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Human Umbilical Cord Stem Cell Xenografts Improve Cognitive Decline and Reduce the Amyloid Burden in a Mouse Model of Alzheimer's Disease

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Abstract

Introduction—Alzheimer's disease (AD) is the most common cause of dementia. The search for new treatments is made more urgent given its increasing prevalence resulting from the aging of the global population. Over the past 20 years, stem cell technologies have become an increasingly attractive option to both study and potentially treat neurodegenerative diseases. Several investigators reported a beneficial effect of different types of stem or progenitor cells on the pathology and cognitive function in AD models. Mouse models are one of the most important research tools for finding new treatment for AD. We aimed to explore the possible therapeutic potential of human umbilical cord mesenchymal stem cell xenografts in a transgenic (Tg) mouse model of AD.

Methods—APP/PS1 Tg AD model mice received human umbilical cord stem cells, directly injected into the carotid artery. To test the efficacy of the umbilical cord stem cells in this AD model, behavioral tasks (sensorimotor and cognitive tests) and immunohistochemical quantitation of the pathology was performed.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Results—Treatment of the APP/PS1 AD model mice, with human umbilical cord stem cells, produced a reduction of the amyloid beta burden in the cortex and the hippocampus which correlated with a reduction of the cognitive loss.

Conclusion—Human umbilical cord mesenchymal stem cells appear to reduce AD pathology in a transgenic mouse model as documented by a reduction of the amyloid plaque burden compared to controls. This amelioration of pathology correlates with improvements on cognitive and sensorimotor tasks.

Keywords

Alzheimer; Stem Cell; Behavior; Histology; Animal Model; Amyloid Beta

INTRODUCTION

Globally more than 47 million people suffer from dementia, with the likelihood that this number will greatly increase in the coming years [1]. Of all neurodegenerative disorders, Alzheimer's disease (AD) is the most prevalent. The current global cost of care for AD is approximately $605 billion, or about 1% of the entire world's gross domestic product. AD is a neurodegenerative disease defined in the brain by pathological accumulation of amyloid β (Aβ) into extracellular plaques in the brain parenchyma and in the vasculature (known as congophilic amyloid angiopathy [CAA]), and abnormally phosphorylated tau that accumulates intraneuronally forming neurofibrillary tangles (NFTs); lesions are associated with neuronal loss in specific brain regions [2]. Currently there are no effective means to treat or slow down the pathology of AD; although, there are a number of potential therapies in preclinical development and in clinical trials [3]. In recent years, mesenchymal stem cells (MSCs) have been considered a promising therapeutic strategy for both acute injury and progressive degenerative diseases of the central nervous system [4–8]. It has been proposed that stem cell therapies can replace lost cells by differentiating into functional neural tissue; modulate the immune system to prevent further neurodegeneration and effect repair or provide trophic support for the diseased central nervous system (CNS) [7, 8]. Moreover, MSCs greatly enhance autolysosome formation and clearance of Aβ in AD models, which may lead to increased neuronal survival in the setting of Aβ pathology [9]. MSCs have also been shown to be protective against cell death induced by misfolded tau protein [5]. One source of stems cells is human umbilical cord blood mesenchymal stem cells (hUCB-MSCs). In this study, we tested the efficacy of hUCB-MSCs tested the efficacy of hUCB-MSCs in an APP/PS1AD mouse model using an intra-carotid route, following injection of mannitol to transiently open the blood-brain barrier (BBB).

MATERIAL AND METHODS

Animal Model

All the procedures were done according to the guidelines of the institutional animal care committee of NYU School of Medicine. The AD animal model used in this study was a well characterized double transgenic mouse line expressing mutated APP and PS1 [10]. The mice were used at 9–10 months of age a point at which they have extensive amyloid deposition.

All the mice were females. Animals were divided into 2 groups. Five animals served as controls and 5 received human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) by intra-carotid artery, immediately following a 200 μl injection of 20% mannitol in phosphate buffer saline (PBS) to transiently open the BBB, as we have previously published [11, 12].

