NEURAL REGENERATION RESEARCH
Volume 7, Issue 4, February 2012
www.nrronline.org

Cite this article as: Neural Regen Res. 2012;7(4):245-250.

Effects of lateral ventricular transplantation of bone marrow-derived mesenchymal stem cells modified with brain-derived neurotrophic factor gene on cognition in a rat model of Alzheimer's disease☆

Ping Zhang[1], Gangyong Zhao[2], Xianjiang Kang[3], Likai Su[1]

1 *Department of Neurology, Affiliated Hospital of Hebei University, Baoding 071000, Hebei Province, China*
2 *Research Center for Bioengineering Technology, Hebei University, Baoding 071000, Hebei Province, China*
3 *College of Life Science, Hebei University, Baoding 071000, Hebei Province, China*

Abstract

In the present study, transplantation of bone marrow-derived mesenchymal stem cells modified with brain-derived neurotrophic factor gene into the lateral ventricle of a rat model of Alzheimer's disease, resulted in significant attenuation of nerve cell damage in the hippocampal CA1 region. Furthermore, brain-derived neurotrophic factor and tyrosine kinase B mRNA and protein levels were significantly increased, and learning and memory were significantly improved. Results indicate that transplantation of bone marrow-derived mesenchymal stem cells modified with brain-derived neurotrophic factor gene can significantly improve cognitive function in a rat model of Alzheimer's disease, possibly by increasing the levels of brain-derived neurotrophic factor and tyrosine kinase B in the hippocampus.

Key Words: Alzheimer's disease; bone marrow-derived mesenchymal stem cells; brain-derived neurotrophic factor; lateral ventricle; electrotransfection; neural regeneration

Ping Zhang☆, Doctor, Chief physician, Department of Neurology, Affiliated Hospital of Hebei University, Baoding 071000, Hebei Province, China

Corresponding author: Ping Zhang, Department of Neurology, Affiliated Hospital of Hebei University, Baoding 071000, Hebei Province, China
zhangpingbd@sina.com

Received: 2011-09-24
Accepted: 2011-12-22
(N20110517002/WJ)

Zhang P, Zhao GY, Kang XJ, Su LK. Effects of lateral ventricular transplantation of bone marrow-derived mesenchymal stem cells modified with brain-derived neurotrophic factor gene on cognition in a rat model of Alzheimer's disease. Neural Regen Res. 2012;7(4): 245-250.

www.crter.cn
www.nrronline.org

doi:10.3969/j.issn.1673-5374.2012.04.001

INTRODUCTION

Alzheimer's disease (AD) is mainly characterized by amyloid beta 25–35 ($A\beta_{25-35}$)-induced neuronal and synaptic degeneration[1-3], which affects long term potentiation and memory consolidation[4-5]. AD generally responds poorly to treatment. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic factor family, plays an important role in nervous system development and synaptic plasticity, and promotes neural regeneration after injury[6-10]. Some researchers have proposed that gene therapy may have positive effects on AD, involving BDNF, nerve growth factor (NGF) and Aβ catabolic enzyme genes[11]. BDNF is a large molecule and cannot permeate the blood-brain barrier, but bone marrow-derived mesenchymal stem cells (MSCs) are multipotential cells and can transfect genes, thus serving as carriers in gene therapy. Tyrosine kinase B (TrkB) is a receptor tyrosine kinase and functional receptor of BDNF, and plays an important role in signal transduction of cells[12].

In the present study, a rat model of AD was established by injection of $A\beta_{25-35}$ in the bilateral hippocampi and transplanted with MSCs carrying the BDNF gene through the lateral ventricle, to investigate the effects of the BDNF gene-modified MSCs on AD rats[13-16].

RESULTS

Quantitative analysis of experimental animals

A total of 40 Sprague-Dawley rats were randomly assigned to five groups, with eight animals in each group. All the rats were included in the final analysis.

Group	Treatment
Sham-surgery	Injection of normal saline in bilateral hippocampi
Model (AD)	Establishment of AD model by injection of $A\beta_{25-35}$ in bilateral hippocampi
MSCs	MSCs transplantation in AD rats
BDNF	Transplantation of pIRESneo-EGFP-BDNF- transfected MSCs in AD rats
Control	Transplantation of pIRESneo-transfected MSCs in AD rats

Morphology of MSCs before and after BDNF transfection

Inverted phase contrast microscopic observation showed that cells at passage three were aligned in a whirlpool-shape and

were identified as MSCs, as described previously[17] (Figure 1A). Fluorescence inverted phase contrast microscopic observations showed successfully transfected MSCs with green fluorescence (Figure 1B).

