



Melatonin-pretreated adipose-derived mesenchymal stem cells efficiently improved learning, memory, and cognition in an animal model of Alzheimer's disease

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Abstract

Currently, mesenchymal stem cells (MSCs) based therapy has extensive attraction for Alzheimer's disease (AD). However, low survival rate of MSCs after transplantation is a huge challenging. The current study aimed to improve adipose-derived MSCs (AD-MSCs)-based therapy by their pre-treatment with melatonin (MT) 'a well-known antioxidant' in an animal model of AD. In this study, after isolating rat AD-MSCs from the epididymal white adipose tissues, the cells were pretreated with 5 μM of MT for 24 hours. Forty male Wistar rats were randomly allocated to control, sham, amyloid-beta (Aβ) peptide, AD-MSCs and MT-pretreated ADMSCs groups. The novel object recognition, passive avoidance test, Morris water maze and open field test were performed two months following the cell transplantation. The rats were sacrificed 69 days following cell therapy. The brain tissues were removed for histopathological analysis and also immunohistochemistry was performed for two Aβ1-42 and Iba1 proteins. It has been revealed that both AD-MSCs and MT-AD-MSCs migrated to brain tissues after intravenous transplantation. However, MT-ADMSCs significantly improved learning, memory and cognition compared with AD-MSCs (P<0.05). Furthermore, clearance of Aβ deposition and reduction of microglial cells were significantly increased in the MT-ADMSCs compared with AD-MSCs. Although stem cell therapy has been introduced as a promising strategy in neurodegenerative diseases, however, its therapeutic properties are limited. It is suggested that pretreatment of MSCs with melatonin partly would increase the cells efficiency and consequently could decrease AD complication including memory and cognition.

Keywords Alzheimer's disease · β-amyloid · AD-MSCs · Melatonin

Introduction

Alzheimer's disease (AD), which is recognized as a neurodegenerative disorder, is associated with the progressive loss of

neurons, leading to memory impairment and interference in daily activities (Tong et al. 2015). Extracellular deposition of amyloid-β (Aβ) and Tau hyperphosphorylation, as two major factors in neuronal loss (Ruzicka et al. 2016; Zhao et al. 2017), reduce the size of frontal and temporal lobes, which are involved in memory and learning. Therefore, abnormalities, such as progressive learning dysfunction, memory decline and cognitive impairment occur in patients with AD (Lemmens et al. 2011; Yan et al. 2014).

There are only a few therapeutic strategies for AD, which just relieve the symptoms with no effects on the disease progression or factors involved in its pathogenesis (Ager et al. 2015; Choi et al. 2014; Tong et al. 2015). Cell therapy is one of the most advanced and novel approaches that have been suggested in treatment of neurodegenerative disorders. According to recent researches, mesenchymal stem cells (MSCs) transplantation improves cognitive performance in

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animal AD models (Eftekharzadeh et al. 2015; Kan et al. 2011; Munoz et al. 2005). MSCs produce neurotrophic factors and neuroprotective cytokines, leading to improve dentate gyrus proliferation of endogenous neural stem cells and enhancement of behavioral outcomes (Kan et al. 2011; Munoz et al. 2005). Also, they have pivotal effects on A β clearance in animal AD models (Eftekharzadeh et al. 2015). Adipose-derived MSCs (AD-MSCs) is promising among other sources of MSCs because of their easy access, culture and expansion and also immunomodulatory Properties. It is noteworthy noted that AD-MSCs promote neurogenesis in vitro (Yan et al. 2014).

Transplantation of AD-MSCs reduces A β deposits and restores memory in AD models (Kim et al. 2012; Lee et al. 2010). Nevertheless, considering the noxious microenvironment of the brain, viability reduction of transplanted cells is a potential problem in AD (Rockenstein et al. 2015). Direct MSCs transplantation is not efficacious, and nearly 80–90% of cells die following transplantation within three days (Liu et al. 2009). Multiple factors may be involved in the early eradication of transplanted cells, such as inflammation, hypoxia, thermal stress, and free radical activity (Khatibi et al. 2017; Roushandeh et al. 2017; Tang et al. 2005). Consequently, establishing a strategy to improve cell survival and homing is necessary in cell therapy. Cell preconditioning is a promising approach for increasing cell survival and homing after transplantation; it also represents multiple therapeutic benefits (Haider and Ashraf 2012; Tang et al. 2014; Yu et al. 2013).

Melatonin (MT), a pineal gland hormone, regulates multiple physiological processes. It also controls different regulatory functions of the cells, including cell signaling, immune responses, and inhibition of nuclear DNA damage and oxidation of fatty acids. MT induces antiapoptotic effects on normal cells and exhibits significant anti-aging properties (Esteban-Zubero et al. 2016; Gholami et al. 2014). It is also a powerful free radical scavenger, which activates cellular antioxidants in different cell types (Carpentieri et al. 2016; Liu et al. 2016). Pretreatment with MT can enhance the homing of MSCs after transplantation (Mortezaee et al. 2016). Evidence suggests that MT also protects human AD-MSCs against oxidative stress and apoptosis (Rafat et al. 2018; Tan et al. 2016).

Therefore, The purpose of the present study was to investigate the antioxidant potential of MT and AD-MSC-base therapy on the histological properties, cognition and memory in a rat model of Alzheimer's disease.

Materials and Methods

AD-MSCs isolation and expansion

Epididymal white adipose tissues were removed aseptically from six-week-old male Wistar rats. Phosphate-buffered saline (PBS; Invitrogen, USA), containing 1% penicillin/streptomycin

(Invitrogen, USA), was used twice to wash the tissues at 37°C. Adipose tissues were then treated with 0.1% collagenase type I (Gibco, USA), which was dissolved in 1% bovine serum albumin (BSA) in warm PBS. The samples were kept in a water bath for half an hour to achieve total digestion and homogenization.

The supernatants were removed after five minutes of centrifugation at 1200 rpm, and the pellets were resuspended in 1% BSA. This step was repeated in order to remove red blood cells (RBCs) using RBC lysis buffer. Finally, the harvested cells were cultured in DMEM/Ham F-12 medium at 37°C, consisting of 1% penicillin/streptomycin, besides 10% fetal bovine serum (FBS). The medium was changed every three days, and the cells sub cultured with trypsin/EDTA (Sigma, USA) after 90% confluences.

Flow cytometry

AD-MSCs between 3–5 passages were harvested and probed for 20 minutes with CD44-FITC (550974), CD90-PerCPCY5 (557266), CD45-FITC (554877), and CD34-PE (551387) antibodies (BD bioscience, USA). After rinsing the cells twice in PBS, an NxT Flow Cytometer (Attune™; Thermo Fisher Scientific, USA) was used.

Preconditioning with MT

The passage 4 AD-MSCs with concentration of 10⁶ cells /flask were cultured with 5 μ M of MT (Sigma, USA) for 24 hours.