Stem Cell Preparation

Human umbilical cord-derived mesenchymal stem cells were purchased from American Type Culture Collection (ATCC® PCS500010™, Manassas City, VA, USA), together with their growth-supporting media, growth factors, enzymes and antibiotics. The cells were prepared and sub-cultured according to the manufacturer's guidelines and procedures. Cells from the 5^{th} and 6^{th} passage were utilized for injection. Cells were suspended in 0.5 ml of PBS (GIBCO Invitrogen Corporation, Cat# 14190-094, United Kingdom) at a density of 1×10^6 cells/ml. 5×10^5 cells were injected. The morphology of the injected hUCB-MSCs is shown in Fig. (1).

ANIMAL BEHAVIOR ASSESSMENTS

Sensorimotor Tests

Rotarod—Animals were placed onto the rod to measure forelimb and hindlimb motor coordination and balance. The trials were conducted as previously published [13, 14].

Locomotor Activity—A Hamelton-Kinder photobeam system was used to measure the activity of the animals. A video camera recorded the horizontal movements on the circular open field chamber (75 × 75 cm). Each animal were tested for 15 minutes, the total distance (cm), the mean resting time and the velocity (mean and maximum) were recorded, as previously published [13, 14].

Cognitive Test

Object Recognition: The object recognition test was used to measure changes in short term memory. This was done by allowing the mice to explore a square-shaped open field box (48 cm square, 18 cm high) with two novel objects at diagonal corners. The time was recorded via a tracking system. After 3 hours upon which retention delay occurs, the mice were placed into the box with a novel object placed. The duration to explore the novel object is 6 minutes. The percentage short term memory score is calculated as previously published [13, 14].

Immunohistochemistry: The right hemisphere was immersion-fixed overnight in periodate-lysine-paraform-aldehyde. Following fixation, the brain was moved to a phosphate buffer solution containing 20% glycerol and 2% dimethylsulfoxide (DMSO) and stored at 4°C until sectioned. Serial coronal brain sections (40 μm) were, placed in ethylene glycol cryo-protectant and stored at −20°C until they were used. Sections were immunolabeled with a monoclonal anti-Aβ antibody 6E10 as previously described [13, 14]. To detect the presence of the human stem cells in the mouse brains, the sections were immunolabeled with a mouse monoclonal antibody to human nuclear antigen (Cat. No.: NBP2-34342, Novus Biologicals)

that is specific for human cells. To examine the brain inflammation levels of treated mice, we assessed the degree of astrocytosis and microgliosis with GFAP and Iba-1 antibodies respectively. Astrogliosis and microgliosis were analyzed at 10X magnifications in the cortex and in the hippocampus. Reactive astrocytosis was rated on a scale from 0 to 4. The rating for astrogliosis was based on the extent of GFAP immunoreactivity (number of GFAP immunoreactive cells and complexity of astrocytic branching) as previously published [14]. The assessment of microgliosis was based on the extent of immunoreactivity with Iba-1 antibody. Rating with rating from 0 (few resting microglia) to 4 (numerous ramified/ phagocytic microglia) in increments of 0.5. The rating was done by an observer blinded to the treatment status of the mice [14]. The images of the immunostained tissue sections with the monoclonal antibody 6E10 were acquired by Nikon Eclipse E Fluorescence microscope (Nikon Instruments, Melville, NY) and processed by NIS elements for advanced research, with the input levels adjusted to span the range of acquired signal intensities. Total Aβ burden (defined as the percentage of test area occupied by Aβ) was quantified for the cortex and for the hippocampus on coronal plane sections stained with the monoclonal antibodies 6E10. For each mouse five adjacent sections were selected, and from each section 5 random fields were analyzed. Total area of pixel intensity was measured with the automated measurements tools in NIS Elements software. The total intensity was averaged and expressed as normalized, corrected values. All procedures were performed by an individual blinded to the experimental condition of the study [14].

RESULTS

Behavioral studies (Rotarod, Locomotor Activity and Object Recognition)

One month following the injection of hUCB-MSCs, the mice were subjected to behavioral testing (sensorimotor and cognitive tasks). On rotarod testing, treated animals navigated better than controls which received only PBS ($p=0.0024$, by two-tailed student's t-test) Fig. (2A). On locomotor activity testing no significant differences were seen between the two groups in terms of the distance traveled (Fig. 2B), Vmax (Fig. 2C), Vmean (Fig. 2D) or in rest time (Fig. 2E).