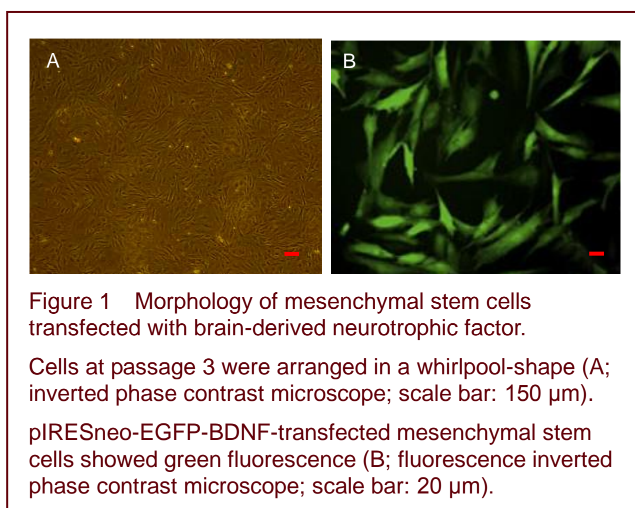

Figure 1 Morphology of mesenchymal stem cells transfected with brain-derived neurotrophic factor.

Cells at passage 3 were arranged in a whirlpool-shape (A; inverted phase contrast microscope; scale bar: 150 μm).

pIRESneo-EGFP-BDNF-transfected mesenchymal stem cells showed green fluorescence (B; fluorescence inverted phase contrast microscope; scale bar: 20 μm).

Learning and memory ability of AD rats transplanted with BDNF-transfected MSCs

Morris water maze was used to evaluate the learning and memory ability of rats in each group. In the navigation test, the average escape latency was significantly shortened in the BDNF group compared with the model group ($P < 0.01$ or $P < 0.05$; Figure 2).

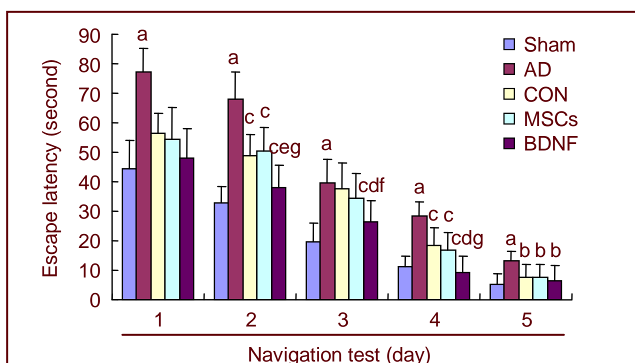

Figure 2 Changes in average escape latency (time that a rat took to search the platform) in the navigation test.

The mean value of escape latency of each rat in one day was regarded as the average escape latency. Data were expressed as mean ± SD of eight rats in each group.

Data of homogeneity of variance were analyzed with one-way analysis and Student-Newman-Keuls *q* test; data of heterogeneity of variance were analyzed with Welch and Dunnett T3 test.

$^aP < 0.01$, *vs.* sham group; $^bP < 0.05$, $^cP < 0.01$, *vs.* AD group; $^dP < 0.05$, $^eP < 0.01$, *vs.* MSCs group; $^fP < 0.05$, $^gP < 0.01$, *vs.* CON group.

Sham: Sham-surgery group; AD: Alzheimer's disease model group; CON: rats treated with pIRESneo-transfected bone marrow-derived mesenchymal stem cells (MSCs); MSCs: rats treated with MSCs; BDNF: rats transplanted with pIRESneo-EGFP-BDNF-transfected MSCs.

In the spatial probe test, the number of times the test animal crossed the platform was increased in animals in the BDNF group compared with animals in the model group ($P < 0.05$ or $P < 0.01$; Figure 3).

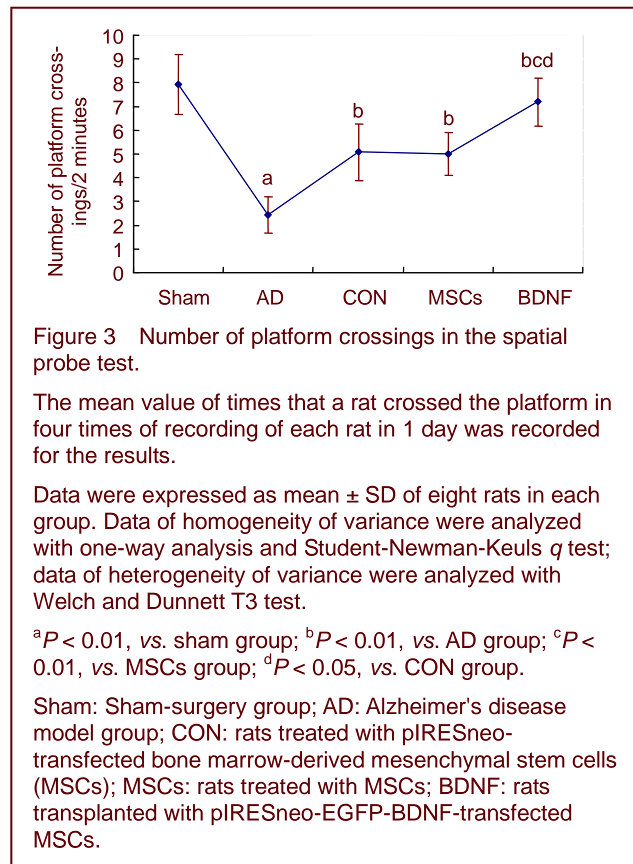

Figure 3 Number of platform crossings in the spatial probe test.

