Int J Clin Exp Med 2015;8(7):10471-10480
www.ijcem.com /ISSN:1940-5901/IJCEM0007857

Original Article

Folic acid in combination with adult neural stem cells for the treatment of spinal cord injury in rats

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Received March 12, 2015; Accepted May 5, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: Purpose: To observe the therapeutic effect of folic acid in combination with adult neural stem cells on spinal cord injury and to investigate the possible mechanism. Methods: A total of 120 Wistar rats were randomly assigned to six groups: normal, model, sham-surgery, folic acid injection, adult neural stem cell transplantation, and combination (folic acid injection + adult neural stem cells transplantation) groups. Morphology of neural stem cells was observed by inverted microscopy. Expression of CD105, CD45, CD44, and CD29 were detected by flow cytometry; expression of neuron-specific enolase and glial fibrillary acidic protein were determined by immunofluorescence. Motor coordination and integration capabilities were assessed using BBB scores; Morphology of spinal cord tissues was observed by hematoxylin-eosin staining and 5-bromodeoxyuridine immunohistochemistry. GDNF, BDNF and NT-3 expression in spinal cord tissues were determined by ELISA; while expression of the apoptosis-related proteins BCL-2, Bax and caspase-3 was detected using western blotting. Results: Flow cytometry showed that the isolated cells were positive for CD44 and CD29 and negative for CD105 and CD45. Combination treatment significantly improved the behavior of model rats with spinal cord injury, attenuated inflammatory reaction of spinal cord tissues, restored injured nerve cells, and increased expression of GDNF, BDNF and NT-3 in spinal cord tissues, up regulated BCL-2 expression, and down regulated Bax and caspase-3 expression. Conclusions: Folic acid in combination with adult neural stem cells significantly improved nerve function and plays a key role in maintaining microenvironment homeostasis in the neurons of rats with spinal cord injury.

Keywords: Folic acid, adult neural stem cells, spinal cord injury

Introduction

Spinal cord injury is characterized by a high incidence and high morbidity. The consequences following spinal cord injury are lifelong and devastating due to massive neuronal death. Over one hundred years, a series of strategies such as surgery, drugs, physical therapy, and genetic therapy have emerged to treat spinal cord injury, but these treatments fail to cure the patient's paralysis at varying degrees [1, 2]. Therefore, finding an effective and safe treatment for spinal cord injury is currently crucial in medical field. Exogenous stem cells transplantation is a novel hotspot in the existing studies of spinal cord injury, adult neural stem cells are a class of pluripotent cells that can differentiate into neurons, astrocytes and oligodendrocytes, also have the ability of self-renewal and maintenance [3, 4]. These neural stem cells are mainly located in the sub-granular zone of dentate gyrus and the subventricular zone of lateral ventricle [5, 6]. Folic acid (folate, pteroylglutamic acid or vitamin B9) is an essential water-soluble B vitamin for the body health, growth and development, collectively known as a group of chemical structure produced by pteridine ring structure and/or the number of glutamic acid residues. Folic acid is not only associated with hematopoietic system, but also involves in the nervous system growth and development. Accumulating evidence suggests that folic acid plays a crucial role on the proliferation and differentiation of neural stem cells [7, 8]. However, the underlying mechanism remains unclear. Preliminary work by our research group found

that, folic acid significantly reduced the expression of inflammatory proteins in nerve cells and prevented Ca^{2+} overload under hypoxia condition, significantly up regulated Notch1 mRNA and protein expression, and exerted significant protective effects on nerve cells. We speculate that the combination of folic acid with adult neural stem cells is a feasible treatment for spinal cord injury in rats.

Materials and methods

Materials

Wistar rats were provided by the Medical Laboratory Animal Center of Hubei Province (license No. SCXK (e) 2008-0004). Calcium folinate was purchased from Guangdong Lingnan Pharmaceutical Ltd., Guangdong, China; Dulbecco's modified eagle's medium (DMEM)/F12, epidermal growth factor, basic fibroblast growth factor, and penicillin-streptomycin solution were purchased from Sigma, USA; N2, B27 and fetal calf serum purchased from Gibco, USA; neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); the goat anti-rabbit Cy3 fluorescent secondary antibody was purchased from KPL, USA; CD105, CD45, CD44 and CD29 fluorescent antibodies were purchased from BD; GDNF, BDNF and NT-3 enzyme-linked immunosorbent assay kits were purchased from R&D; BCL-2, Bax and caspase-3 antibodies were purchased from BioLegend Company.