In addition to locomotor evaluations, the two groups of mice underwent cognitive testing using the Novel Object Recognition test. Mice treated with hUC-MSCs spent more time with the novel object compared to that of the old object (Fig. 3, one tailed t-test, $p= 0.0463$, comparing first and second bars). The time spend with the novel object differed significantly between the of hUCB-MSCs treated and control Tg groups (Fig. 3, one-tailed t-test, $p=0.0427$, comparing first and third bars).

Histology

After the behavioral tasks, both groups were sacrificed at 10 to 11 months of age and the brains were extracted and further processed for histology. Morphological analysis showed that treated animals had fewer plaques than controls receiving only PBS as detected by 6E10. To determine the efficacy of stem cell therapy in the APP/PS1 Tg mouse model, the total amyloid burden was quantified by stereological technique using random unbiased sampling in the serial sections. The amyloid burden is shown in the cortex and hippocampus

in Fig. (4). Significant reduction of the amyloid burden was observed in the total cortex by 26% (two tailed t-test, p=0.0084) and in the hippocampus by 38% (two tailed t-test, p=0.0011).

Astrogliosis

To evaluate the effect of stem cells on brain inflammation, serial sections were stained with GFAP and Iba-1 antibodies. Semi–quantitative analysis of astrocytes (GFAP immunolabeling) didn't show a significant difference between both treated and untreated groups (Fig. 5). Reduction or activation of astrocytes in treated animals versus controls was not statistically significant. However, a significant difference were observed with microglia immunoreactivity in the cortex and hippocampus of treated animals versus controls, respectively (one tailed Mann-Whitney test p=0.0011 and p=0.042 in the cortex and hippocampus, respectively, Fig. 6).

Assessment for the presence of hUCB-MSCs in the Brain

To assess for the presence of the human stem cells in the mouse brains, the sections were immunolabeled with a mouse monoclonal antibody to human nuclear antigen (Cat. No.: NBP2-34342, Novus Biologicals) that is specific for human cells. Immunolabeled cells were detected scattered throughout the brain of all hUCB-MSCs injected mice, while in control mice no immunolabeled cells could be detected (Fig. 7).

DISCUSSION

Neurodegenerative diseases create a tremendous social burden due to their devastating nature, cost, and lack of effective therapies. There are many ongoing clinical trials to treat Alzheimer's disease; however, so far the results of these clinical trials has been disappointing [15]. Cellular therapies offer great promise for the treatment of these diseases, and research progress to date supports the utilization of stem cells to offer cellular replacement and/or provide environmental enrichment to attenuate neurodegeneration [4–8]. In diseases where specific subpopulations of cells or widespread neuronal loss are present, cellular replacement may reproduce or stabilize neuronal networks. In addition, environmental enrichment may provide neurotrophic support to remaining cells or prevent the production or accumulation of toxic factors that harm neurons. Bone marrow-derived MSCs (BM-MSCs) have great potential as therapeutic agents since they are easily obtained from bone marrow and can be expanded rapidly *ex vivo* for autologous transplantation. They are allogeneic and non-immunogenic, thus eliminating the risk of rejection [16]. The potential of BM-MSCs in the treatment of AD was shown in an acute AD mouse model, where the intracerebral transplantation of BM-MSCs promoted the reduction of Aβ through the microglial activation [17]. This AD model consisted of the acute injection of Aβ peptide into the dentate gyrus (DG) of the hippocampus in wild-type C57BL/6 mice. It was also suggested that BM-MSCs can ameliorate Aβ-induced neurotoxicity and cognitive decline by inhibiting apoptotic cell death and oxidative stress in the hippocampus [18] The intracerebral transplantation of BM-MSCs into amyloid precursor protein (APP)/presenilin 1 (PS1) double-transgenic AD model mice modulated the immune/inflammatory responses resulting in a reduction of pathology and improvements in the cognitive decline [19]. It has also been

shown that BM-MSC injected to the tail vein of the AD model rats not only migrate through the blood–brain barrier, but survive in the hippocampus with associated cognitive benefits [20]. Single intra-cerebral injection of BM-MSCs produced an acute reduction in Aβ deposits and facilitated changes in key proteins required for synaptic transmission in young AD mice [21].