Animals and experimental design

The adult male Wistar rats (250–300 g) were purchased from Pasteur Institute of Iran, which were kept in a 12:12 h light/dark cycle with free access to food and water at 22°C. The Research Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1396.544) approved all the procedures, which were in line with the NIH guidelines. In a random manner, the rats were classified into five groups (N=8): control; sham; A β ; experimental group 1 receiving AD-MSCs through the tail vein one week post-A β injection; and experimental group 2 receiving MT-ADMSCs from the tail vein one week post-A β injection. In the sham group, a Hamilton syringe needle was stereotaxically inserted in the brain and removed without any injections (Fig. 1).

AD induction with A β injection

After A β 1–42 dissolving (Tocris Bioscience, UK) in 100 μ L of PBS, incubation was performed at 37°C for seven days before application. For inducing deep anesthesia, ketamine (87 mg/kg), along with xylazine (13 mg/kg), was injected intraperitoneally (Van 1977). Bilateral intracerebroventricular injections of A β (5 μ g) were performed using a 5- μ L Hamilton microsyringe

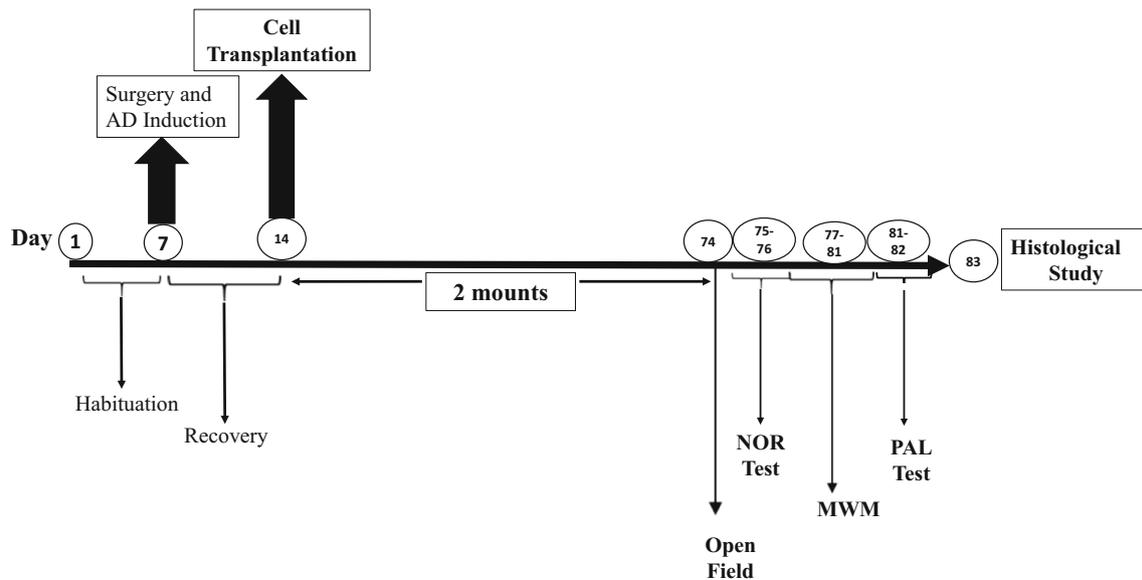


Fig. 1 Study design diagram; Alzheimer was induced by ICV injection of A β . Cell transplantation was done 7 days after the injections of A β . Finally, behavioral studies (Open field, NOR test, MWM, PAL test) were performed and brain tissue samples were studied histologically

(USA) according to the stereotaxic coordinates (AP: 1.2 mm; ML: 2 mm; DV: 4 mm from the Bregma) on a stereotaxic device (Stoelting Co., USA). The animals recovered in cages after suturing the skin (Cetin et al. 2013).

AD-MSc transplantation

In line with the manufacturer's protocol, the fluorescent DiI Stain (Invitrogen) was used to label AD-MSCs and MT-ADMSCs. Incubation of the cells was performed for 20 minutes in DiI solution (1 μ g/mL) at 37°C. After washing, the cells were resuspended in PBS. Seven days following the injection of A β , AD-MSCs or MT-ADMSCs (10⁶ cells/200 μ L) were injected in the tail vein of rats.

Behavioral studies

Different tests including the novel object recognition (NOR) test, open field (OF) test, Elevated plus maze (EPM) test, passive avoidance learning (PAL) test, and Morris water maze (MWM) were conducted two months after cell transplantation (Kim et al. 2015; Kommaddi et al. 2018).

OF test This test was used for evaluating the animals' locomotor activity. It uses a white acrylic field (surface area, 50 \times 50 cm; height of walls, 38 cm) with low ambient light and records the time spent in the peripheral and central zones. After placing the rats in the middle of open field, they were given time to explore for 10 minutes. A video camera was used to record the number of animals' entries to the center, spent time in the peripheral and central regions, and total traveled distance (Etaee et al. 2017).

Elevated plus maze (EPM) test: This test was designed to measure anxiety behavior in rodent. Briefly, the maze consisted of two open arms (50 \times 10 cm each), two enclosed arms (50 \times 10 \times 50 cm each) and a central platform (10 \times 10 cm). The maze was elevated 100 cm above the floor and was illuminated with two 100 W lamps. During the 10 min test period, each rat was allowed to explore the maze, and their behavior was checked by a digital camera above the maze. The time spent in the open arms, the number of open and enclosed arms entries, and time spent in open and enclosed arms were recorded. The number of entries and time spent in the closed arms indicate anxiety. The time spent and number of entries in the open arms show anxiolytic behavior in rodent (Campos et al. 2013; Lister 1987).

Novel object recognition test One day before the test, the animals were put in an apparatus (70 \times 50 \times 40 cm) for 20 minutes so that their exploratory behaviors would not interrupt their object interactions. Two round or square bowls (familiar objects) were placed in the box on the following day (familiarization phase). After placing the rat next to the front wall and at the midpoint for 10 minutes (in front of the objects), it was moved back to its cage. One hour later in the testing phase, a novel object replaced a familiar object, and the animal was given time to explore for five minutes. The distinction ratio was determined as the ratio of spent time with the novel object to the total spent time on either object. Using 70% ethanol, all the areas and objects were cleaned between trials so that the rats could perceive the olfactory cues (Komaki et al. 2014).

MWM test This test examines the animals' reference and spatial working memory. Briefly, a circular black pool (diameter, 180

cm; height, 60 cm) was divided into four quadrants (north, east, west, and south), and at the center of the north quadrant, a black platform was placed. The pool was placed in the room with low light and sound insulated. The geometric shapes were pasted at the walls for visual cues. Also, the room has various cues such as racks, sweep, sink, water hoses and shelves.

The animals were assessed by a five-day protocol at the same time (10:00 and 12:00). They were placed in a quadrant and given time to find the platform in 90 seconds during the first four days. If the animal did not find the platform at the set time, it handler directed to the platform in training. The platform was removed on day five (probe trial), and the rats were allowed 60 seconds to swim in order to evaluate their reference memory. Thirty seconds of rest was determined between the two trials, and a five-minute rest was allowed between the two blocks. Then, escape latency, time in the target quadrant, average swimming speed and travelled distance were measured (Asadbegi et al. 2017).

