Scientific Research

Effect of BDNF and Adipose Derived Stem Cells Transplantation on Cognitive Deficit in Alzheimer Model of Rats

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ABSTRACT

In this study, the potential for recovery mediated by co-treatment of brain-derived neurotrophic factor (BDNF) and adipose tissue derived stem cells (ASCs) on functional recovery after Ibotenic acid (Ibo) lesion of the nucleus basalis magnocellularis (NBM) was examined. Ibotenic acid was injected bilaterally into the NBM of experimental rats, then the animals received treatments as follows: ASCs (500×10^3), BDNF (5 ug/ul) and a combination of BDNF and ASCs. Two months after the treatment, cognitive recovery was assessed by the Morris water-maze. These results showed that ASCs transplantation may have therapeutic value in disease and conditions that result in memory loss, and co-treatment with BDNF doesn't offer more efficacious cognitive function.

Keywords: Alzheimer's Disease; Adipose Stem Cells; Brain-Derived Neurotrophic Factor; Learning and Memory

1. Introduction

Alzheimer's disease (AD) is a progressive dementia associated with cholinergic cell deterioration in the nucleus of Meynert, which results in the loss of cognitive functions [1,2]. In AD, the severity of cognitive deficits correlates with synaptic loss in cholinergic neurons supplying neocortex and hippocampus [3].

Although adult brain has limited regenerative capability, to devise a method for manipulating and reimplanting neural stem cells is difficult. Stem cell therapy is one of the most interesting approaches for the treatment of neurodegenerative diseases. Our previous work showed that infusion of bone marrow mesenchymal stem cells in Alzheimer model of rats lead to improve learning and memory ability [4]. Recent studies have raised the possibility that adipose derived stem cells (ASCs) could be a good candidate for brain repair, due to their accessibility [5], and potency to differentiate into neurons [6,7]. However the main problem in stem cells transplantation strategy is how to improve cell survival *in vivo*. It has been known that the migration of stem cells is controlled by a set of special genes introducing the neurotrophic growth factors [8]. Studies show that BDNF, a member of the neurotrophin family, which is wildly expressed in adult brain areas, prevents neuronal degeneration during development [9] and promotes *in vivo* neurogenesis in adult forebrain

[10]. A new strategy on manipulating components of the niche that facilitates cross-talk between stem cells and the dysfunctional brain may offer more efficacious neurotransplantation [11]. The hypothesis that BDNF promotes survival and differentiation of grafted neural stem cells [12,13] triggered us to investigate whether the co-treatment of BDNF with adipose stem cells increases the effects of transplanted cells in restoring cognitive deficit. Since the ultimate goal for cell therapy is functionality and few studies have examined a cognitive endpoint, here we focused on changes in learning and memory recovery.

2. Materials & Methods

2.1. Animals

Fifty-seven male Wistar rats weighing 250 - 300 g were used for this study. Animals were housed with free access to food and water in a 12-h light/dark cycle and constant temperature. All procedures concerning animal care were in accordance with Guilan University of Medical Sciences ethical committee article (DEC No. 2719).

2.2. Surgical Procedures & Behavioral Test

To establish the AD animal model, we infused Ibotenic acid into the nucleus basalis magnocellularis. On the day of surgery, the animals were anesthetized with ketamine/

xylazine (50 mg/kg, i.p.) and placed in a computerized stereotaxic apparatus (Neurostar, Germany). The incisor bar was set at: −1.14 mm posterior and ±2.46 mm lateral to the bregma and 7.9 below the top of the skull to reach the nucleus basalis magnocellularis [14], then guide cannula was implanted bilaterally for further infusions. Rats received bilateral infusions of 0.5 ul or Ibotenic acid (10 ug/ul) using a 5 ul Hamilton syringe. After 14 days, rats were tested in the Morris Water Maze (MWM) in order to test learning ability. Animals who showed memory impairment were distributed into 4 groups: IBO + BDNF (5 ug/ul), IBO+ASCs (500 × 10^3 cells), IBO + (ASCs/ BDNF) and IBO+PBS. The control intact animals received only PBS. BDNF infusion was repeated twice for two weeks after the initiation of treatment in BDNF-receiving groups.