Another source of stem cells is human umbilical cord blood mesenchymal stem cells (hUCB-MSCs). It has been shown that co-culture of hUCB-MSCs with BV2 microglia exposed to Aβ42 induced a reduction of the Aβ in the medium in association with an increased expression of the Aβ-degrading enzyme neprilysin (NEP) in the microglia [22]. When hUCB-MSCs were transplanted into the hippocampus of a 10-month-old transgenic mouse model of AD (with extensive pathology) for 10, 20, or 40 days, NEP expression was increased in the mice brains, in association with increased production of soluble intracellular adhesion molecule-1 (sICAM) [22]. Moreover, Aβ plaques in the hippocampus and other brain regions were decreased associated with active migration of hUCB-MSCs toward Aβ deposits. This data suggests that hUCB-MSC-derived sICAM-1 decreases Aβ plaques by inducing NEP expression in microglia through the sICAM-1/LFA-1 signaling pathway. Injecting hUCB-MSCs by intracerebral cannula into the hippocampus of APP/PS1 AD model transgenic mice resulted in a significant improvement of spatial learning and memory [23]. These investigators also reported a reduction in tau hyperphosphorylation. The effects were associated with reversal of disease-associated microglial neuroinflammation, as evidenced by decreased microglia-induced proinflammatory cytokines, elevated alternatively activated microglia and increased anti-inflammatory cytokines. They concluded that hUCB-MSCs exerted a neuroprotective effect through modulation of microglial activation state, thereby ameliorating disease pathophysiology and reversing the cognitive decline associated with amyloid deposition in AD mice. hUCB-MSCs have proven to be more beneficial than bone marrow-derived MSCs in term of cell procurement, storage and transplantation [24].

For stem cell delivery to the brain, systemic administration is preferable. Intracranial stem cell delivery may be associated with side effects such as bleeding and local tissue injury. However, systemic injection requires overcoming the blood brain barrier (BBB) in order to achieve adequate entry of stem cells into the CNS. Mannitol injection has been extensively used to transiently open the BBB and has also been shown to enhance delivery of stem cells to the brain [11, 25]. In our study, we used this method to open the BBB, followed immediately by injection of 1×10^5 hUC-MSCs via the carotid artery. After one month, sensorimotor and cognitive tasks were performed. Significant difference was observed with rotarod testing, where treated animals navigated better than controls that received PBS only, suggesting an improvement in the coordination of treated mice. Importantly, locomotor activity testing did not show any significant differences between the groups, suggesting that basic locomotor function and the state of alertness was similar in both groups. The presence of differences in locomotor activity could confound the interpretation of cognitive testing. On novel object recognition testing we found that the hUC-MSCs treatment led to a significant cognitive improvement. Furthermore, the quantification of the amyloid burden performed on serial brain sections by immunohistochemistry shows a reduction of the amyloid burden in the cortex and the hippocampus of treated animals. The histopathology results correlated with the effect observed in the object recognition test. There was no

evidence of inflammatory toxicity with the use of the hUC-MSCs as judged by GFAP immunoreactivity, which showed no significant differences between treated and control Tg mice. Whereas, significant difference were observed with microglia immunoreactivity in the cortex and in the hippocampus of treated versus controls. Recently, similar data obtained by systemic transplantation of bone marrow-derived mesenchymal stem cells in an AD mouse model were reported, supporting our findings [26].

The mechanisms involved are unclear, but there are a number of non-mutually exclusive possibilities. It has been reported that hUCB-MSCs secrete high levels of sICAM-1, which induces NEP expression, a key Aβ-degrading enzyme in microglia. Furthermore, hUCB-MSC-derived sICAM-1 interrupted the CD40/CD40L interaction on microglia through down regulation of CD40 expression in microglia [22]. It has also been reported that galectin-3 secreted by hUCB-MSCs protects against Aβ42 neurotoxicity [22]. Finally, hUCB-MSCs may participate simultaneously in Aβ clearance and neuronal survival through a paracrine mechanism in the AD microenvironment [22].