PAL test A step-through apparatus was used for the PAL test. It consisted of two compartments (20×20×30 cm), including a transparent plastic compartment and a dark compartment of opaque plastic, separated by a rectangular opening (6×8 cm). Stainless steel rods were applied on the floor of compartments, enabling the delivery of an electric shock by the generator (Behbood Pardaz, Iran).

For training, the door was lifted after placing the rats in the light chamber for five seconds. As soon as the rat entered the dark chamber, the guillotine door was closed, and they were kept in the chamber for 30 seconds; this trial was repeated after a 30-minute interval. The entrance latency to the dark chamber (STLa) was also measured. The guillotine door was shut for 30 seconds after the animal's entry into the dark compartment; then, an electric shock was induced for three seconds (0.5 mA). After two minutes, this procedure was repeated. The training ended after the rat's two-minute stay in the light chamber. In addition, the number of entries to the dark chamber was determined.

Moreover, in the retention test, the guillotine door was lifted after putting the rats in the light chamber for five seconds. The time spent in the dark compartment (TDC), as well as the step-through latency in retention (STLr), was recorded for five minutes. The retention test ended if the rat failed to enter the dark chamber in this period (Hasanein and Shahidi 2011).

Histopathological and immunohistochemistry studies

Sixty-nine days after cell therapy, the animals were sacrificed, and the brain tissues were stained using antibodies against A β 1-42 and IBA1. Using xylazine and ketamine, deep anesthesia was induced, and then, 150–200 mL of PBS and 4% paraformaldehyde were used for perfusion. The brain tissues were then harvested and

fixed using 4% paraformaldehyde. After tissue processing, they were paraffin-embedded (Merck, Germany).

Using a microtome, 5 μ m transverse sections of brain tissues were prepared. When the sections were cleared and dehydrated, antigen retrieval was performed. Incubation was performed overnight at 4°C using a blocking solution, followed by anti-beta-amyloid (ORB10087) and anti-Iba1 (ORB336635) primary antibodies. Afterwards, the sections were washed with PBS. Incubation was performed for two hours using a secondary anti-rabbit antibody (406403) at room temperature. DAPI was used for counterstaining the nuclei for 45 minutes after PBS washing; the sections were then mounted.

Data analysis

Kolmogorov–Smirnov test was used to analyze normality of data in SPSS v 16. Non-parametric Kruskal–Wallis H-test was used to assess the significance among non-normality of data for some variables. One-way ANOVA and Tukey's tests were applied for the analysis of normal data. All data were presented as the mean \pm S.E.M. The significance level was 0.05 in all analyses.

Results

AD-MSCs were expanded easily in culture and presented their surface markers

The cells from rat adipose tissues were attached to a flask during 2–3 weeks and formed a spindle shape (Fig. 2a). The flow cytometry findings confirmed the positivity of cells for CD44 and CD90 markers and their negativity for CD45 and CD34 (Fig. 2b, c, e, and f).

Behavioral studies

MT pretreatment of AD-MSCs had no effects on behavioral parameters in the OF test:

Kolmogorov–Smirnov test indicated nonparametric of data for the total traveled distance ($p < 0.001$) and moving velocity ($p < 0.01$). Kruskal–Wallis test revealed no significant differences between groups for total traveled distance (H value = 4.476, $P = 0.214 > 0.05$; Fig. 3a) and moving velocity (H value = 6.021, $P = 0.111 > 0.05$; Fig. 3b).

MT pretreatment of AD-MSCs had no effects on anxiety behavioral parameters in the EPM test:

Kolmogorov–Smirnov test indicated nonparametric of data for the time spent in open and closed arms, and number of

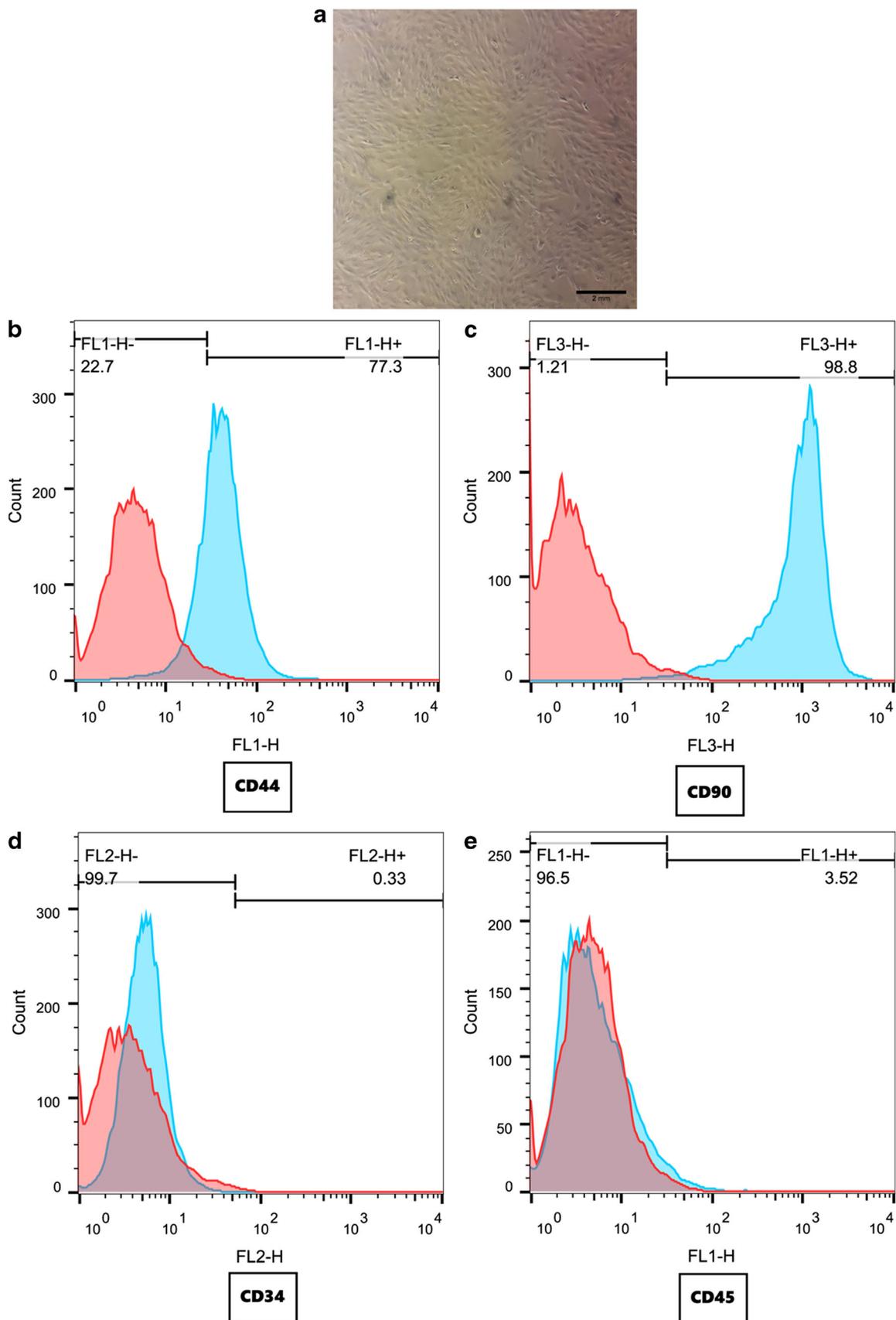
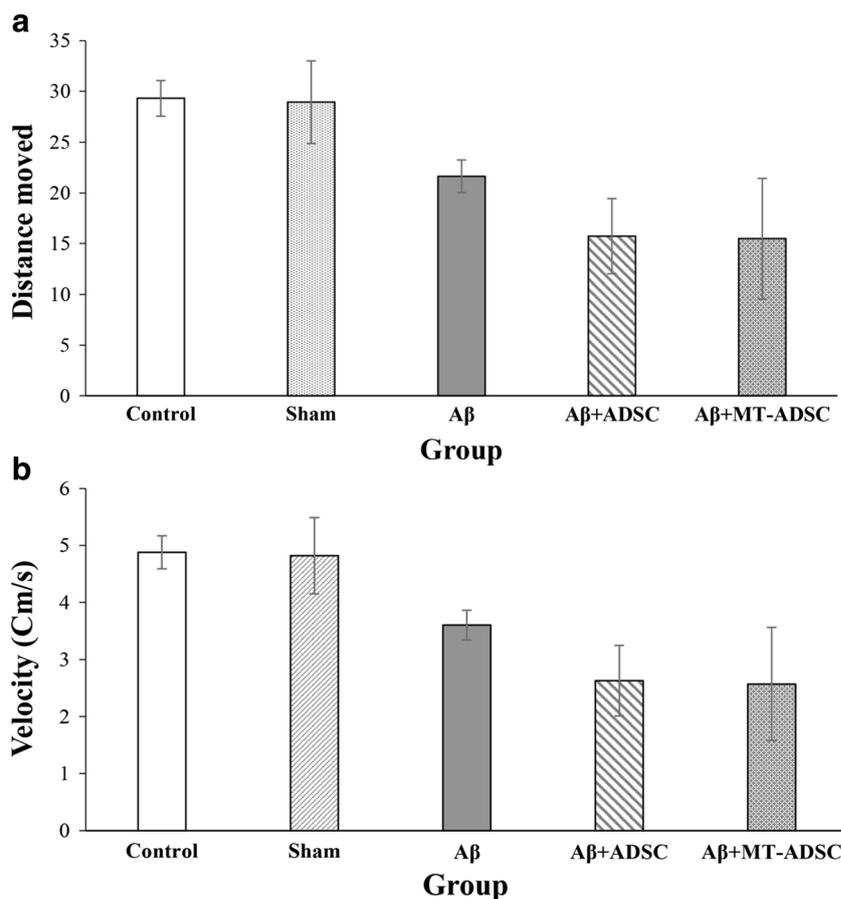