The mean value of times that a rat crossed the platform in four times of recording of each rat in 1 day was recorded for the results.

Data were expressed as mean ± SD of eight rats in each group. Data of homogeneity of variance were analyzed with one-way analysis and Student-Newman-Keuls *q* test; data of heterogeneity of variance were analyzed with Welch and Dunnett T3 test.

$^aP < 0.01$, *vs.* sham group; $^bP < 0.01$, *vs.* AD group; $^cP < 0.01$, *vs.* MSCs group; $^dP < 0.05$, *vs.* CON group.

Sham: Sham-surgery group; AD: Alzheimer's disease model group; CON: rats treated with pIRESneo-transfected bone marrow-derived mesenchymal stem cells (MSCs); MSCs: rats treated with MSCs; BDNF: rats transplanted with pIRESneo-EGFP-BDNF-transfected MSCs.

Histomorphology in hippocampal CA1 region of AD rats transplanted with BDNF-transfected MSCs

Hematoxylin-eosin (Figure 4) and Nissl body (Figure 5) staining results of the hippocampal CA1 region showed that the structures were disorderly, number of nerve cells was reduced, and the number of Nissl bodies was decreased and even absent, and were accompanied with karyopyknosis.

Uranyl acetate-lead citrate staining (Figure 6) of the hippocampal CA1 region showed that the neuronal membrane was shrunken, the cytoplasm was swollen, the mitochondrial crista and partial membrane fusion were obscure or absent, rough endoplasmic reticulum granule fusion and degranulation were observed, the number of free ribosomes was significantly decreased, and there was double-layer nuclear membrane fusion. In addition, the above pathological changes were improved in the control, MSCs, and BDNF groups (Figures 4–6). In particular, transplantation of pIRESneo-EGFP-BDNF-transfected MSCs significantly improved the pathological changes.

BDNF and TrkB mRNA and protein expression in the hippocampus of AD rats transplanted with BDNF-transfected MSCs

Reverse transcription-PCR and western blot analysis showed BDNF and TrkB mRNA and protein expression

in the hippocampal CA1 region of each group.

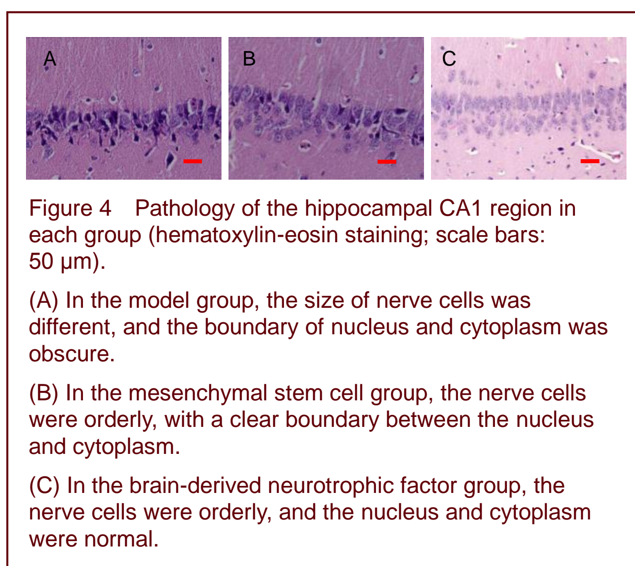

Figure 4 Pathology of the hippocampal CA1 region in each group (hematoxylin-eosin staining; scale bars: 50 μm).

(A) In the model group, the size of nerve cells was different, and the boundary of nucleus and cytoplasm was obscure.

(B) In the mesenchymal stem cell group, the nerve cells were orderly, with a clear boundary between the nucleus and cytoplasm.

(C) In the brain-derived neurotrophic factor group, the nerve cells were orderly, and the nucleus and cytoplasm were normal.