Isolation, primary culture and passage of adult NSCs

Six-week-old Wistar rats were sacrificed by over-anesthesia induced by sodium pentobarbital. The brain tissues were harvested sterilely, and subgranular zone and subventricular zone were dissociated in Hanks balanced solution, and collected in a centrifuge tube containing 2 mL NSC culture solution (DMEM/ F12 culture solution supplemented with 1% N2, 2% B27, 20 μg/L epidermal growth factor and 20 μg/L basic fibroblast growth factor). Cell suspensions were prepared by trituration 30 times using a 1-mL transferpette and filtrated through a 75-μm filtration mesh. Culture solution was added to cell suspensions to a total volume of 10 mL and mixed evenly. Then, 10 μL of a cell suspension was mixed with an equal volume of trypan blue solution, and the total number of cells was quantified using a blood cell counting plate. According to the cell count, the cell suspension concentration was adjusted to 1×10^5 cells/mL and the suspension was incubated in CO_2. Half of the culture solution was renewed every 3 days. Cells were triturated and passaged every 2 weeks. The NSC spheres formed from NSCs of passage two were used for subsequent experiments.

Identification of adult NSC [9]

NSCs of passage one were harvested, washed with PBS twice after discarding the culture solution, digested with mixture of 0.25% trypsin and 0.2% ethylenediamine tetraacetic acid (1:1), and washed with PBS containing 20% bovine serum albumin. A single cell suspension at a concentration of 1.0×10^6/mL was prepared, and 500 μL of cell suspension was placed in each of three Eppendof tubes: tube 1, negative control (no antibody); tube 2, isotype control (5 μL of anti-rat IgG1-FITC, APC, PE, and PerCP/Cy5.5); and tube 3, detection tube (5 μL of anti-human CD105-PerCP/Cy5.5, CD45-APC, CD44-FITC and CD29-PE). Incubations were maintained at room temperature in the dark for 30 minutes, followed by flow cytometry.

Induced differentiation of adult NSCs [10]

When cells of passage two formed neural spheres, 10% fetal calf serum was added into the culture media to induce adult NSC differentiation. At 7 days, cells were fixed with 4% paraformaldehyde at room temperature for 15 minutes, washed with PBS three times, blocked with 5% bovine serum albumin (containing 0.2% Triton X-100) at room temperature for 1 hour, incubated with rat-derived GFAP and NSE antibody (1:100) at 37°C for 2 hours, followed by tetramethylrhodamine isothiocyanate-labeled anti-mouse secondary antibody (1:200) at 37°C for 1 hour. Three PBS washes each of 5 minutes were performed between each step. Cells were stained with 4'-6-diamidino-2-phenylindole at room temperature for 5 minutes, washed with PBS and photographed using a fluorescence microscope.

Animal grouping and treatment

The 120 specific pathogen-free male Sprague-Dawley rats, aged 8 weeks, weighing 200 ± 10

g, were randomly assigned to six groups: normal, model, sham-surgery, NSC transplantation, folic acid injection, and combination (NSC transplantation + folic acid injection) groups. Subsequent experiments were performed at 1, 14 and 28 days following NSC transplantation.

Establishment of spinal cord injury model

An impact device for falling injury was composed of the bar, peripheral sleeve, the hammer and an external fixator according to the methods of Wamil et al [11]. A 10-g force was fell from the 1.25 cm height, obtaining 10 × 1.25 g/cm falling impact. The rats were weighed and anesthetized with 30-50 mg/kg of 1% sodium pentobarbital via intraperitoneal injection at 25°C, then fixed on the holder. A median incision was made on the dorsal skin along spinal cord, exposing dorsal skin and subcutaneous tissue, 4 cm. After the T11 vertebral spinous process was positioned, the paravertebral muscle of T9-11 were bluntly dissected and fixed using a distractior, exposing T9-11 vertebral body. Subsequently the laminotomy was performed. The impact bar was placed on T10 segment of the spinal cord, the sleeve was fixed, and the hammer was allowed to fall freely along the sleeve from the height of 1.25 cm, causing T10 spinal cord contusion injury. The rats were allowed freely to food and water postoperatively, and underwent urinary bladder extrusion 4-5 times daily for 1-2 weeks, until the micturition reflex recovered.

Adult NSC transplantation

At 72 hours following model establishment, the rats were anesthetized and placed on the stereotactic apparatus. An adult NSC suspension (1×10^5 NSCs, 10 μL) was harvested using a sterile glass microsyringe, and injected at a point 3 mm posterior and 1.1 mm lateral to the bregma, to depths of 2 and 4 mm, with each point of 5μL suspension, at 1 μL/min. The needle was maintained for 20 minutes after injection, and removed at 0.5 mm/min. The incision was sutured, followed by intramuscular injection with 100 000 U penicillin for 1 week postoperatively.

Behavior experiment

In a predetermined observation time, 1 d, 14 d, 28 d after injury, 8 rats in each group were evaluation of moving ability with BBB [12] score on flat ground.