Fig. 2 Undifferentiated ADSCs (**a**) under phase contrast microscopy displayed a flattened fibroblast-like morphology. Flow cytometric analysis of ADSCs showed that they were positive for CD44 and CD90 (**b** and **c**, respectively) and negative for CD34 and CD45 (**d** and **e**, respectively)

Fig. 3 Effect of cell transplantation in A β rat model on distance move (**a**) and velocity (**b**) in open field test. Data are expressed as mean \pm SEM.



entries into open and closed arms ($p > 0.001$). Kruskal–Wallis test revealed no significant differences between groups in time spent in the open arms (H value = 2.72, $P = 0.436 > 0.05$) and number of entries into open arms (H value = 5.217, $P = 0.157 > 0.05$). Also, Kruskal–Wallis test showed that there was no significant difference in the time spent in closed arms [H value = 0.149, $P = 0.985 > 0.05$] and number of entries into closed arms [H value = 1.691, $P = 0.639 > 0.05$] between groups (Fig. 4).

ADSCs and MT-ADSCs had positive effects on the novel object recognition test:

Kolmogorov–Smirnov test showed normality of data ($p = 0.20 > 0.05$). One-way ANOVA showed that there was significant difference in discrimination index between groups [F (4,39) = 10.259, $p > 0.001$]. Compared to the A β +ADMSC, A β +MT-ADMSC, sham, and control groups, the results of this index were lower in the AD group ($P < 0.01$). Both AD-MSc and MT-ADMSC transplantations had positive effects on discrimination indices in contrast with the A β group ($P < 0.05$). Interestingly, MT-ADMSC-based therapy showed more ameliorative activities in contrast with the AD-MSc therapy ($P < 0.05$) (Fig. 5).

Transplantation of MT-ADSCs ameliorated memory impairments, as well as context and spatial learning

Based on the MWM test, the effects of A β injection and cell transplantation on spatial memory were examined. Data on acquisition trials are listed in Table 1. The A β group spent less time in the target quadrant in comparison with the controls in the probe trial; also, it took more time for this group to find the platform. Transplantation of MT-ADMSCs majorly increased the spent time in the target quadrant, while it reduced the escape latency in contrast with A β ($P < 0.05$). Nevertheless, the swimming speed was not significantly different between the test groups (Fig. 6).

MT-ADSCs effect on the swimming speed at MWM test

The swimming speed data on acquisition trials are showed in Fig. 7. One-way ANOVA showed that there was significant difference between groups at first day [F (4,39) = 77.854, $p < 0.001$]. Tukey post revealed that control group has more speed compare to sham, A β , A β +ADMSC and A β +MT-ADMSC groups ($p < 0.001$). The A β animals had more speed time compare to sham group ($p < 0.001$). One-way ANOVA analysis in second day showed that there was significant difference between groups [F (4, 39) = 75.308, $p < 0.001$]. Tukey post