The Morris water maze [15] consisted of a black pool (148 cm diameter) filled with water (26°C ± 2°C). A circular black platform was submerged 2 cm below the water surface, in the middle of the target quadrant. The behavior of the rats in the pool could be tracked with a camera connected to Ethovision system (Noldus, EX 6.1, Netherlands) allowing us to measure swim speed, distance and latency to find the platform. Rats were trained with a protocol of four trials per day, with an interval of 20 min, for 5 consecutive days. A probe trial was administered on the fifth day, when each subject was placed into the water diagonally opposite the target quadrant, and allowed 90 s to search the water, from which the platform had been removed.

2.3. Adipose Stem Cells Isolation and Culture

Adipose tissue was obtained from the abdomen. One gram adipose tissue was incubated with 1.5 mg collagenase type II in 10 ml saline at 37°C for one hour. Digested adipose tissues were centrifuged for ten minutes at 1500 rpm, and pellets were washed with 10 ml and plated in 25 ml cultured flask containing DMEM, 10% FBS and antibiotics. Then cells were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. On reaching confluency, the adherent cells were detached by 0.05% trypsin and 0.02% EDTA for 5 min at 37°C, harvested and washed with DMEM and 10% FBS and finally resuspended in complete medium. After 2 - 3 passages, the morphologically homogeneous population of ASCs was analyzed for the expression of cell surface molecules using histology staining. Flow cytometry test was used for detecting stem cells markers of CD44, CD 105, CD90 according to standard protocols [5]. Cell suspensions with viability more than 90%, were made at a density of 500 × 10^3 cells/μl and were kept on ice to optimize cell viability until infusion.

2.4. Statistical Analysis

The data is expressed as means ± SEM. Group differences in the escape latency in the Morris water maze probe task were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The repeated ANOVA measure for multiple group comparison was used to analyze group differences of the data collected during the training days.

3. Results

Adipose stem cells were successfully culture-expanded and a morphologically homogeneous population of fibroblast-like cells was seen after 14 days. Flow cytometry analysis showed uniformly positive stem cells for CD44, CD105, CD90 as shown in **Figure 1**.

Since the ultimate measure of stem cells transplantation into the brain is functionality, we tested animals two months after transplantation. As seen in **Figure 2**, there was no initial difference between treated animals during the first days of acquisition in the Morris water maze.

Treated rats acquired the task rapidly in all groups, and latency to escape diminished over time (p < 0.001). Since the experimental groups didn't differ in swim speed (Ibo: 22 ± 0.6 cm/s, cell/BDNF: 22.7 ± 0.4 cm/s, ASCs: 21.8 ± 0.93, F (4, 170) = 0.85, p > 0.05), we used latency to find the platform as an indicator of learning performance. The improvement in acquisition was found in ASCs-transplanted group across trials ($F_{9,387}$ = 91.3; p < 0.0001). Cell transplanted rats spent shorter time (11.3 ± 0.9 sec; **Figures 3** and **5**) to reach the platform in probe test, and also spent more time in the target quadrant (25 ± 1.34 sec; **Figure 4**) compared to other treated groups, except unlesioned control group.

There was no statistical difference in the ability of learning and memory between BDNF and Ibo + PBS (p = 0.13); however, the BDNF group showed better performance (**Figure 2**). Although water maze acquisition was improved by adipose stem cells transplantation, but none of the treated groups reached to the level of the naive group (**Figure 2**).