Immunohistochemistry

Spinal cord tissues were harvested for immunohistochemistry at 1, 14 and 28 days following NSC transplantation. The rats were anesthetized by intraperitoneal injection with 10% chloral hydrate, rapidly perfused with 150 mL heparinized NaCl (0.9%) through the ascending aorta, followed by 200 mL cold paraformaldehyde (4%) and 300 mL paraformaldehyde in 2 hours. The spinal cord tissues were harvested, fixed in 4% paraformaldehyde at 4°C for 48 hours, dehydrated with alcohol, paraffin embedded, and then prepared into 5-μm coronal sections for 5-bromodeoxyuridine immunohistochemistry.

Western blotting for expression of the apoptosis-related genes BCL-2, Bax and Caspase-3

Spinal cord tissues were placed in pre-cooled whole cell lysate at 4°C, homogenized in a 0.6-mL ice bath, centrifuged at 12000 r/min at 4°C for 5 minutes, and the supernatant was discarded. The amount of protein was quantified using the Coomassie brilliant blue G250 technique and samples were stored at -80°C. Protein samples (15 μg) were placed in 2 × sodium dodecyl sulfate gel loading buffer, boiled for 5 minutes, and centrifuged at 6000 r/min for 3 minutes. The supernatant was harvested for sampling. The protein on gels after electrophoresis was transferred to polyvinylidene difluoride membranes. The membranes were blocked at room temperature for 2 hours, placed in hybridization bags, incubated with primary antibody (BCL-2, Bax, caspase-3; 1:800) overnight at 4°C, then with horseradish peroxidase-labeled goat anti-rabbit IgG (1:1500) and pre-stained protein marker, and shaken at room temperature for 1 hour. The membranes were washed, colorized in developer, and exposed to X-ray film. Results were scanned by computer and semiquantitatively analyzed using automatic image analysis system. Absorbance values for target bands were determined in the same condition, and the absorbance ratio of target band to β-actin of samples from each group was calculated.

Enzyme-linked immunosorbent assay for GDNF, BDNF and NT-3 level in the Spinal cord

Spinal cord tissue homogenate (10%) was prepared and centrifuged at 10000 r/min for 10