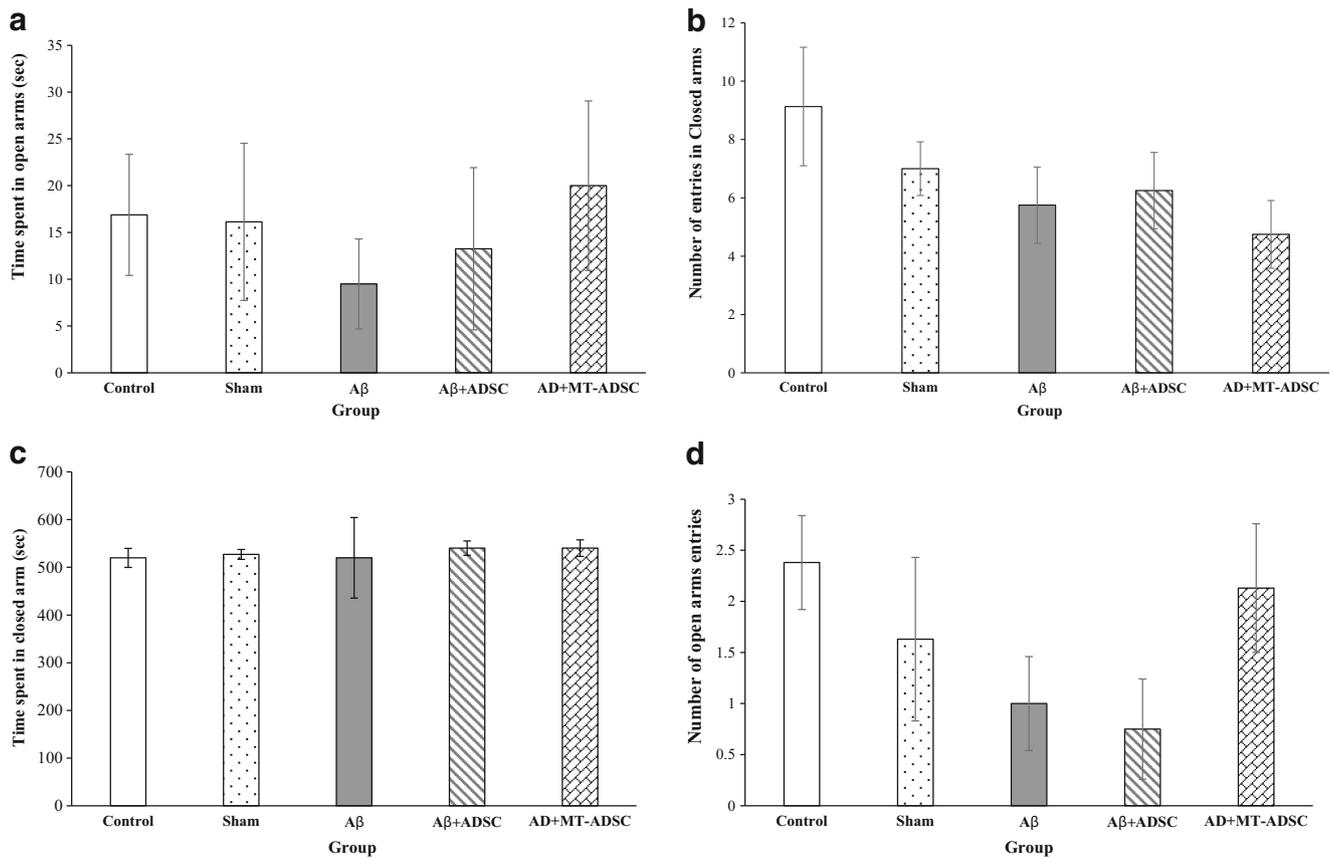


Fig. 4 Effect of MT pretreatment of AD-MSCs on elevated plus maze test performance: (a) Time spent in the open arms; (b) number of entries in closed arms; (c) Time spent in the close arms; and (d) number of total

entries into the open arms. Data were analyzed by Kruskal–Wallis test. Data are means ± SEM (*n* = 8 per group)

revealed that control group has higher speed compare to sham, Aβ, Aβ+ADMSC and Aβ+MT-ADMSC groups (*p* < 0.001). Furthermore, One-way ANOVA showed that there was significant difference between groups at third day [*F* (4,39) = 68.936, *p* < 0.001] and forth day [*F* (4,39) = 144.482, *p* < 0.001]. Tukey post showed that control group has higher speed compare to

sham, Aβ, Aβ+ADMSC and Aβ+MT-ADMSC groups at third and fourth days (*p* < 0.001).

The results of the swimming speed in the control group by one-way ANOVA showed that there was significant difference between groups on the different days [*F* (3,27) = 11.15, *p* < 0.001]. Tukey post showed that first day of learning day has high speed

Fig. 5 Effect of cell transplantation in Aβ rat model on the discrimination index in new object recognition test (**P* < 0.01 when compared with the control group, ***P* < 0.05 when compared with Aβ group, #*P* < 0.05 when compared with Aβ+ADSC group). Data are expressed as mean ± SEM

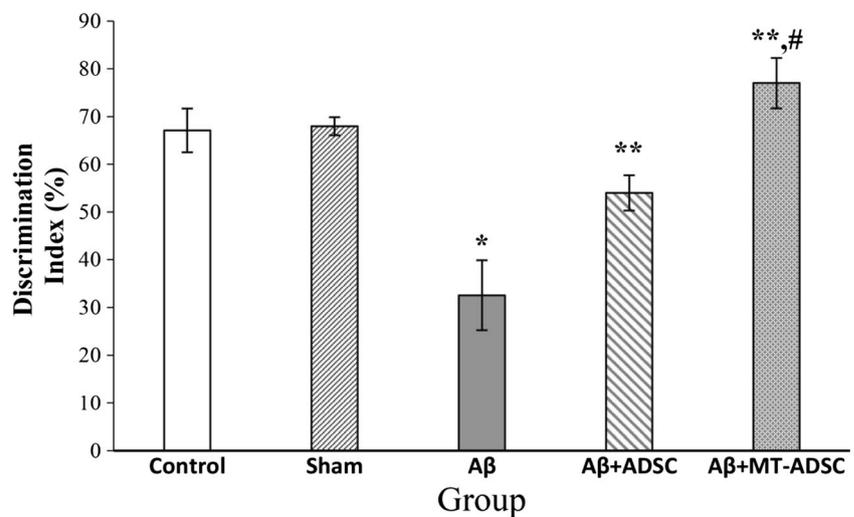


Table 1 Effect of cell transplantation in A β rat model on scape latency during acquisition trials. A) Comparison between groups in each day B) Comparison between the performances of each group in four days

A					
	Day 1	Day 2	Day 3	Day 4	
Control vs. Sham	Ns	Ns	Ns	Ns	
Control vs. A β	Ns	Ns	Ns	Ns	
Control vs. A β + ADSC	*	****	Ns	*	
Control vs. A β + MT-ADSC	****	Ns	Ns	Ns	
Sham vs. A β	*	Ns	Ns	Ns	
Sham vs. A β + ADSC	**	****	*	*	
Sham vs. A β + MT-ADSC	****	Ns	Ns	Ns	
A β vs. A β + ADSC	Ns	**	Ns	Ns	
A β vs. A β + MT-ADSC	****	Ns	Ns	Ns	
A β + ADSC vs. A β + MT-ADSC	****	****	Ns	Ns	
B					
	Control	Sham	A β	A β +ADSC	A β +MT-ADSC
Day-1 vs. Day 2	***	**	****	Ns	Ns
Day-1 vs. Day 3	****	****	****	****	Ns
Day-1 vs. Day 4	****	****	****	****	Ns
Day 2 vs. Day 3	Ns	Ns	Ns	***	Ns
Day 2 vs. Day 4	Ns	*	**	****	Ns
Day 3 vs. Day 4	Ns	Ns	Ns	Ns	Ns

Ns not significant

compare to other days ($p < 0.001$). There was not significant difference between second, third and fourth days.