4. Discussion

In the present study, the transplanted group showed an improvement in learning performance, indicating effectiveness of ASCs in restoring learning capability of AD animals two months after transplantation. This finding is in agreement with the results of similar studies on bone marrow mesenchymal stem cells [4], neural stem cells [13,16], and umbilical cord blood stem cells transplantation in AD animals [17]. Although the mechanisms of recovery are not completely understood, a first explanation could be the capacity of ASCs to dif ferentiate into
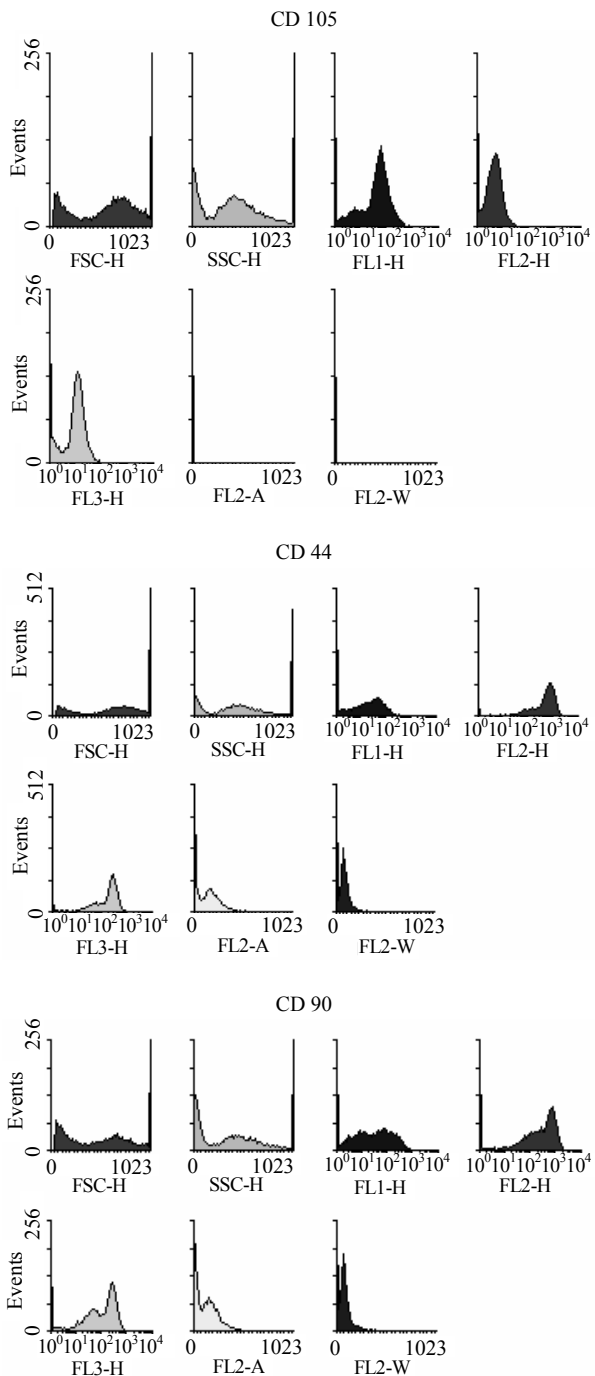
Figure 1. Flow cytometric analysis of ASCs indicated cell surface phenotype characteristic CD44, CD90, CD105.

the new cholinergic neurons to compensate cholinergic deficit. The potency of these cells to differentiate into the neuron has been reported in previous studies [3,6]. However we cannot exclude the possibility that stem cells may provide therapeutic effects by keeping the integrity of neurons through exerting protective chaperone effect [12,18] or neurochemical modulation.

Moreover, we showed that infusion of recombinant
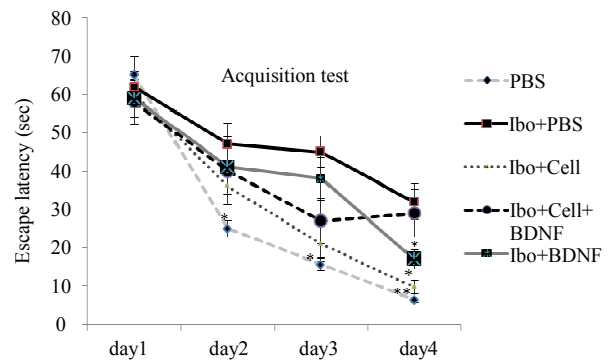
Figure 2. The ability of spatial learning in MWM. PBS control (n = 13), Ibo + PBS (n = 10), IBO + BDNF (n = 8), IBO + ASCs (n = 14), IBO + (ASCs + BDNF, n = 8) *p < 0.01, **p < 0.001 compared with Ibo + group.
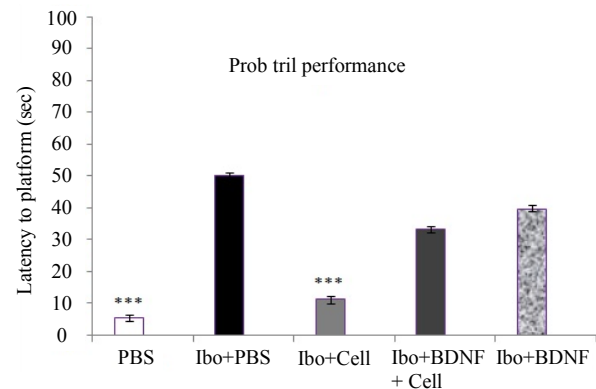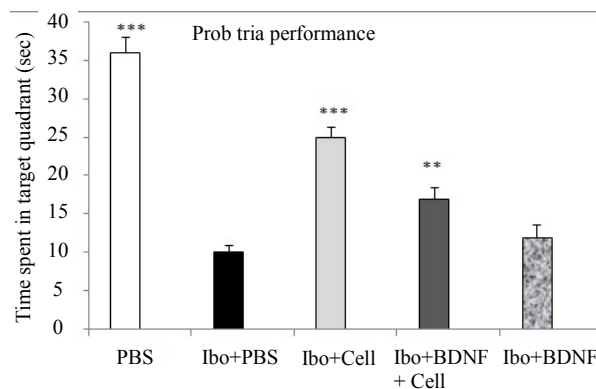

