufer and 1U taq DNA polymeras (Fermentas, Canada). PCR reactions were performed inside a programmable thermocycler (Bio-rad, USA) under the following temperature profile: 94 °C for 5 min, 35 cycles at 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, and a last extension at 74 °C for 10 min. We also ran1μL of the PCR product on a 1.5 % agarose gel (Alizadeh et al., 2019b; Salehi et al., 2019). The Image J software was used to quantify the density of PCR bands.

2.6. BMSC transplantation

We used fluorescent CM-Dil Stain (Invitrogen) to label BMSCs and MT-BMSCs in compliance with the manufacturer’s protocols. The cells were incubated in Dil solution (1 μg/mL) at 37 °C for 20 min. They were then washed, and suspended in PBS. Seven days after the injection of Aβ into rats, BMSCs or MT-BMSCs (10⁶ cells/200 μL) were injected in rats via the tail vein (Nasiri et al., 2019; Yang et al., 2015b).

2.7. Animals

The sample consisted of 45 male Wistar rats weighing 250–300 g, which were procured from the Medical Faculty of Hamedan University of Medical Sciences. Rats were placed under standard lighting conditions (12-h light-dark cycles), temperature (22 °C) and humidity (55–65 %). Animals had convenient access to food and water ad libitum for one week to become acclimatized to the new environment. All procedures of animals were submitted to a stereotaxic apparatus (Stoelting, USA). The animals were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg), then washed, and suspended in PBS. Seven days after the injection of Aβ into rats, BMSCs or MT-BMSCs (10⁶ cells/200 μL) were injected in rats via the tail vein (Nasiri et al., 2019; Yang et al., 2015b).

2.8. Surgery and injection of Aβ (1–42)

The procedure was conducted as described in previous studies (Nikkhah et al., 2014; Shahidi et al., 2017a). To sum up, after being anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg), animals were submitted to a stereotaxic apparatus (Stoelting, USA). The coordinates for intra-ventricular injections of Aβ matched the rat brain in stereotaxic (AP: 1.2 mm, ML: 2 mm, DV: 4 mm from Bregma) (Paxinos (2007)). Aβ (Tocris Bioscience, UK) solution (1 μg/μL

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Length (bp)</th>
</tr>
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<tbody>
<tr>
<td>β-actin</td>
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<tr>
<td>Melatonin Receptor1 (MT1)</td>
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<tr>
<td>MT2</td>
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<td>199</td>
</tr>
<tr>
<td>Bax</td>
<td>S: 5'-AACAACTGGACGGTCCAGAGG-3' A: 5'-GAAGTGTCGCGCCTGCAAATC-3'</td>
<td>304</td>
</tr>
<tr>
<td>Bel-2</td>
<td>S: 5'-TGACCTGGTCTGCTGTTGAC-3' A: 5'-GACAACTCCTCCCCAGTTCA-3'</td>
<td>116</td>
</tr>
</tbody>
</table>
Fig. 1. Study design chart; Alzheimer was triggered by ICV injection of Aβ, followed by cell transplantation 7 days after the injections. In the end, behavioral studies were assessed. In the end, behavioral and histological study of brain tissue samples were undertaken.

2.9. Experimental design

Intra-ventricular injection of Aβ1-42 aimed to induce AD. Animals were divided randomly into five groups (n = 8) prior to the operation procedure. Control group (without Aβ injection and treatment), sham group (only surgery without injection of Aβ), Aβ group (injection of Aβ-peptide without any treatment), experimental group 1 (injected by Aβ-peptide and followed for one week before receiving BMSC via tail vein), and experimental group 2 (injected by Aβ-peptide and followed for one week before receiving MT-BMSC via tail vein). After transplantation of the BMSC and MT-BMSC, behavioral studies were assessed. In the end, all animals were sacrificed following treatment for the histological study (Fig. 1).

2.10. Behavioral studies

2.10.1. Open field test

The open field test evaluated the animals’ locomotor activity. The apparatus is made of a white acrylic field (surface area: 50 × 50 cm and wall height: 38 cm) with low ambient lighting. We first placed rats in amid the open field, and they were given some time to explore the surrounding area for 10 min. The velocity and total traveled distance was recorded by a video camera (Nasiri et al., 2019).