The results of the swimming speed in the sham group by one-way ANOVA showed that there was significant difference between different days [F (3,27) =23.47, $p < 0.001$]. Tukey post showed that first day of learning day has high speed compare to third and fourth days ($p < 0.01$). Also, second day of learning day has high speed compare to third and fourth days ($p < 0.01$).

The results of the swimming speed in the A β group by one-way ANOVA showed that there was significant difference between days [F (3,27) =12.2, $p < 0.001$]. The second day of learning day has high speed compare to fourth days ($p < 0.01$).

One-way ANOVA showed that there was not significant difference between acquisition days in the ADMSC group [F (3,27) =2.04, $p = 0.131 > 0.05$].

The results of the swimming speed in the A β +MT-ADMSC group by one-way ANOVA showed that there was significant difference between different days [F (3,27) =10.92, $p < 0.001$]. Tukey post showed that first day of learning day has low speed compare to third and fourth days ($p < 0.01$ and $p < 0.05$, respectively). Also, second day of learning day has low speed compare to third day ($p < 0.05$).

MT-ADMSCs enhanced memory in the PAL test

The results of the PAL learning phase are shown in Fig. 7. During the first acquisition trial, the test groups were

not significantly different regarding STLa (Fig. 8a). The number of acquisition trials was different between the test groups. The frequency of trials was higher in the A β group versus the sham, control, and experimental groups ($P < 0.05$; Fig. 8b). Fig. 6.C depicts the PAL retention phase. The test groups were significantly different in terms of STLa; in comparison with the sham and control groups, the A β group had lower STLa ($P < 0.05$). However, the AD-MSC and A β groups were not significantly different.

In comparison with the A β and AD-MSC groups, MT-ADMSC transplantation significantly increased STLa ($P < 0.01$; Fig. 8c). Furthermore, the MT-ADMSC group spent less time in the dark chamber in contrast with the AD-MSC and A β groups (Fig. 8d). On the other hand, TDC was longer in the A β group, compared to the MT-ADMSC, control, and sham groups. TDC was not significantly different between the A β group and AD rats receiving AD-MSCs ($P < 0.05$; Fig. 8d).

MT-AD-MSCs-based therapy considerably decreased A β deposit in comparison with the AD-MSCs group

The AD-MSCs and MT-ADMSCs reduced the brain A β deposition. However, reduction was significant only in the MT-ADMSC group (Fig. 9a-d; $P < 0.05$).

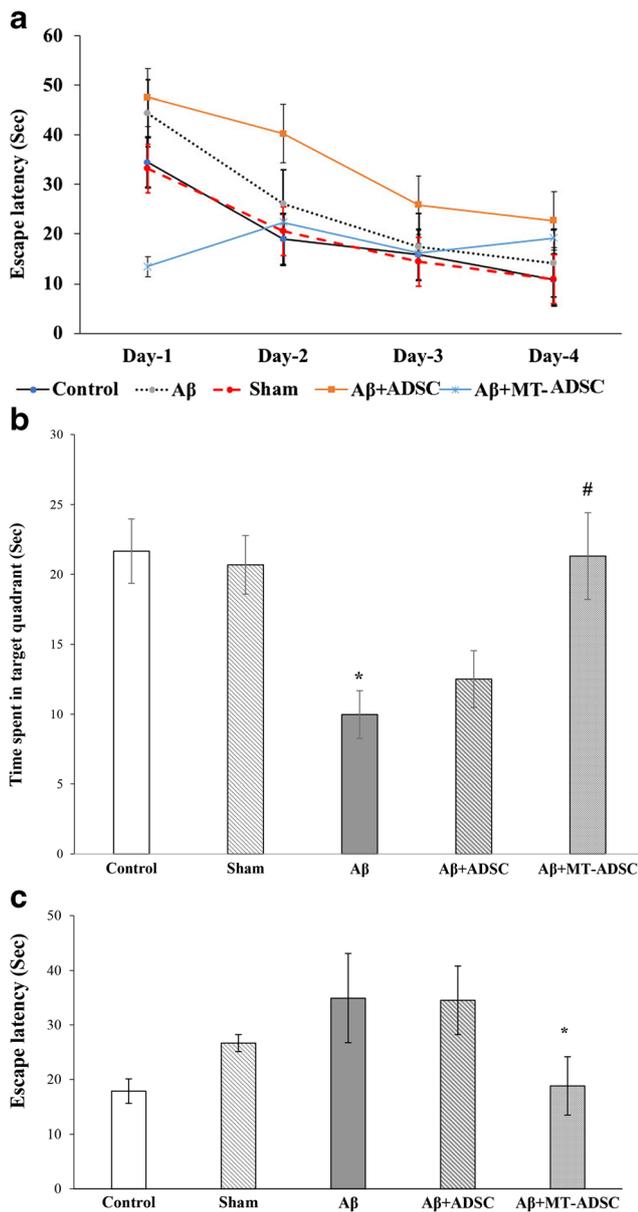


Fig. 6 Effect of cell transplantation in A β rat model on mean escape latency to find the hidden platform during acquisition trials (a), time spent in target quadrant (b), and mean escape latency to find the hidden platform at probe trial (c) in the Morris water maze (* $P < 0.05$ when compared with the control group in B, # $P < 0.05$ when compared with the A β group, * $P < 0.05$ when compared with the A β group in C). Data are expressed as mean \pm SEM

Transplanted AD-MSCs and MT-AD-MSCs were detected in the brain and differentiated to microglia

The histological examination demonstrated the migration of Dil-labeled AD-MSCs and MT-ADMSCs from the delivery site to the brain. The MT-ADMSC group had a significantly higher number of transplanted cells in comparison with the AD-MSC group. The immunohistochemistry study using Iba1 antibody showed that

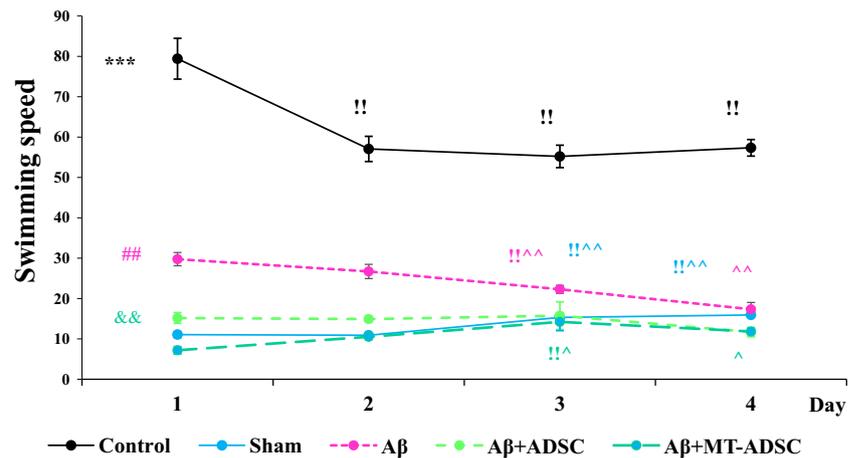
transplanted AD-MSCs and MT-ADMSCs differentiated to microglia in the brain. The microglia count in the brain significantly decreased in both experimental groups in comparison with the controls. However, the number of microglia was significantly lower in the MT-ADMSC group, compared to the AD-MSC group (Fig. 9e-h; $P < 0.05$).