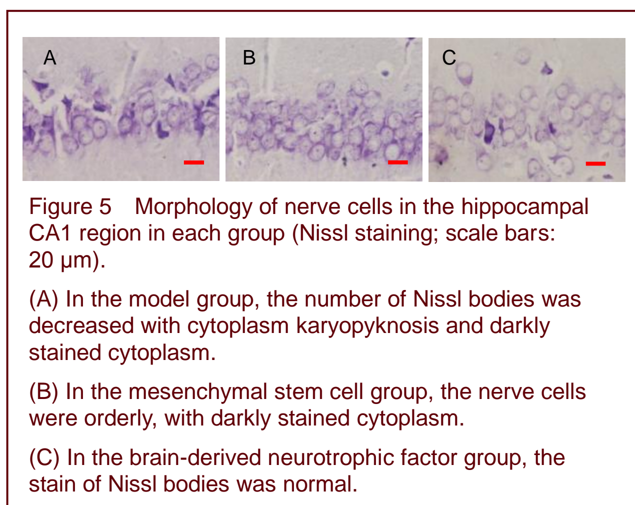

Figure 5 Morphology of nerve cells in the hippocampal CA1 region in each group (Nissl staining; scale bars: 20 μm).

(A) In the model group, the number of Nissl bodies was decreased with cytoplasm karyopyknosis and darkly stained cytoplasm.

(B) In the mesenchymal stem cell group, the nerve cells were orderly, with darkly stained cytoplasm.

(C) In the brain-derived neurotrophic factor group, the stain of Nissl bodies was normal.

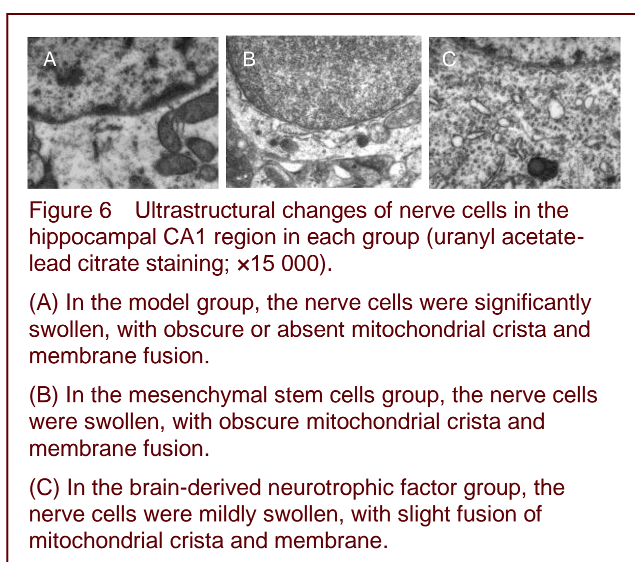

Figure 6 Ultrastructural changes of nerve cells in the hippocampal CA1 region in each group (uranyl acetate-lead citrate staining; ×15 000).

(A) In the model group, the nerve cells were significantly swollen, with obscure or absent mitochondrial crista and membrane fusion.

(B) In the mesenchymal stem cells group, the nerve cells were swollen, with obscure mitochondrial crista and membrane fusion.

(C) In the brain-derived neurotrophic factor group, the nerve cells were mildly swollen, with slight fusion of mitochondrial crista and membrane.

The expression levels were minimal in the model group, and maximal in the sham-surgery group; the levels of BDNF and TrkB mRNA and protein were increased in the control, MSCs, and BDNF groups compared with the model group ($P < 0.01$). In particular, BDNF and TrkB mRNA and protein expression were significantly increased in the BDNF group compared with the control and MSCs groups ($P < 0.05$; Figure 7).