Our results suggest that a single application of hUC-MSCs administered by a systemic route are a promising therapy that can modulate the CNS environment to ameliorate AD related pathology and its associated cognitive symptoms.

Acknowledgments

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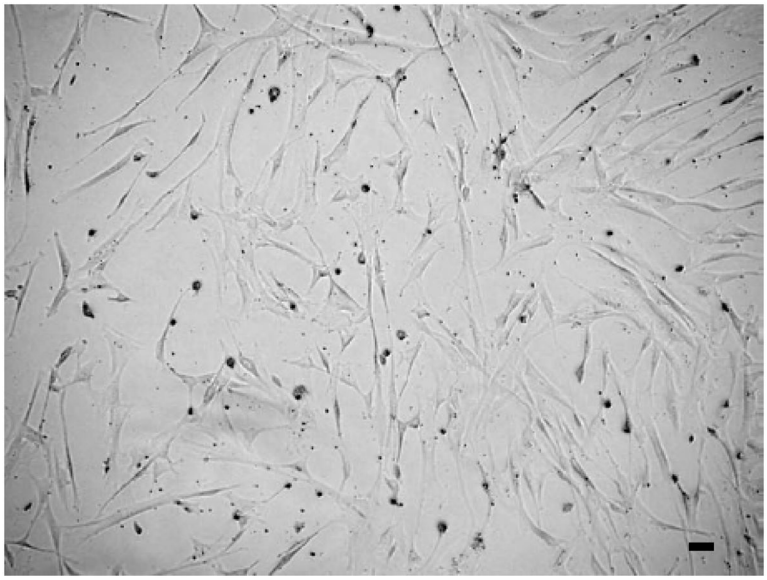

Fig. (1).
Shows representative morphology of the human umbilical cord Stem cells obtained at the 5 passage. These stem cells were injected via the carotid artery after mannitol infusion to transiently open the blood brain barrier. (Magnification 10X and Scale bar = 25μm).

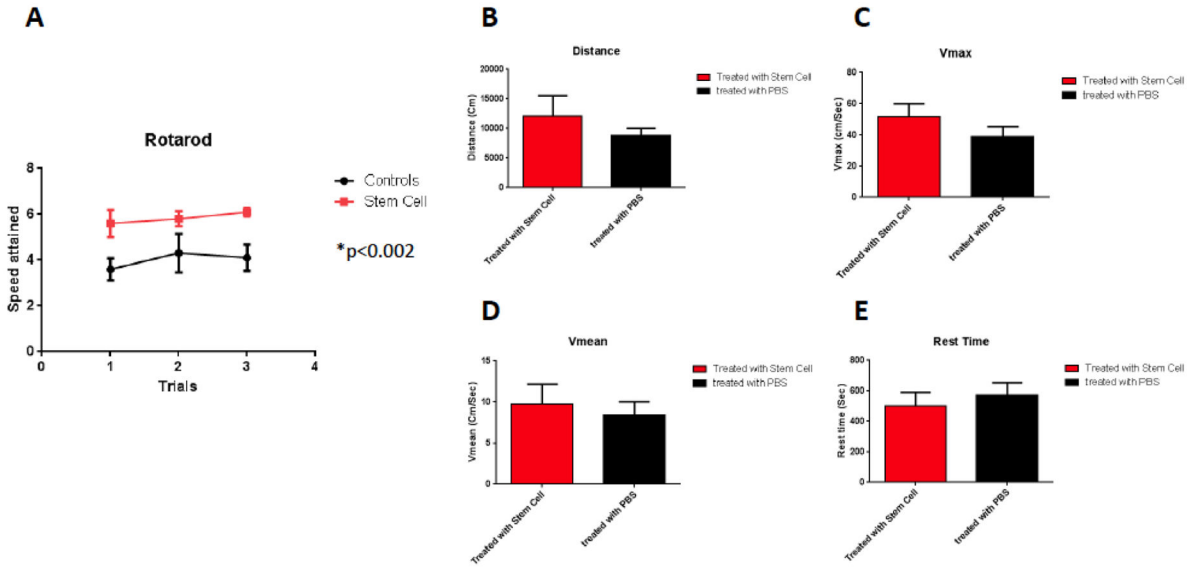

Fig. (2).