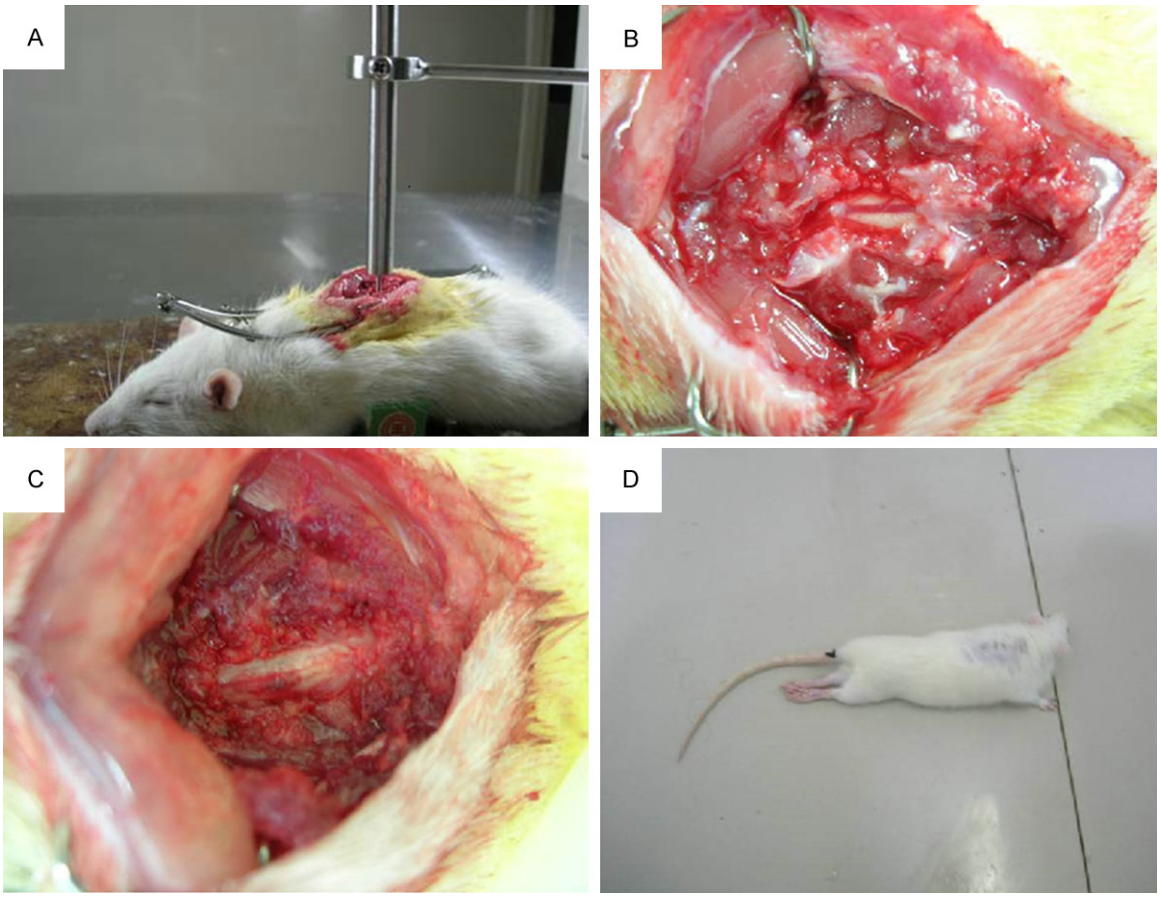

Figure 1. Establishment of animal model of spinal cord injury. A: Represents preparation of spinal cord injury; B: Represents ruddy tissue before injury; C: Represents subdural bluing, red after injury; D: Represents paralysis of lower extremities paraplegia after spinal cord injury.

minutes. The supernatant was harvested and the protein concentration was determined. Protein samples (50 μL) were harvested, and GDNF, BDNF and NT-3 contents were determined according to the manufacturer's instructions (R&D). The absorbance value at 405 nm was determined using a microplate reader to calculate GDNF, BDNF and NT-3 content in spinal cord tissues.

Statistical analysis

Experimental data are expressed as means ± SD and were analyzed using one-way analysis and t-test by SPSS13.0 software.

Results

Establishment of spinal cord injury model

During surgery, the appearance of flapping body, tail flicking and apnea indicates the successful modeling. After surgery, hemostasis was achieved using gelatin sponge, the muscle, subcutaneous tissue and skin were sutured (**Figure 1**).

Morphological changes in adult NSCs

Inverted microscopy showed that cultured cells were first scattered, with small cell bodies, round shape and good refraction (**Figure 2A**); at 3 days, cell spheres consisting of several cells were observed (**Figure 2B**); at 5 days, large cell spheres formed by tens, even hundreds of cells were observed (**Figure 2C**). After passages, single cells and small cell groups were observed in the culture medium. Some single cells exhibited division phases and gradually formed cell spheres. After induction with 10% fetal calf serum, processes were observed at 1 day, with good refraction (**Figure 2D**); typical nerve cells were found at 3 days (**Figure 2E**); cell bodies were plump, with halation in the surroundings,