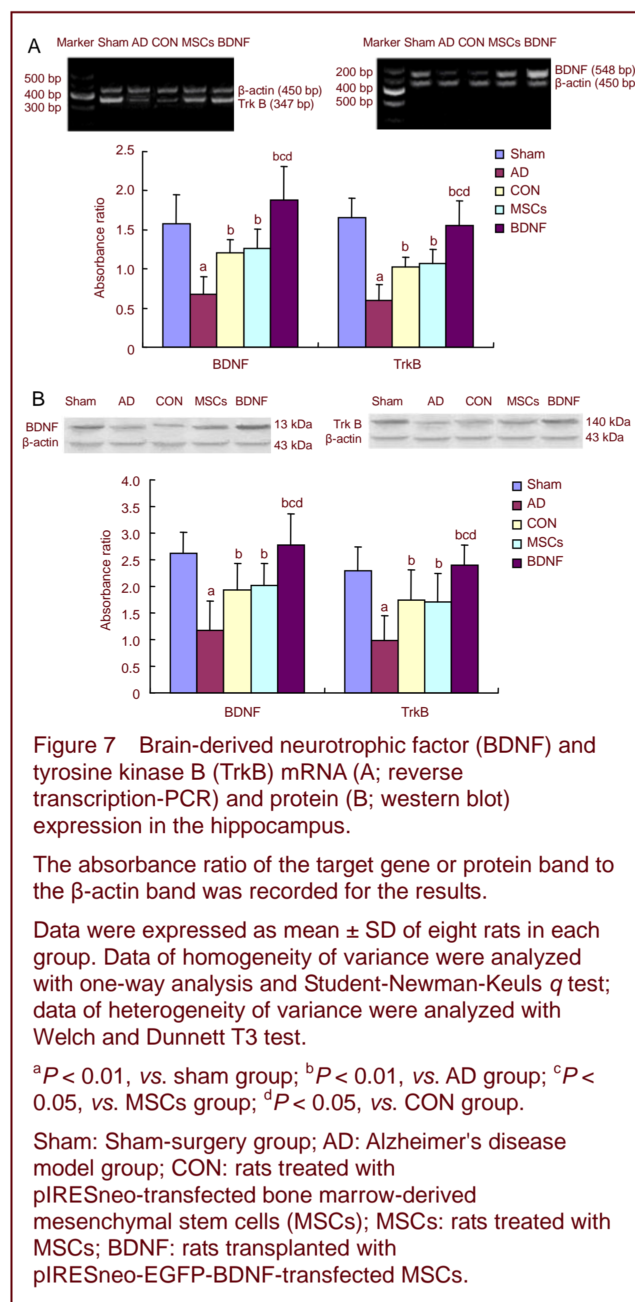

Figure 7 Brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) mRNA (A; reverse transcription-PCR) and protein (B; western blot) expression in the hippocampus.

The absorbance ratio of the target gene or protein band to the β-actin band was recorded for the results.

Data were expressed as mean ± SD of eight rats in each group. Data of homogeneity of variance were analyzed with one-way analysis and Student-Newman-Keuls *q* test; data of heterogeneity of variance were analyzed with Welch and Dunnett T3 test.

$^aP < 0.01$, *vs.* sham group; $^bP < 0.01$, *vs.* AD group; $^cP < 0.05$, *vs.* MSCs group; $^dP < 0.05$, *vs.* CON group.

Sham: Sham-surgery group; AD: Alzheimer's disease model group; CON: rats treated with pIRESneo-transfected bone marrow-derived mesenchymal stem cells (MSCs); MSCs: rats treated with MSCs; BDNF: rats transplanted with pIRESneo-EGFP-BDNF-transfected MSCs.

DISCUSSION

BDNF plays an important role in cognition, learning, and memory formation[18-19]. It promotes cholinergic neuron differentiation and survival[20-22], and enhances synaptic plasticity[23-24]. Decreased hippocampal BDNF in AD patients reduces retrograde transport in the cholinergic system and induces cognitive impairment[25-26]. TrkB is a BDNF functional receptor, comprising of one single transmembrane peptide chain encoding a

proto-oncogene, and participates in cell differentiation, attachment, and proliferation[12]. It is highly correlated with normal cognition and mild cognitive dysfunction, and from mild cognitive dysfunction to dementia[27-28]. Results from the present study showed that lateral ventricular transplantation of MSCs modified with the BDNF gene significantly attenuated neuronal injury in the hippocampal CA1 region of AD model rats, and increased BDNF and TrkB expression compared with MSCs transplantation alone. We presume that the transplanted MSCs modified with BDNF migrated to the hippocampus and secreted BDNF. Increased BDNF levels in the hippocampus can activate the neuronal surface receptor TrkB[29-30] and increase TrkB receptor expression, which promotes TrkB homodimer formation, triggers tyrosine kinase phosphorylation, and transfers signals to downstream adapter proteins and enzymes, such as mitogen activated protein kinase, phospholipase C-γ, and phosphatidylinositol 3-kinase. Thus, preventing cholinergic neuron degeneration and death and facilitating learning and memory function[31-32].

Lateral ventricular transplantation simulated subarachnoid space injection in humans, which has been shown to result in minimal injury, allows for repetitive injections. This can help overcome problems such as low transplantation rate and difficulty in passing through the blood-brain barrier. Adenovirus has been frequently used as a gene vector, but it can induce an immune response and has carcinogenic potential. The present study utilized electrotransfection, a method which exhibits minimal effects on cell viability[33-34].

In conclusion, lateral ventricular transplantation of MSCs modified with the BDNF gene significantly improved cognition of AD model rats possibly by upregulating hippocampal BDNF and TrkB expression.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiment was performed at the Central Laboratory of Hebei University Medical Department and Laboratory of Cell Biology, Hebei University College of Life Sciences, China, from October 2008 to March 2010.

Materials

A total of 40 healthy, male, adult Sprague-Dawley rats, weighing 280–300 g, and six healthy Sprague-Dawley rats (used for cell culture), aged 2–3 weeks, weighing 80–120 g, were provided by the Laboratory Animal Center of Hebei University (license No. SCXK (Ji) 2008-1-003; 812095). The experimental procedures were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China[35].

Methods

MSCs isolation and culture

Six rats, aged 2–3 weeks, were anesthetized and sacrificed. The medullary canal was rinsed with 10 mL L-DMEM containing 10% serum. The bone marrow was placed in a culture flask and cultured in 5% CO_2 and saturated humidity at 37°C. Half of the culture medium was replaced after 24 hours, and totally replaced at 72 hours. The culture medium was replaced every 3 days thereafter. Cells were passaged after reaching confluency[17].