Rotarod: A significant difference was observed between controls (n=5) and treated mice with stem cells (n=5) (Two tailed t-test, p=0.0024) (Fig. 2A). Locomotor activity: No significant difference was observed between treated animals and controls in their distance traveled (Fig. 2B), maximum velocity (Fig. 2C), Average speed (Fig. 2D) or resting time (Fig. 2E).

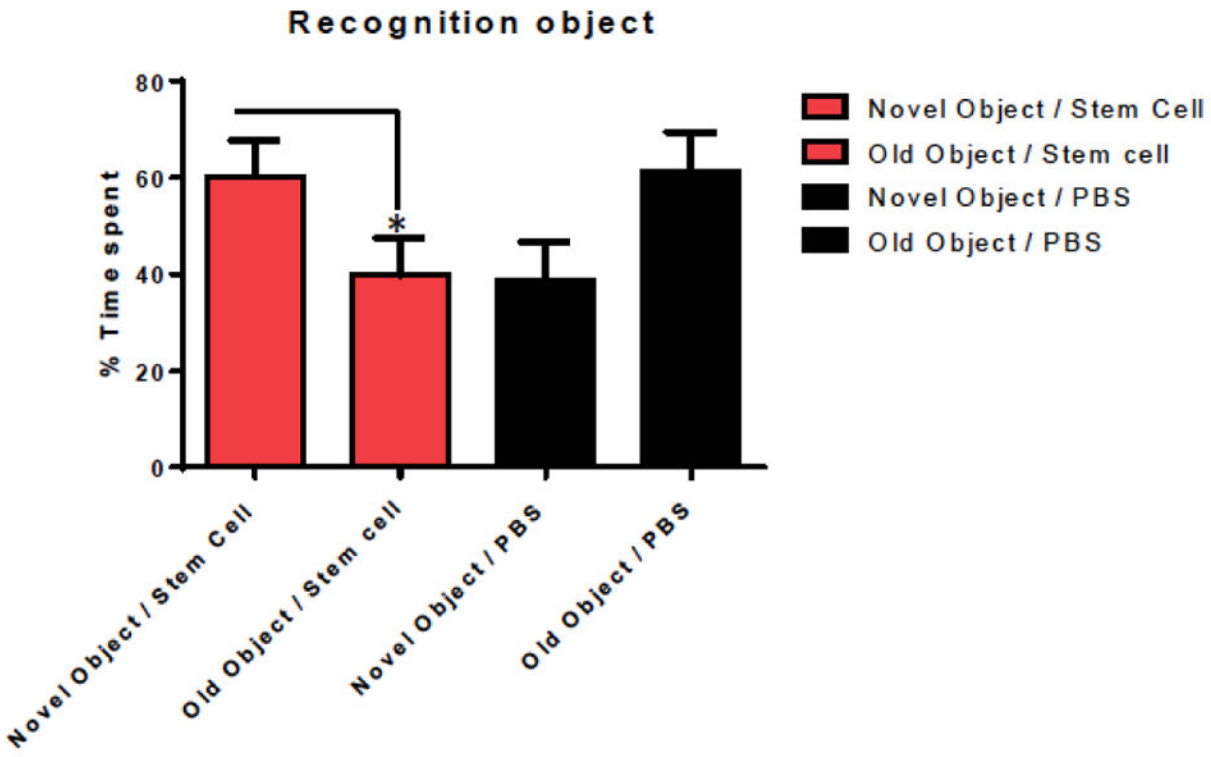

Fig. (3).

Shows working memory improvement using a short term memory test (Novel Object Recognition Test). The bars depict the percentage of amount of time spend with the novel or old object. Mice treated with hUC-stem cells spent more time with the novel object compared to the old object (one tailed t-test, p= 0.0463). The time spend with the novel object differed significantly between the stem cell treated and control Tg groups (n=5 per group) (one-tailed t-test, p=0.0427).