2.10.2. Novel object recognition test

A day before the test, animals were placed in the apparatus (70 × 50 × 40 cm) without any object for acclimation phase for 20 min. In the next day, we inserted two square or round bowls (known objects) inside the box to be used in the familiarization phase for 5 min (in front of the objects). An hour within the testing phase, one of the familiar objects was replaced by a novel object. The animals were given sufficient time to explore the familiar and novel objects for 5 in. The ratio of time spent with the novel object to the total time spent on each object was calculated as the distinction index. After each testing session, all areas and objects were rinsed with 70 % ethanol to eliminate any residual odors (Komaki et al., 2014).

2.10.3. Morris water maze (MWM)

Learning and spatial memory was evaluated with MWM. Briefly, a circular black pool (180 cm in diameter and 60 cm in height) was placed in the sound-proof room. The pool was divided into north (N), east (E), west (W) and south (S) quadrants with a hidden black escape platform in the northern quadrant. Rat training lasted for 4 days, and each day consisted of two blocks of four trials. During the learning days, the time spent to reach the hidden platform was recorded by a video camera mounted over the pool, which connected to a computer. During the probe test, the platform was removed, and rats had to swim for 60 s. The time spent in the target quadrant was calculated.

2.10.4. Passive avoidance learning (PAL)

The apparatus consisted of light and dark compartments. The former (dimensions: 20 cm × 20 cm × 30 cm) was constructed using a transparent plastic. The latter was built using dark opaque plastic (dimensions: 20 cm × 20 cm × 30 cm) whit a stainless steel rod (3 mm in diameter) in the floor of compartments. A shock generator (BehboodFardaz Co. Tehran, Iran) induced electricity in the dark compartment floor. An opaque guillotine door (6 cm × 8 cm) was placed between the two compartments.

Training

At first, the apparatus was presented to each rat in two trials. To do so, the rats were placed in the light compartment away from the door for 5 s. Then, the guillotine door was raised. The rats head for the dark environment instinctively. When the rats moved into the dark compartment, the guillotine door was shut and the animals remained in this compartment for 30 s. This trial was repeated with 30 min intervals. In another scenario, when the rats were inserted in the dark compartment, we tested the delay of entering the dark compartment (STLs). Following the spontaneous entering of rats into the dark compartment (i.e. staying in the light compartment) and the amount of time spent in the dark compartment (TDC) was up to 5 min. The retention test was assumed to be complete when the rat failed to enter the dark chamber within 3 s (Hasanein and Shahidi, 2011).

2.11. Tissue preparation and immunohistochemistry

Two months after transplantation, the rats were heavily anesthetized with xyloseinead and ketamine and then perfused transcardially with 150–200 ml PBS followed by 4% paraformaldehyde. The animal brains were harvested and fixed with paraformaldehyde and used for succeeding histological processing. In the end, we embedded tissue specimens in paraffin (Merck, Germany) and prepared 5 μm transverse sections.

Immunohistochemistry dropped after deparaffinization, antigen retrieval and rinsing of the sections with PBS. Then, blocking solution was used to treat sections before incubation with anti-GFAP (Ab4674), anti Iba1(Ab178846) and anti-Beta tubulin (Ab41489) overnight at 4 °C. In the following next, sections were washed with PBS before being incubated with the secondary antibody: goat anti-rabbit (IgG) (Ab67617) for 2 h at room temperature. We used DAPI to counterstain the nuclei for 45 min after rinsing it with PBS. Afterwards sections were mounted (Alizadeh et al., 2015; Moayeri et al., 2017). For quantitatively analysis of the expression of the proteins IHC method was performed. In addition, BMSCs and MT-BMSCs were labeled by CM-DiI before transplantation. The stained tissue were observed directly by fluorescence microscopy and analysed by Image J software.