MSCs transfection with pIRESneo-EGFP-BDNF

MSCs at passage 3 or 5 at 70–80% confluency[22] were trypsinized, mixed with serum-containing culture medium, and centrifuged at 500 r/min for 5 minutes. Electrotransfection was performed according to the manufacturer’s instruction using NeuroPORTER transfection reagent (GTS, San Diego, CA, USA) with an electrotransfection system (Bio-Rad, Hercules, CA, USA) at 280 V for 20 ms. Plasmids pIRESneo-EGFP-BDNF or pIRESneo 20 μg were provided by Sangon, Shanghai, China. Cells were cultured for 48 hours after electrotransfection and screened using culture medium containing 15% fetal bovine serum and 100 μg/mL G418. Half of the culture medium was replaced every 2–3 days. The screening lasted for 10–14 days. After discarding G418, the cells were cultured in DMEM containing 15% fetal bovine serum for 5–7 days. Cell morphology was observed by a fluorescence inverted phase contrast microscope (Olympus, Tokyo, Japan).

Establishment of rat AD model and intervention

The rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (0.35 mL/100 g) and placed in a stereotaxic instrument (Chengdu Taimeng, Sichuan, China). According to Rat Brain Stereotaxic Coordinates described by George Paxinos[4], the cranium was drilled at 3.5 mm posterior to Bregma and 2 mm adjacent to the median. $A\beta_{25-35}$ (diluted to 2 μg/μL with normal saline at 37°C for 6 days) was slowly injected using a vertically oriented microsyringe to 3 mm below the dura mater, *i.e.* hippocampal CA1 region, with 5 μL (10 μg) for each side of the hippocampus for at least 20 minutes. The microsyringe was maintained for 5 minutes, with 10 minute intervals between two injections. The wound was washed with gentamicin and sutured. The rats were allowed free access to food and water after they awoke[12].

In the MSCs group, 10 days after $A\beta_{25-35}$ injection, the rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (0.35 mL/100 g) and placed in a stereotaxic instrument. According to the Rat Brain Stereotaxic Coordinates[4], the left ventricle was localized at 1.0 mm posterior to Bregma and 1.5 mm adjacent to the median, and 10 μL MSCs (5×10^6) were injected using a vertically oriented microsyringe to 4.0 mm below the dura mater. The injection lasted for at least 15 minutes. The microsyringe was maintained for 10 minutes. In the control group, the rats were injected with 10 μL pIRESneo-transfected MSCs (5×10^6), and the BDNF group was injected with 10 μL pIRESneo-EGFP-BDNF-transfected MSCs (5×10^6).

Morris water maze for learning and memory ability

Morris water maze task was used to evaluate rat learning and memory ability[5]. The test was performed at 16 days and was completed at 20 days. In the navigation test, the pool was divided into four quadrants of equal size. The rats in each group were placed in water with the head facing the wall. The duration taken for the rats to search and climb the platform in the water was recorded and regarded as the escape latency. If an animal failed to climb onto the platform within 120 seconds, it was manually guided onto the platform and made to stay for 10 seconds. The escape latency was recorded as 120 seconds. The four periods of training for each rat in the morning or afternoon were performed continuously, with 60 seconds between two sessions. The mean value of escape latency of each rat in one day was regarded as the average escape latency, reflecting the learning and memory ability of the rats to the platform in water. In the spatial probe test, each rat was trained for 5 days. The platform in the water was removed the day after the navigation test. The rats were placed in water at a random point, and the times that the rats crossed the position where the platform was placed in 120 seconds were recorded. The mean value of four recordings was regarded as the number of times of platform crossing for each rat (times/2 min).

Preparation of hippocampal samples

Four rats from each group were sacrificed after the Morris water maze test. The left cerebral hemisphere was rapidly harvested, and cut coronally into three parts from the anterior and posterior of the hippocampus. The segment containing the intact hippocampus was saved. Hippocampal CA1 region was cut into pieces, 1 mm^3, fixed in 4% glutaraldehyde, postfixed in 1% osmic acid for 1 hour, dehydrated with alcohol and acetone, and embedded with epoxy resin Epon812 to prepare samples for electron microscopic observation. Following ultrathin sectioning, the sections were stained with uranyl acetate and lead citrate, and tissue ultrastructure was observed by a transmission electron microscope (Hitachi, Tokyo, Japan). The remaining tissues from the hippocampal CA1 region were fixed in 4% paraformaldehyde for 24–48 hours, dehydrated, paraffin embedded, and evenly sectioned to prepare coronal sections that were 5 μm thick. Hematoxylin-eosin and Nissl-stained sections were observed by light microscopy (Olympus).