Figure 3. Performance of animals in prob trial. Means and standard errors of the latency to find the platform, in sec are depicted PBS control (n = 13), Ibo + PBS (n = 10), IBO + BDNF (n = 8), IBO + ASCs (n = 14), IBO + (ASCs/BDNF, n = 8) **p < 0.001 compared with Ibo-group.

Figure 4. The ability of spatial learning in MWM. PBS control (n = 13), Ibo + PBS (n = 10), IBO + BDNF (n = 8), IBO + ASCs (n = 14), IBO + (ASCs + BDNF, n = 8) *p < 0.01, **p < 0.001 compared with Ibo + group.

BDNF into the NBM, 14 days afte the lesion, slightly reverses the deficit in memory caused by Ibo. It has been documented that BDNF is implicated in learning and memory [19,20]. Several investigators have reported pro-
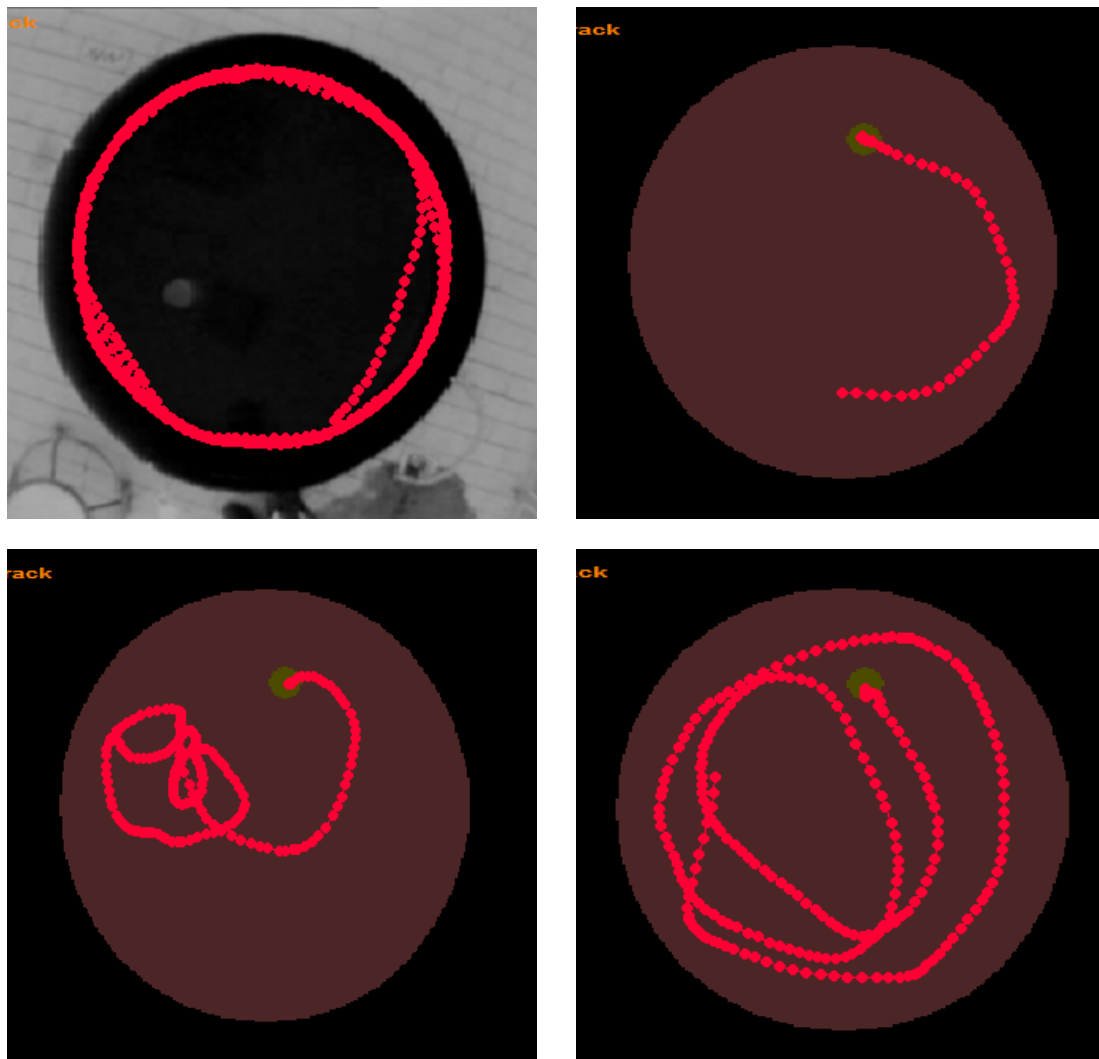
Figure 5. Shows a sample of computer tracking from probe trial (90 sec duration). Top left: Ibotenic acid, top right: ASCs transplanted, down left: BDNF and down right: ASCs + BDNF. The rat of Ibo group swims in a concentric pattern, whereas cell treated group swims in a short direct pattern toward the hidden platform.