Discussion

Stem cell therapy, especially MSC-based therapy, is promising for AD (Bali et al. 2017). The current study took some initial steps towards the improvement of MSC-based cell therapy for AD. The current study, for the first time, pretreated AD-MSCs with MT and then transplanted MT-AD-MSCs in an experimental AD model and investigated the healing effects. It was observed that MT improved ADMSC-based therapy in AD. Also, MT-ADMSCs improved the learning ability, memory function, and cognition significantly more than AD-MSCs. These beneficial effects were showed without change in the locomotion activity or anxiety behavior in the experimental groups.

The avoidance and spatial memory of all rats were examined by PAL and MWM test. Also, the MWM test is a negatively reinforced task and reaching the platform is considered rewarding (Paul et al. 2007). The average swimming speed is best variable for measured of animal motivation to reach reward in the water tank of MWM. The AD rat exhibited cognition and memory impairment without higher speed and more time of swimming in acquisition days of MWM test. This result is accordance to previous study in which A β micro-injection into brain has been considered as suitable model for induction of amnesia and reward learning impairment (Malin et al. 2001). The AD rats unable to distinguish various cues to reaching to platform as reward in learning. Current result is similar to reported study while the behavioral task and method was different (Malin et al. 2001; Shi et al. 2015; Shi et al. 2012). The A β +ADSC, A β +MT-ADSC groups presented better cognition and memory in NOR, PAL and MWM tests. The potential effect of MT and MT-ADSC did not affect in locomotion and anxiety behavior. However, the shorter distances have specials for β +ADSC and A β +MT-ADSC groups in the open field test (no significant). Therefore, the EPM test was chosen for to better understanding the effect of MT and MT-ADSC in anxiety. The result of EPM test revealed that the lower speed of β +ADSC and A β +MT-ADSC rats did not cause by anxiety. Also, the AD rats did not exhibit anxiolytic or anxiogenic behavior in EPM test.

Fig. 7 Effect of cell transplantation in A β rat model on mean of swimming speed (Cm/S) to find a hidden platform in the Morris Water Maze. Data are expressed as mean \pm SEM. ***= P<0.001 as compare control group. ##= P<0.01 as compare sham group. &&= P<0.01 as compare A β group. != P<0.01 as compare to first day. ^= P<0.01 as compared to second day. ^= P<0.05 as compared to second day



Of noted, the histopathological study showed more migration and presence of MT-AD-MSCs in brain tissues. Pretreatment of AD-MSCs with MT resulted in the significant clearance of A β deposition (Jahnke et al. 1999; Sánchez-Barceló et al. 2010). According to previous studies, MT treatment could improve MSC treatment for different diseases. In a study by Lee et al., treatment with MT improved MSC mobility in the umbilical cord blood, besides skin wound healing (Lee et al. 2014).

Based on several studies, MT treatment improves AD-MSCs treatment for sepsis-induced kidney damage, acute ischemia-reperfusion damage, and acute interstitial cystitis (Chen et al. 2014a; Chen et al. 2014b; Yip et al. 2013). Moreover, MT, which can promote the osteogenic differentiation of bone marrow MSCs, is applied in the treatment of osteoporosis (Amstrup et al. 2013; Zhang et al. 2010). As previously reported, MT exhibits major ROS elimination potential, inhibits the p53 pathway (Zhang and Zhang 2014), and regulates intracellular signaling

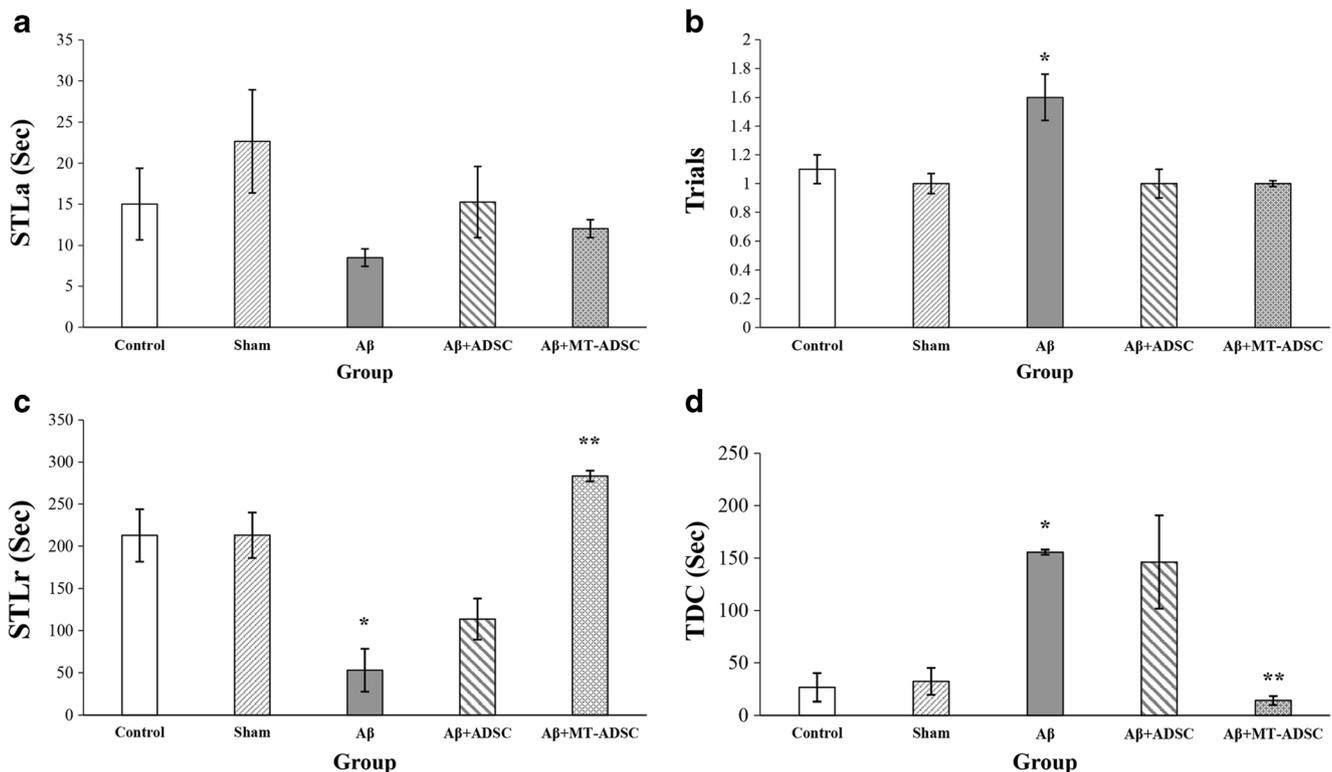


Fig. 8 Effect of cell transplantation in A β rat model on the step-through latency in acquisition trial (a), the number of trials to acquisition (b), the step-through latency in recognition trial (c), and the time spent in the dark

compartment (d) in passive avoidance learning task (*P <0.01 when compared with the control group, **P <0.05 when compared with the A β group). Data are expressed as mean \pm SEM