Reverse transcription-PCR for BDNF and TrkB mRNA expression in the hippocampus

The rats from each group were sacrificed after the Morris water maze test. The right cerebral hemisphere was rapidly harvested, and the hippocampus was isolated in an ice bath. Total RNA from the brain samples were prepared with Trizol reagent (Life Technologies, Carlsbad, CA, USA), in accordance with the manufacturer's protocol. RNA integrity was analyzed using 1% agarose gel electrophoresis and reverse transcribed into cDNA according to the instructions from the reverse transcription kit (Promega, Madison, Wisconsin, USA). The cDNA was subjected to PCR amplification. BDNF, TrkB and internal reference β-actin primers were designed according to rat target gene mRNA sequences in GenBank.

Reaction conditions were as follows: predenaturation at 94°C for 5 minutes, 94°C for 45 seconds, 72°C for 30 seconds, annealing at 53°C for 45 seconds for 30 cycles in total, followed by extension at 72°C for 10 minutes. PCR products, 6 μL, were subjected to 1.2% agarose electrophoresis. After ethidium bromide staining, the electrophoresed images were analyzed using a Bio-Rad gel imaging analysis system (Hercules, CA, USA) under an ultraviolet lamp. The absorbance ratio of the target gene band to β-actin band was analyzed.

Gene	Primer sequence	Product size (bp)
BDNF	Upstream: 5'- GTT ATT TCA TAC TTC GGT TGC-3' Downstream: 5'- ATG GGA TTA CAC TTG GTC TCG- 3'	548
TrkB	Upstream: 5'-TGG GAC GTT GGG AAT TTG GTT-3' Downstream: 5'- CAG CCG TGG TAC TCC GTG TG-3'	347
β-actin	Upstream: 5'- AGG GAA ATC GTG CGT GAC AT-3', Downstream: 5'- CAT CTG CTG GAA GGT GGA CA -3'	450

Western blot analysis for BDNF and TrkB protein expression in the hippocampus

The hippocampus was isolated in an ice bath using the above mentioned method. Total protein was extracted and protein content was determined using a bicinchoninic acid kit. Proteins were separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, with the concentration of stacking gel at 5%. Protein sample, 20 μL, was transferred to polyvinylidene difluoride membranes at 100 V for 45 minutes. Membranes were blocked with 1% bovine serum albumin at room temperature overnight, incubated with primary antibody (rabbit anti-rat BDNF, TrkB or rat anti-mouse β-actin monoclonal antibody; Beijing Zhongshan Goldernbridge, Beijing, China; 1: 1 000) at 4°C overnight, washed with Tris-buffered saline/Tween for 5 minutes × 3, incubated with secondary antibody horseradish peroxidase-labeled goat anti-rabbit or goat anti-mouse (1: 3 000; Beijing Zhongshan Goldenbridge) at room temperature for 1 hour, and washed with Tris-buffered saline/Tween-20 for 5 minutes × 3. After a 1 minute incubation in polyclonal rabbit anti-BDNF antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1: 400 dilution in Tris-buffered saline/Tween-20), the membranes were washed and incubated in enhanced chemiluminescence at room temperature, to develop the membrane. With β-actin as internal reference, images were analyzed using Quantity One software (Bio-Rad). Results were represented by the absorbance ratio of target protein band to the β-actin band.

Statistical analysis

Measurement data were expressed as mean ± SD and analyzed using SPSS 13.0 software (SPSS, Chicago, IL,

USA). Data were subjected to homogeneity test for variance. Data of homogeneity of variance were analyzed with one-way analysis and mean between two groups was compared using Student-Newman-Keuls *q* test. Data of heterogeneity of variance were analyzed with Welch, and mean between two groups was compared using Dunnett T3 test. $P < 0.05$ was considered statistically significant.

Author contributions: Ping Zhang was in charge of funding, conceived and designed this study, conducted the experiments, wrote the manuscript and analyzed the data. All authors participated in data integration and data analysis.

Conflicts of interest: None declared.

Funding: This study was financially sponsored by Science and Technology Support for Major Projects of Hebei Province, No. 09276103D; Hebei Province Science and Technology Research and Development Program, No. 08206120D.

Ethical approval: This experiment was approved by the Experimental Animals Ethics Committee of Affiliated Hospital of Hebei University, China.