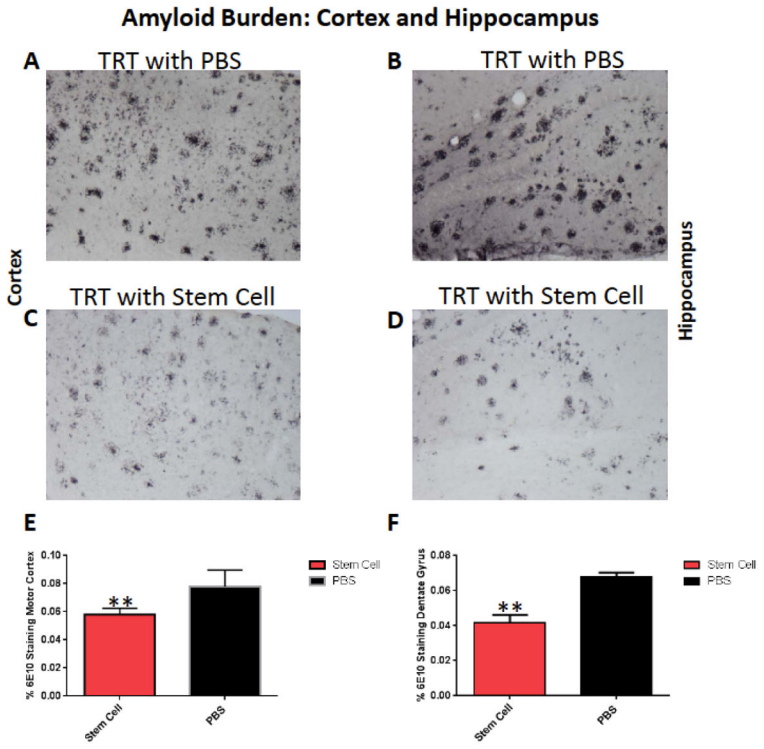

Fig. (4).

Shows representative brain sections from both group (n=5 per group) showing Aβ immunostaining in the cortex (A and C) and in the hippocampus (B and D) in PBS treated mice (A and B) and hUC-stem cells treated animal (C and D). A significant reduction of the amyloid burden was observed in the cortex by 26% (E, two tailed t-test, p=0.0084) and in the hippocampus by 38% (F, two tailed t-test, p= 0.0011).

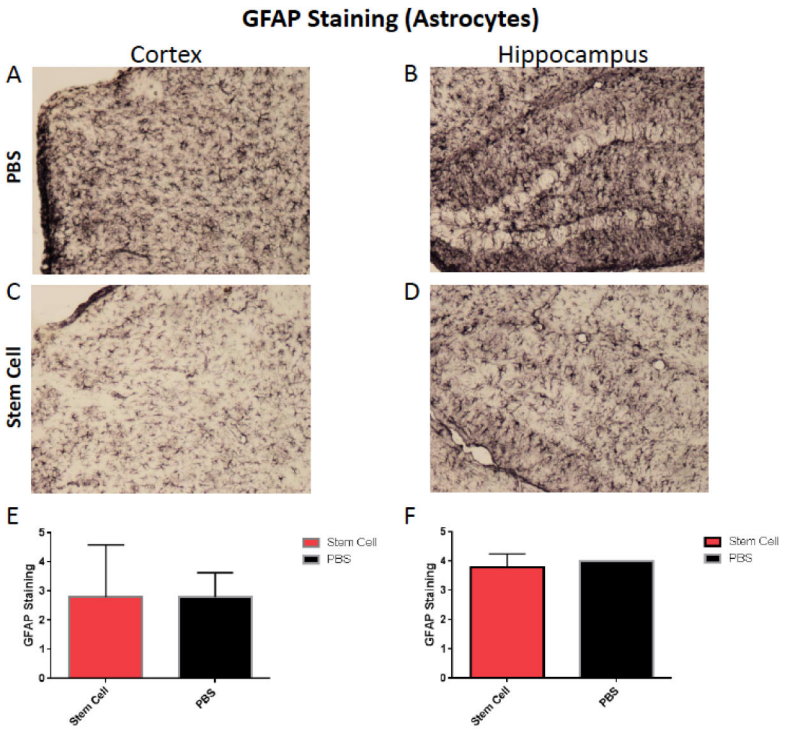

Fig. (5).