tective and anti-apoptotic effects of BDNF on neural cells [9,12,21] and that it induces neurogenesis [22]. It is possible that BDNF induces the survival of some but not all of the degenerating cholinergic neurons in the NBM.

We initially assumed that adding BDNF to stem cells will increase learning and memory performance. Much to our surprise, the present study shows that adding BDNF to ASCs leads to improvement in learning ability, but in a lesser extent compared to cells alone. Our results appear to contradict results of Xuan [13], who reported that BDNF improves the effects of neural stem cells on the rat model of Alzheimer's disease. The reasons for this discrepancy might be the source of stem cells which in their studies was neural stem cells, and also method of lesion, which was unilateral fimbria-fornix lesioning which causes axonal damage [23], whereas IBO induces neuronal necrosis which is confined to somata of neurons, without

affecting on myelinated axons or blood vessels [24].

To explain why adding BDNF to stem cells didn't lead to an enhancement in learning performance compared to stem cells infusion, one can point to the concentration of the BDNF. There are studies showing that Brain-derived neurotrophic factor in high concentration might induce adverse effects. Although we didn't measure the BDNF levels in NBM after the transplantation, the possible release of BDNF by injured brain [2], glia cells [25] and also transplanted ASCs (Kang *et al*., 2003; McCoy *et al*., 2008), might lead to increase in the level of BDNF, and this neurothrophine, in high concentration causes learning deficits [26,27]. BDNF plays different roles in learning and memory depending on receptors; the receptor P75NTR, involves in, LTD and cell death, however Trkβ is responsible for LTP and survival of neurons [26].

To answer this question that how much stem cells ther-

apy is efficient for Alzheimer treatment, we should mention that although enhancement of learning ability was achieved after the ASCs transplantation, but none of the treated groups reached the control levels performance. This indicates that adding stem cells to neural network is not sufficient to restore learning and memory completely. It is well known that the learning process, as a complex phenomenon in CNS, needs an orchestrated chain of translational and transcriptional events in functional synapses in order to encode, store, and recall information appropriately [20].

One limitation of this study is not being able to assess the fate of the transplanted stem cells and follow up the functional outcome of grafted cells over three months. Although transplanted cells take over a month *in vivo* to develop, the electrophysiological responsiveness of mature neurons form long projecting axons to the forebrain, but it takes at least 3 months for a memory to manifest [21,28].

5. Discussion

Our results showed that ASCs treatment significantly increased learning and memory ability in Alzheimer model of rats. Addition of BDNF to ASCs doesn't improve the effectiveness of cells in restoring cognitive function.

In a clinical point of view, and considering less ethical problems and ease of accessibility, adipose stem cells could be a valuable therapeutic tool in treating patients suffering from neurodegenerative diseases. More broadly, this study supports the view that manipulating components of the niche affects on cross-talk between stem cells and the dysfunctional brain, but not always bring efficacious neuro transplantation outcome.

6. Acknowledgements

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