REFERENCES

[1] Lacor PN, Buniel MC, Furlow PW, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J Neurosci. 2007;27(4):796-807.
[2] Walsh DM, Selkoe DJ. A beta oligomers-a decade of discovery. J Neurochem. 2007;101(5):1172-1184.
[3] Ono K, Yamada M. Lown oligomers as therapeutic targets of Alzheimer's disease. J Neurochem. 2011;117(1):19-28.
[4] Lee CC, Kuo YM, Huang CC, et al. Insulin rescues amyloid beta-induced impairment of hippocampal long-term potentiation. Neurobiol Aging. 2009;30(3):377-387.
[5] Xia W. Brain amyloid β protein and memory disruption in Alzheimer's disease. Neuropsychiatr Dis Treat. 2010;6:605-611
[6] Young KM, Merson TD, Sotthibundhu A, et al. p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. J Neurosci. 2007;27(19):5146-5155.
[7] Hennigan A, O'Callaghan RM, Kelly AM. Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. Biochem Soc Trans. 2007;35(Pt 2):424-427.
[8] Reichardt L. Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci. 2006;361(1473):1545-1564.
[9] Lopatina T, Kalinina N, Karagyaur M, et al. Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. PLoS One. 2011;6(3):e17899.
[10] Gordon T. The role of neurotrophic factors in nerve regeneration. Neurosurg Focus. 2009;26(2):E3.
[11] Nilsson P, Iwata N, Muramatsu SI, et al. Gene therapy in Alzheimer's disease potential for disease modification. J Cell Mol Med. 2010;14(4):741-757.
[12] Ojeda SR, Romero C, Tapia V, et al. Neurotrophic and cell-cell dependent control of early follicular development. Mol Cell Endocrinol. 2000;163(1-2):67-71.
[13] Shen YX, Yang J, Wei W, et al. Induction of learning and memory dysfunction by β-amyloid peptide fragment 25-35 in rats. Zhongguo Yaolixue Tongbao. 2001;17(1):26-29.
[14] Yang C, Zhang Q, Wei YN, et al. The study of β-amyloid protein induced alzheimer disease model rats. Henan Yixue Yanjiu. 2003;12(4):294-296.
[15] Pákáski M, Kálmán J. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. Neurochem Int. 2008;53(5):103-111.
[16] Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. Behav Brain Res. 2011;221(2):555-563.
[17] Chen K, Kang XJ, Zhang P, et al. Bone marrow-derived mesenchymal stem cells of SD rat cultured in vitro and identification of the phenotype and purity. Shangdong Yiyao. 2010;50(11):34-36.
[18] Schindowski K, Belarbi K, Buée L. Neurotrophic factors in Alzheimer's disease: role of axonal transport. Genes Brain Behav. 2008;7 Suppl:43-56.
[19] Webster NJ, Pirrung MC. Small molecule activators of the Trk receptors for neuroprotection. BMC Neurosci. 2008;9 Suppl 2:S1.
[20] Hefti F, Knusel B, Lapchak PA, Protective effects of nerve factor and brain- derived neurotrophic factor on basal forebrain cholinergic neurons in adult rats with partial fimbrial transections. Prog Brain Res. 1993;98:257-263.
[21] Nilbratt M, Porras O, Marutle A, et al. Neurotrophic factors promote cholinergic differentiation in human embryonic stem cell-derived neurons. J Cell Mol Med. 2010;14(6B):1476-1484.
[22] Alderson RF, Alterman AL, Barde YA, et al. Brain derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. Neuron. 1990;5(3):297-306.
[23] Waterhouse EG, Xu B. New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. Mol Cell Neurosci. 2009;42(2):81-89.
[24] Chapleau CA, Carlo ME, Larimore JL, et al. The actions of BDNF on dendritic spine density and morphology in organotypic slice cultures depend on the presence of serum in culture media. J Neurosci Methods. 2008;169(1):182-190.
[25] Peng S, Garzon DJ, Marchese M, et al. Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. J Neurosci. 2009;29(29):9321-9329.
[26] Laske C, Stransky E, Leyhe T, et al. BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. J Psychiatr Res. 2007;41(5):387-394.
[27] Ginsberg SD, Che S, Wuu J, et al. Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. J Neurochem. 2006;97(2):475-487.
[28] Chen Z, Simmons MS, Perry RT, et al. Genetic association of neurotrophic tyrosine kinase receptor type 2 (NTRK2) With Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2008;147(3):363-369.
[29] Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol. 2010;70(5):304-322.
[30] Numakawa T, Suzuki S, Kumamaru E, et al. BDNF function and intracellular signaling in neurons. Histol Histopathol. 2010;25(2):237-258.
[31] Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol. 2000;10(3):381-391.
[32] Zhu D, Wu X, Strauss KI, et al. N-methyl-D-aspartate and TrkB receptors protect neurons against glutamate excitotoxicity through an extracellular signal-regulated kinase pathway. J Neurosci Res. 2005;80(1):1104-113.
[33] Wang SB, Sun CX, Yao CG, et al. Progress in the research and application on electroporation. Guowai Yixue: Shengwu Yixue Gongcheng Fence. 2005;28(6):370-373.
[34] Ferreira E, Potier E, Logeart-Avramoglou D, et al. Optimization of a gene electro-transfer method for mesenchymal stem cell transfection. Gene Ther. 2008;15(7):537-544.
[35] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

(Edited by Zhu WJ, Lv HX/Su LL/Song LP)