Shows representative brain sections from both group showing GFAP immunostaining in the cortex (A and C) and in the hippocampus (B and D) in PBS treated mice (n=5) (A and B) and hUC-stem cells treated animal (n=5) (C and D). Semi-quantitative analysis of the GFAP immunoreactivity did not show any significant difference in the cortex (E) or hippocampus (F).

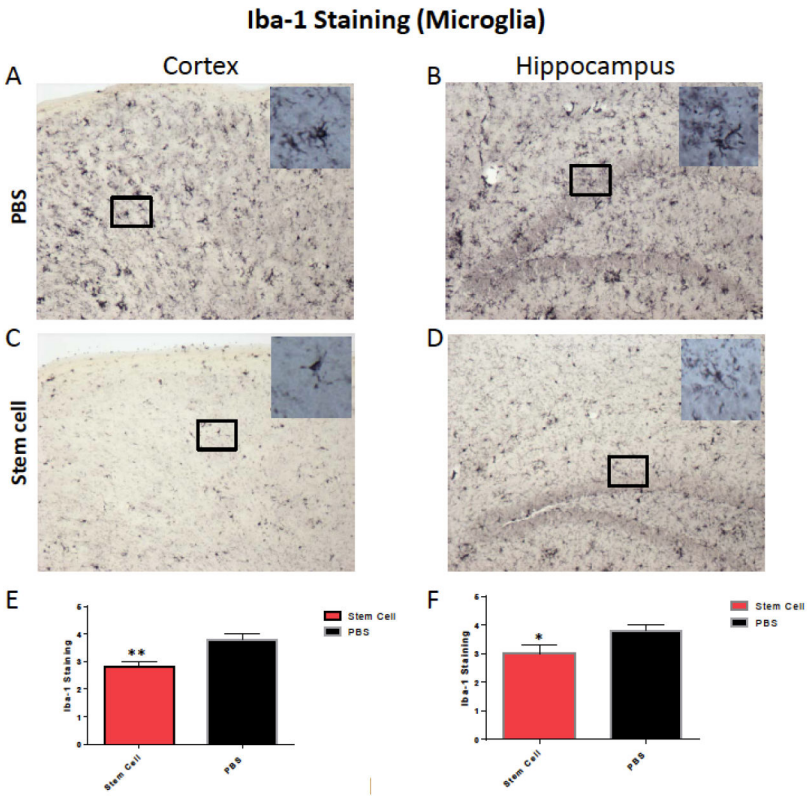

Fig. (6).

Shows representative brain sections from both groups (n=5 per group) showing microglia immunostaining in the cortex (A and C) and in the hippocampus (B and D) in PBS treated mice (A and B) and hUC-stem cells treated animal (C and D). Semi-quantitative analysis of the microglia immunoreactivity shows a significant difference in the cortex (E, one tailed Mann-Whitney test p=0.0011) and in the hippocampus (F, one tailed Mann-Whitney test p=0.042).

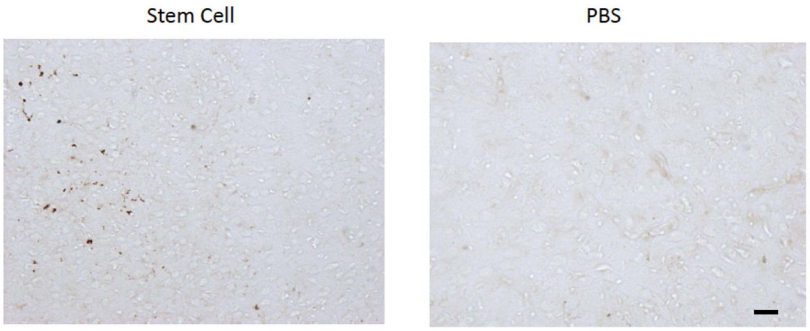

Fig. (7).

Shows an immunolabeled brain section of the cortex using a monoclonal antibody to human nuclear antigen, specific for human cells (Cat. No.: NBP2-34342, Novus Biologicals). Scattered hUCB-MSCs were detected in treated animals throughout the brain and were absent in the control animals. (Scale bar = 25μm).