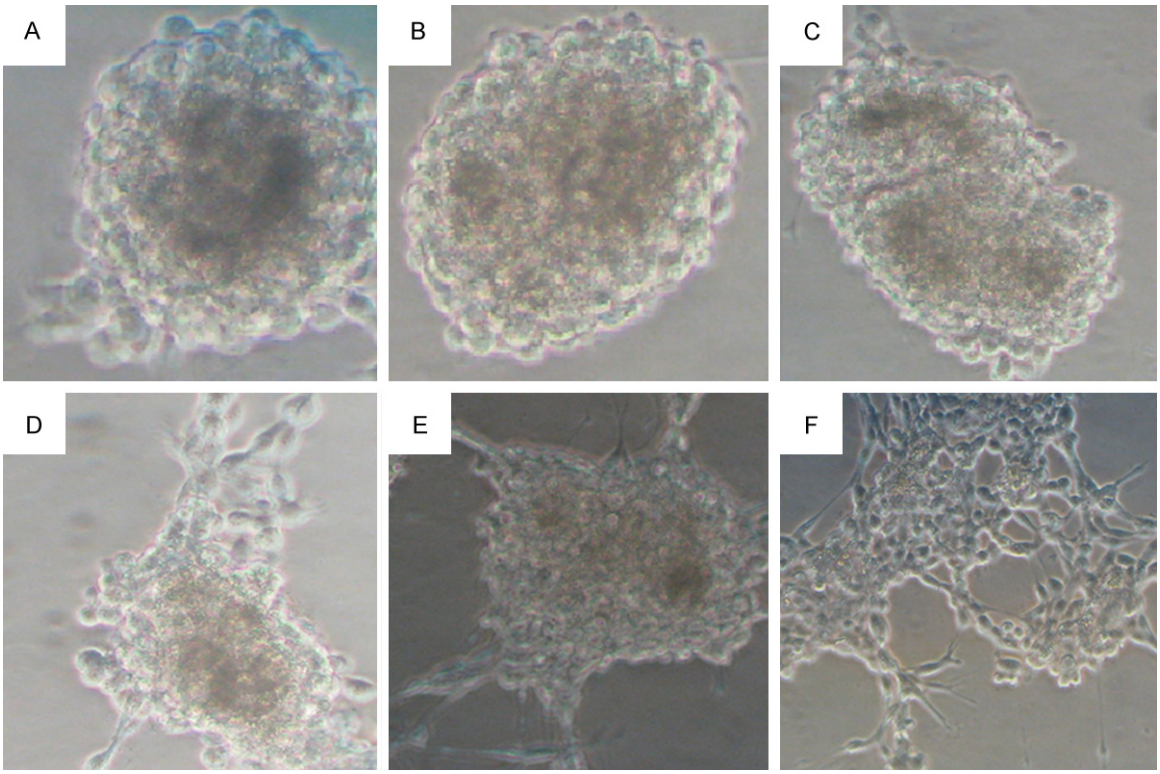

Figure 2. Morphological changes in adult neural stem cells (NSCs) before and after induction. A: NSCs at 1 day before induction; B: NSCs at 3 days before induction; C: NSCs at 5 days before induction; D: NSCs after induction for 1 day; E: NSCs after induction for 3 days; F: NSCs after induction for 5 days.

thin and long axons and dendrites, with interlacing into a network (**Figure 2F**).

Identification of adult NSCs

Flow cytometry of passage one NSCs showed that NSCs were positive for CD44 and CD29 and negative for CD105 and CD45, indicating that the cultured cells displayed properties resembling those of mesenchymal stem cells (**Figure 3**).

Primary cultures of adult neural stem cells were positive for CD44 and CD29, with 99.38% of NSCs expressing these markers, but negative for CD105 and CD45. This indicated that cultured cells have properties resembling those of mesenchymal stem cells as we expected.

Induced differentiation of adult NSCs

NSCs rapidly adhered in culture medium containing 10% fetal calf serum, and some axons were seen to intercross with each other; at 24 hours, processes on the cell surface were observed, and these gradually increased and became extended with prolonged time. The intercrossing processes of different cells formed a network. Immunofluorescence staining at 7 days showed that cultured cells were GFAP or NSE positive, indicating that the cultured cells differentiated into astrocytes or neurons (**Figure 4**).

Results of behavior experiment

As shown in **Table 1**, the beam balance test scores were significantly lower in the model group compared with the normal group at all time points ($P < 0.01$), indicating that the motor coordination abilities of SCI rats were significantly reduced. After 1 day of treatment, no significant difference was found among the NSC transplantation, folic acid injection and combination groups ($P > 0.05$); however, at 14 and 28 days, the scores were significantly increased in the NSC transplantation and combination groups compared with the model group ($P < 0.05$). Moreover, combination treatment significantly improved BBB scores compared with NSC transplantation and folic acid injection alone.