Data analysis

The data analysis was performed using one-way ANOVA and Tukey’s tests in SPSS 22. A significance level of 0.05 was considered for all analyses.
3. Results

3.1. Isolation, expansion and flow cytometric analysis of BMSCs

The cells isolated from rat bone marrow were adhered to the flask within 2–3 days. After adhesion, they became spindle in shape (Fig. 2A). Since the first passage, the flask was filled every 2–3 days and a new passage was used. The flow cytometry results of rat passages 3–5 BMSCs corroborated the existence of CD44 and CD90, which yielded a negative value for CD45 and CD34 (Fig. 2B, C, D, E).

3.2. Expression of pro/anti apoptotic molecules, MT1 and MT2 receptors in BMSCs and MT-BMSCs

The results suggested that melatonin decreased the expression of Bax (pro-apoptotic protein) (Fig. 3A, B). It also upregulated the expression of Bcl2 (anti-apoptotic protein) (P < 0.05) (Fig. 3C, D). Also, the upregulation of MT1 (Fig. 3E,F) and MT2(Fig. 3G,H) melatonin receptors was confirmed by RT-PCR (P < 0.05)(Rafat et al., 2018a).

3.3. Behavioral studies

3.3.1. Effects of MT pretreatment of BMSCs in OF test

The results of statistical analysis using one-way ANOVA exhibited a significant difference between experimental groups with respect to the total traveled distance and velocity. According to the Tukey post-test, Aβ rats and Aβ + BMSC group had the lowest discrimination compared to the control group (P < 0.05). The Aβ + MT-BMSC transplantation treatment increased this index in comparison with the Aβ + BMSC group (P < 0.01).

3.3.2. Effects of MT pretreatment of BMSCs in the novel object recognition test

The results of statistical analysis using one-way ANOVA illustrated that a significant difference between experimental groups in terms of discrimination index (Fig. 5). According to the Tukey posttest, Aβ rats and Aβ + BMSC group had the lowest discrimination compared to the control group (P < 0.05). The Aβ + MT-BMSC transplantation treatment increased this index in comparison with the Aβ + BMSC group (P < 0.01).

3.3.3. Effects of MT pretreatment of BMSCs in the Morris water maze test

The MWM test assessed the impacts of cell transplantation and Aβ injection on learning and spatial memory (Fig. 6). Statistical analysis by ANOVA exhibited that rats in the Aβ group spent a significantly greater amount of time on the hidden platform compared to the control, sham and Aβ + BMSC groups on first day (P < 0.05). The Aβ group spent more time to find the platform compared to the Aβ + MT-BMSC, sham and control groups in second day of training (P < 0.01). In addition, it took a significantly longer time for the Aβ group to reach hidden platform in comparison with sham and Aβ + MT-BMSC groups on the third day of the trial phase (P < 0.05). The results indicated significant differences between sham and Aβ + MT-BMSC groups on the fourth day of training (P < 0.01). There was not difference between control and sham groups in all days of the training trial (P > 0.05). There was no significant difference between Aβ and Aβ + BMSC groups in various days of the training phase (P > 0.05).

According to the results of ANOVA test, the groups were significantly different in terms of swimming time in the target quadrant (Fig. 7). In this regard, Aβ group spent the shortest time in the target quadrant compared to the control, sham, Aβ + BMSC and Aβ + MT-
BMSC groups (P < 0.05, P < 0.05, P < 0.01 and P < 0.01, respectively). On the contrary, Aβ + MT-BMSC group spent the longest time in the target quadrant compared to the Aβ + BMSC group (p < 0.05). There was not significantly different between control, sham, and Aβ + MT-BMSC groups (Fig. 7).

3.3.4. Effects of MT pretreatment of BMSCs on the PAL test

Fig. 8 shows the results of PAL test. At the time of the first acquisition trial, there was no significant difference in step-through latency (Fig. 8A). This result revealed that there is no difference change between groups in the exploratory behavior in the dark compartment. One-way ANOVA demonstrated a significant difference in the number of acquisition trials between groups. Tukey post-test indicated that the frequency of trials in the Aβ group was substantially higher than the sham, control, Aβ + BMSC and Aβ + MT-BMSC groups (P < 0.05; Fig. 8B). There was no significant change in the frequency of trials between Aβ-BMSC groups and Aβ+MT-BMSC. It is indicated that learning of Aβ + BMSC group is same as Aβ + MT-BMSC group.