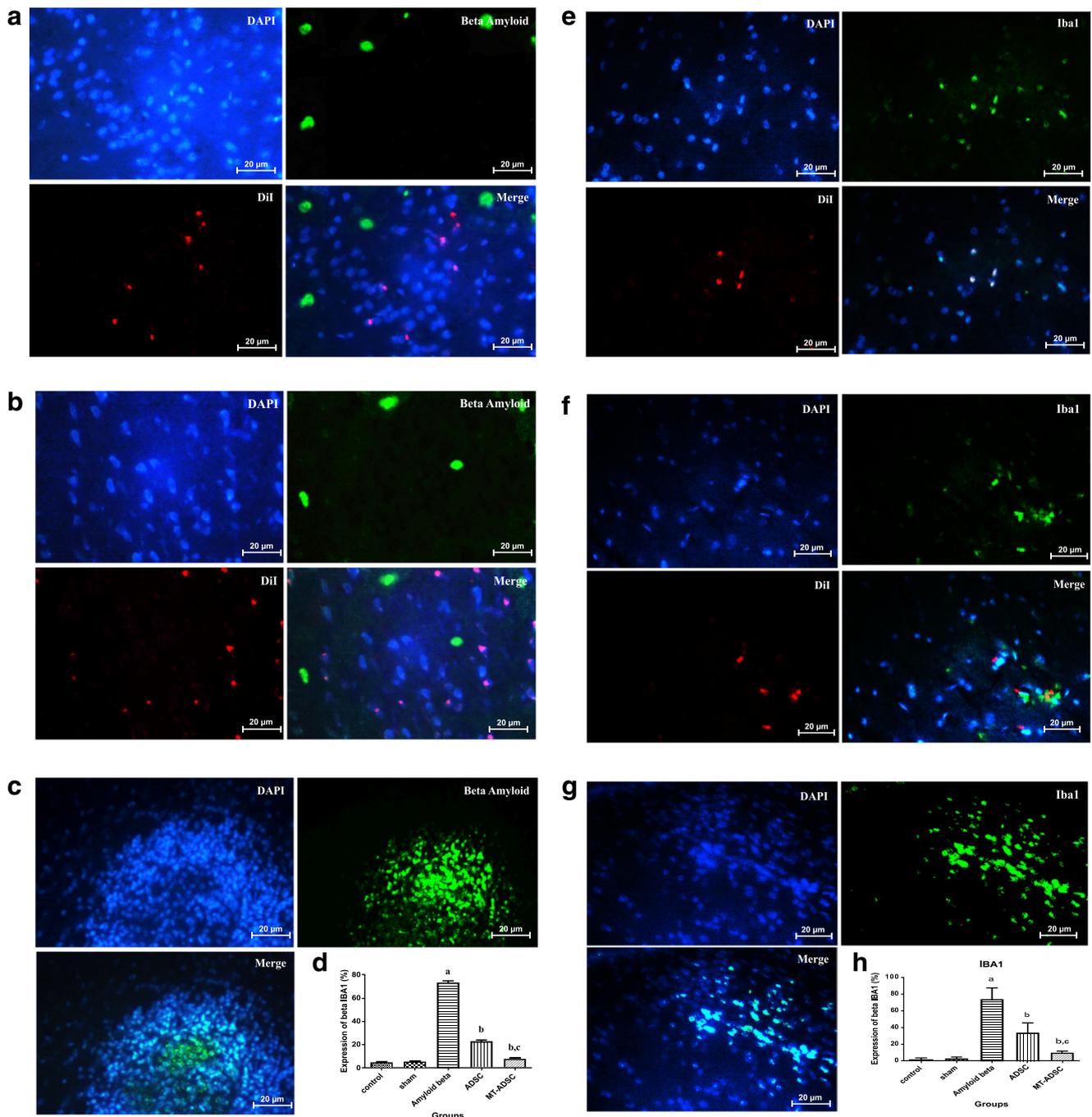


Fig. 9 Effect of cell transplantation on Iba1-positive cells and Aβ deposits in the brain of Aβ rat model detected by immunohistochemistry. ADSC (A, E), MT-ADSC (B, F), AD (C, G), percentage of amyloid beta

protein (D) and Iba1 protein (H). a: compared with the control group; b: compared with the Aβ group; c: compared with the ADSC group (P < 0.05)

(Luchetti et al. 2010). In addition, its effects on immune regulation and senescence delay have been reported (Bali et al. 2017; Cardinali et al. 2008; Espino et al. 2012; Koyama et al. 2002; Son et al. 2014).

In the current study, homing of both AD-MSCs and MT-ADMSCs in the brain was confirmed by an immunohistochemical study 69 days after intravenous cell transplantation. The results showed that preconditioning with MT increased

the transplanted cell count in the brain after this period; this increase can be due to the greater migration or survival of these cells in the brain (or both).

The transplanted cells were detected in the Aβ deposition site. The Aβ plaque studies showed that both AD-MSCs and MT-ADMSCs could clear Aβ deposits, which consequently led to the improvement of cognitive performance in AD rat models. However, the effect of

MT-ADMSCs was significantly greater on the clearance of A β deposits than that of AD-MSCs. Therefore, pretreatment with MT can increase all the outcomes of AD-MSC transplantation (Liu et al. 2016).

The current immunohistochemical study analyzed the differentiation of transplanted cells to microglia using Iba1. The histological results showed the differentiation of AD-MSCs and MT-ADMSCs into microglia at the site of A β deposits. The number of microglia cells was majorly lower in MT-ADMSCs versus AD-MSCs (Ohsawa et al. 2004). Microglia activation can have a dual role in the AD pathogenesis. On one hand, acute microglial activation may reduce the accumulation of A β , and on the other hand, chronic microglia activation may lead to synapse loss and neurotoxicity through stimulation of some proinflammatory cascades (Sarlus and Heneka 2017).

It has been demonstrated that bone marrow MSCs can preserve the resting microglia phenotype and control the activation of microglia (Yan et al. 2013). Also, the number of microglia depends on the plaque and A β count. Therefore, the current findings regarding the few number of microglia in MT-ADMSCs can be related to the enhanced regulatory impact of AD-MSCs and the reduced amount of A β (Mandrekar-Colucci and Landreth 2010).

Conclusion

The current study employed a simple, non-genetic engineering, and versatile strategy to improve AD-MSCs-based therapy, especially for AD. AD-MSCs were pretreated with one of the well-known antiinflammatory and antioxidant agents' Melatonin' and transplanted in a rat AD model. Consistent with our assumption, MT pretreatment increased the survival of AD-MSCs, improved learning, memory, and cognition, and reduced A β plaques, highlighting the positive effects of MT. Nevertheless, future comprehensive research is essential to clarify the mechanisms through which MT induces its positive effects on ADMSC-based therapy.

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