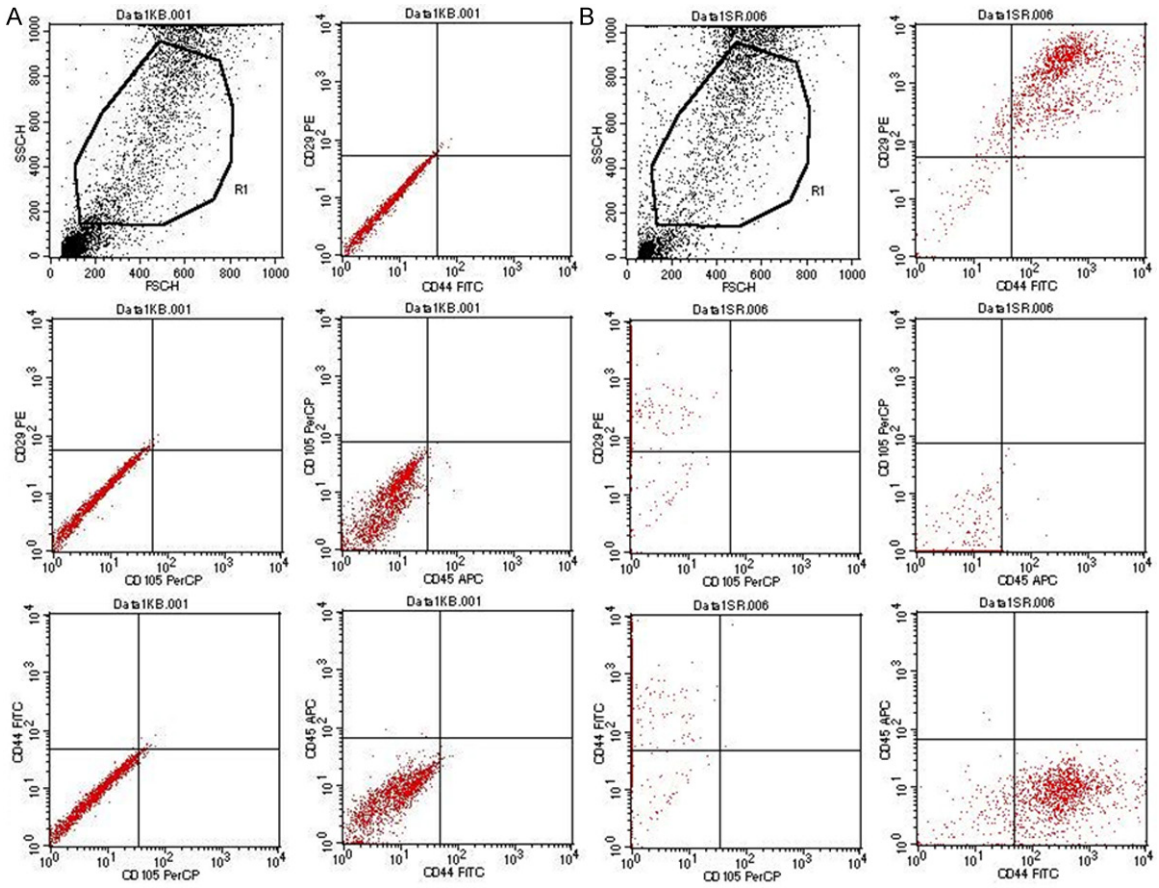

Figure 3. Flow cytometry of cultured neural stem cells. A: Represents isotype control of CD105, CD45, CD44 and CD29; B: Represents flow cytometry detection for CD105, CD45, CD44 and CD29.

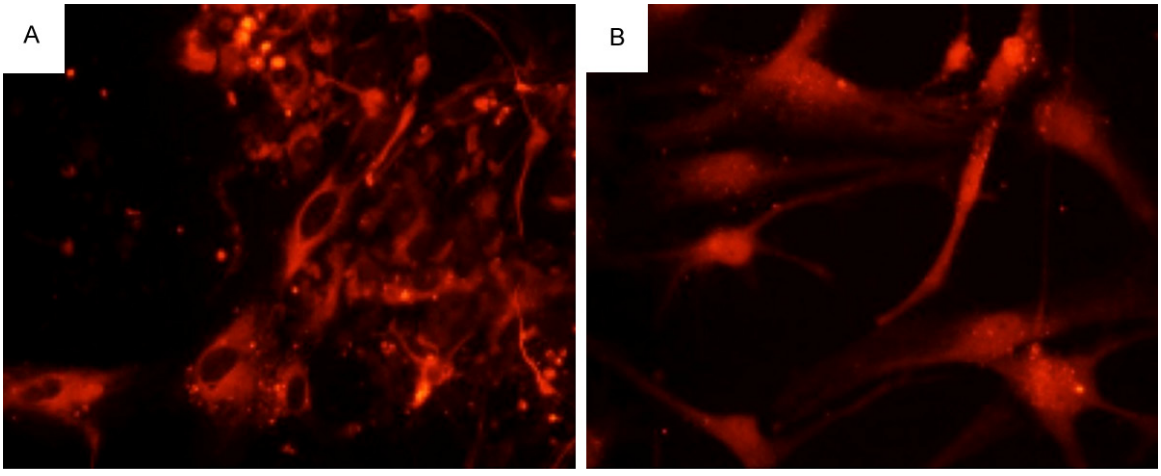

Figure 4. Immunofluorescence staining of cultured cells after induction. After induction with 10% fetal calf serum for 7 days, adult neural stem cells differentiated into glial cells or nerve cells that expressed glial fibrillary acidic protein or neuron-specific enolase. A: Glial fibrillary acidic protein fluorescent staining after induced differentiation; B: Neuron specific enolase fluorescent staining after induced differentiation.

Immunohistochemistry results

Immunohistochemistry showed a large number of 5-bromodeoxyuridine-positive cells around the ependyma at the transplantation site, indicating survival of transplanted NSCs. With prolonged time, the transplanted cells migrated along a certain path, and were mainly distrib-

Table 1. BBB score of rats in different groups (n = 8)

Group	1 day	14 days	28 days
Normal	21.0 ± 0.00	21.0 ± 0.00	21.0 ± 0.00
Model	0.4 ± 0.52[a]	8.8 ± 0.36[a]	14.0 ± 0.761[a]
Sham-surgery	19.9 ± 0.83	21.0 ± 0.00	21.0 ± 0.00
NSCs transplantation	0.5 ± 0.36	11.8 ± 0.57[b]	16.3 ± 0.54[b]
Folic acid injection	0.3 ± 0.46	9.9 ± 0.46b	14.7 ± 0.39
Combination	0.5 ± 0.27	13.8 ± 0.41[c,d,e]	19.3 ± 0.54[c,d,e]

Note: [a]$P < 0.01$, vs. normal group; [b]$P < 0.05$, [c]$P < 0.01$, vs. model group; [d]$P < 0.05$, vs. folic acid injection; [e]$P < 0.05$, vs. NSC transplantation group. NSC: neural stem cell.

uted at the focus. In the combination group, numerous 5-bromodeoxyuridine-positive cells aggregated at the focus, and exhibited a tendency to differentiate into nerve cells. Folic acid promoted NSC proliferation, enhanced restoration of neurological function reconstruction, promoted repair of injured spinal cord tissues, and improved motor function in SCI rats (**Figure 5**).