Fig. 8.C depicts the PAL retention phase. The one-way ANOVA demonstrated a significant difference in terms of STLr. According to the Tukey post-test, Aβ group had a lower STLr in comparison with the control (P < 0.05), sham (P < 0.05), Aβ + BMSC (P < 0.01), and Aβ + MT- BMSC groups (P < 0.01). The Aβ+MT- BMSC group had a greater STLr compared to Aβ + BMSC group (P < 0.05).

In contrast, the results of one-way ANOVA exhibited a significant difference between groups in terms of time spent in the dark compartment (TDC) (Fig. 8D). TDC in the Aβ group was longer than the control (P < 0.05), sham (P < 0.05), Aβ + BMSC (P < 0.01), and Aβ + MT- BMSC groups (P < 0.01). Also, TDC in Aβ+MT- BMSC group was significantly lower than Aβ + BMSC group (P < 0.05).

3.4. Transplanted BMSCs and MT-BMSCs identified in the brain and distinguished to neuron, astrocyte and microglia

Tissue studies have shown the migration of BMSCs and MT-BMSCs into the brain (show with red stain). However, the number of transplanted cells in MT-BMSC group was significantly higher than the BMSC group. According to the immunohistochemical analysis of antibody
beta tubulin (Fig. 9A-H), GFAP (Fig. 9J-Q), and Iba1 (Fig. 9S-Z), BMSCs and MT-BMSCs transplanted into microglia, astrocytes and neurons were differentiated. On the other hand, the differentiated cells of astrocytes and neurons in the MT-BMSCs group were significantly higher than BMSC group but the differentiated microglia were significantly lower than the BMSC group (Fig. 9(1,2,3)).

4. Discussion

This study explored the impact of transplantation of BMSC and MT-BMSC pretreatment on learning and memory in the ICV-amyloid beta rat. The major results of the study are as follows: (1) the MT pretreatment increased the MT1 and MT2 receptors in BMSC; (2) the MT pretreatment reduced the expression of BAX gene, improved Bcl2 in BMSC and increased cell survival under in vitro condition; (3) the migration and differentiation abilities of melatonin-treated cells were fostered under in vitro condition; (4) ICV-amyloid beta pretreatment caused cognition and memory impairment in rats; (5) the MT-BMSC pretreatment improved spatial learning, cognition, avoidance and spatial memory in the ICV-amyloid beta rat. The MT pretreatment did not affect on locomotion activity and the avoidance learning.

In the present study, Aβ was microinjected into ICV to induce AD. The results are in line with previous studies according to which Aβ induced memory deficits and cognition impairment in rat. The preconditioning of cell with melatonin promoted BMSC survival and exerted positive effects on differentiation under in vitro condition. These findings are comparable to previous reports, according to which melatonin has a favorable effect on BMSCs (Mias et al., 2008; Rafat et al., 2018a). The positive effect of melatonin on learning and memory has been reported in normal and AD animals (Jürgenson et al., 2019). The preconditioning of MSC with melatonin improved memory impairment in AD. It is demonstrated the anti-inflammation and anti-apoptosis properties of melatonin (Nasiri et al., 2019). The beneficial effects of MT-BMSCs on the Aβ-treated rat was consistent with the findings of a recent study about the effect of melatonin on brain neuronal deficits and memory impairments in an amyloid-predominant rat (Jürgenson et al., 2019).

It is suggested that stem cell transplantation reinforces cell proliferation and the expression of synaptic proteins in animal models of AD (Bali et al., 2017). BMSCs enhanced migration capacities and decreased cell death following ischemic stroke in mice (Wei et al., 2013). Hypoxic preconditioning attenuated apoptosis and promoted angiogenesis in the brain tissue following ischemic stroke in rats (Jiang et al., 2019). Recent studies have shown the positive effect of preconditioning...
on mesenchymal stem cells. The preconditioned stem cells have greater differentiation potential and learning and memory improvement was expedited after hippocampal injury (Kim et al., 2019). Preconditioning factors such as hypoxia activated the proliferation and migration of mesenchymal stem cells and lowered neuronal death in the ischemic cortex (Wei et al., 2012). However current study did not evaluate the expression of the antioxidant enzyme catalase and the accumulation of reactive oxygen. The biochemical study is necessary to better understanding about the interaction of reactive oxygen species, antioxidant system and cell transplantation in AD animal.