Expression of apoptosis-related proteins, BCL-2, Bax, and caspase-3

Western blotting was used to assess BCL-2, Bax and caspase-3 expression in spinal cord tissues at 28 days after treatment. The internal reference protein actin was highly expressed in each group, demonstrating the reliability of the experimental results. At 28 days, BCL-2, Bax and caspase-3 were expressed in the normal group; BCL-2 expression exhibited a tendency to increase, but Bax and caspase-3 expression levels gradually decreased in the NSC transplantation and folic acid injection groups compared with the model group, although the difference was not statistically significant ($P > 0.05$). BCL-2 expression was significantly elevated, but Bax and caspase-3 expression levels were significantly reduced in the combination group ($P < 0.05$; **Figure 6**).

The gray scale ratios for BCL-2 were 1.138, 0.0892, 0.665, 0.525, 0.685, and 0.883 in the normal, model, sham-surgery, folic acid injection, neural stem cells transplantation, and combination groups, respectively. Those for Bax were 0.644, 1.417, 0.711, 1.003, 0.945, and 0.887, respectively, while those for caspase-3 were 0.232, 0.735, 0.445, 0.559, 0.401, and 0.322, respectively.

GDNF, BDNF and NT-3 contents in spinal cord tissues

Enzyme-linked immunosorbent assays showed that GDNF, BDNF and NT-3 contents in spinal cord tissues were significantly reduced in the model group, indicating that trophic nerve function was reduced in rats. Supplementation of NSCs and intraperitoneal injection of folic acid significantly increased GDNF, BDNF and NT-3 contents and significantly enhanced recovery of neurophysiologic function. In particular, the combination group exhibited significant effects compared with NSCs or folic acid alone (**Tables 2**-**4**).

Discussion

Spinal cord injury is characterized by the high incidence, high morbidity, high treatment cost and onset at young age. This injury is accompanied by wide neuronal death, a large number of axonal degeneration and diffused demyelination, resulting in the loss of working ability and self-care ability as well as a variety of complications. The consequences following spinal cord injury are lifelong and devastating, bringing about great suffering in patients and heavy burden to the family and society. During the past century, a series of treatment strategies targeting spinal cord injury have emerged, such as surgery, drugs, physical therapy and gene therapy, but the patient's paralysis is not completely cured. Therefore, it is urgent and important to find an effective and safe treatment for spinal cord injury. Exogenous stem cells transplantation is currently receiving great attention in the field of spinal cord injury, and neural stem cells appear to be the promisingly therapeutic cells [13, 14]. It is widely recognized that nerve cells in the central nervous system have no regenerative capacity, and the dead cells after injury cannot regenerate like epithelial tissue. Recent studies found the existence of stem cells in the central nervous system, which are adult neural stem cells. Accumulating evidence [15, 16] shows that, neural stem cells are not only present in embryonic brain tissue, but also in the mature brain (including human brain), mainly in subependymal zone and subgranular zone of dentate gyrus. We isolated neural stem cells from adult rat brain and cultured them in vitro. Flow cytometry results revealed positive