Melatonin has antioxidant, anti-apoptotic and neuroprotective effects. Melatonin attenuates mitochondrial dysfunction through the activation of SIRT1 signaling (Yang et al., 2015a). Melatonin is involved in physiological activities via its receptors (MT1/MT2). This finding is in keeping with those reported in previous studies according to which melatonin preconditioning raises survival and efficiency of MSCs (Mias et al., 2008). In addition, the present findings suggest that melatonin preconditioning enhanced MT1/MT2 receptors in BMSCs and...
influenced the anti-apoptosis pathway signaling such as BCL-2 and BAX. The melatonin induces proliferation of MSCs through binding to MT1 and MT2 receptors (Alzahrani, 2019). The melatonin’s actions are mediated through interaction with the G-protein coupled membrane bound melatonin receptors type 1 and type 2 (MT1 and MT2, respectively) or, indirectly with nuclear orphan receptors from the RORα/RZR family (Slominski et al., 2012).

The melatonin boosted cell survival by increasing Bcl-2 expression and decreasing BAX expression and interaction with MT1/MT2 receptors. Melatonin preconditioning of BMSCs via MT1/MT2 receptors improved expression of the antioxidant enzyme catalase and superoxide dismutase-1, boosting MSCs resistance against apoptosis stimulation of specific melatonin receptors. Further, melatonin caused an overexpression of superoxide dismutase-1 and antioxidant enzyme catalase, augmenting the resistance of MSCs to apoptosis induced by hydrogen peroxide (Mias et al., 2008). Also, the melatonin receptors MT1 and MT2 increased PrPC-dependent mitochondrial function, Erk1/2 overexpression and up-regulated phosphorylation of AMPK pathway proteins (Zhao et al., 2020). Mel preconditioning may reinforce MSCs to produce high quality exosomes with higher expression of beneficial RNA cargoes that when transplant to the recipient cells (brain tissues of Alzheimer in that case), they could restore brain tissue through inhibition of certain molecules involve in oxidative stress, apoptosis, and inflammation. Therefore, using preconditioning strategy augment the therapeutic capacity of exosomes compared with the non-preconditioning cells (Alzahrani, 2019).

Furthermore, the results of this study revealed that melatonin preconditioning led to the overexpression of GFAP and Beta tubulin. The HGF and bFGF contain mitogenic and angiogenic factors that regulate the differentiation of mesenchymal stem cells (Xu et al., 2008). Melatonin preconditioning reduced microglial the differentiation of cells from stem cell (Nasiri et al., 2019). Microglia activity exerted different effects on AD pathology. The microglia produce acute and a chronic response into extracellular Aβ deposits AD. Although the acute response of microglia prevents the Aβ accumulation, the chronic response of microglia resulted in the loss of neuron and neurotoxicity (Sarlus and Heneka, 2017).

In conclusion, this study suggested that the preconditioning of BMSCs with melatonin could be a new strategy to reduce apoptosis and increase the survival of stem cells in the migration and differentiation process. This strategy reinforced the differentiation of BMSCs and improved memory impairment in the amyloid beta model of rats.

Ethical statement

The authors declare that all experiments of this study performed under agreement of ethical committee of Hamadan University of medical sciences (No. IR.UMSHA.REC.1394.278).

Author contribution

Alireza Komaki, Keywan Mortezaee and Mahdi Ramezani edited the manuscript
Nasrin Hashemi-Firoozai did data analyzing
Nafiseh Faraji did all of the experiments.
Zeileika Golipoor is supervisor and wrote article

Author statement

We do appreciate from all reviewers for their constructive comments. The authors believe that these comments have made paper better.

Declaration of Competing Interest

The authors disclose no potential conflicts of interest to declare.

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