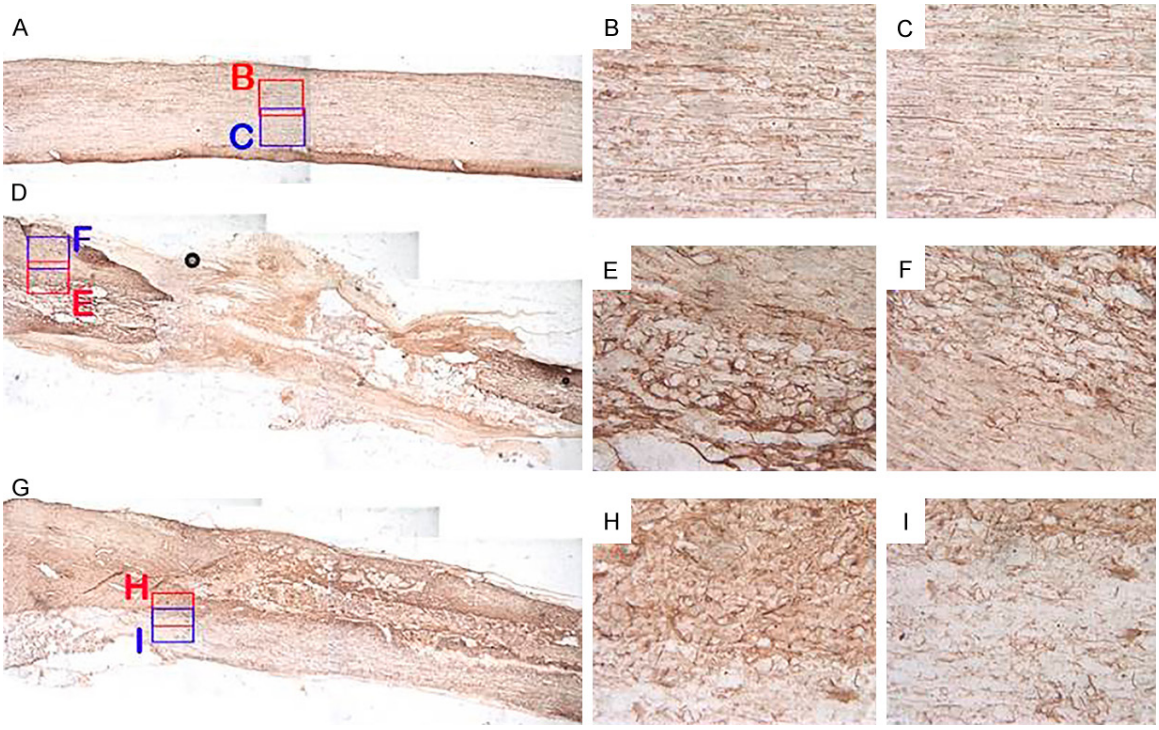

Figure 5. The results of immunohistochemistry. A: Represents Normal group; D: represents NSCT group; G: represents Combination group; B, E, H: represent Gray matter of the spinal cord; C, F, I: represent White matter of the spinal cord.

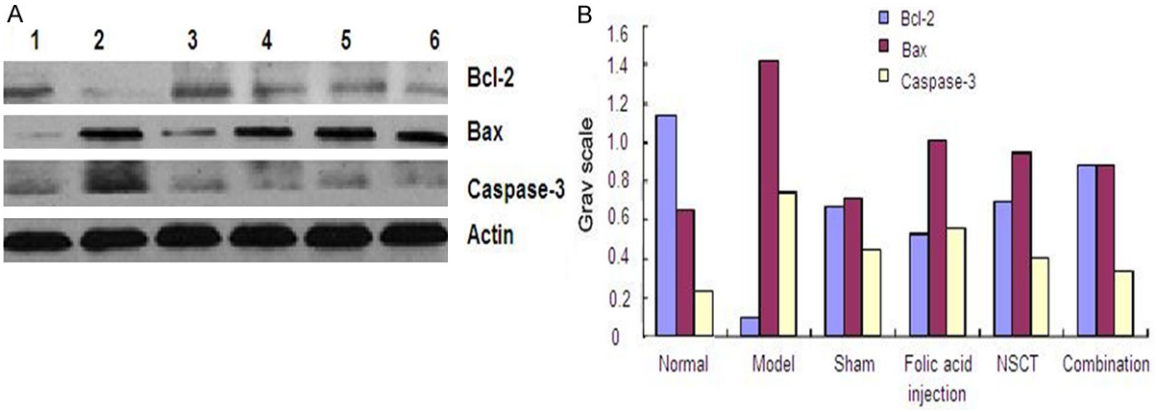

Figure 6. Expression of BCL-2, Bax, and caspase-3. A: Electrophoretogram of a western blot of BCL-2, Bax, caspase-3, and actin in each group at 28 days following treatment; B: Gray scale values of BCL-2, Bax, and caspase-3 in each group. FAI: folic acid injection; NSCT: neural stem cells transplantation.

expression of CD29 and CD44, and negative expression of CD45 and CD105, suggesting that primary cultured neural stem cells have the characteristics of mesenchymal stem cells. Adult neural stem cells at passage 2 were induced with 10% fetal calf serum. As the co-culture time prolonged, the cultured cells began to differentiate and expressed GFAP and NSE detected by immunofluorescence staining, suggesting that the cultured cells are neural stem cells. Experimental results suggest that, 14 days after adult neural stem cells transplantation into the spinal cord, the behavior ability was significantly improved, neuronal apoptosis was decreased, and the secretion of neurotrophic factors was significantly increased, thus

Table 2. The change of GDNF content in spinal cord tissues in different groups (ng/gpr) (n = 4)

Group	1 day	14 days	28 days
Normal	44.66 ± 5.85	47.22 ± 5.76	46.13 ± 7.23
Model	18.37 ± 2.26[a]	30.12 ± 5.12[a]	36.65 ± 6.12[a]
Sham-surgery	43.20 ± 5.13	44.38 ± 6.19	46.65 ± 7.19
NSCs transplantation	16.34 ± 5.24	37.38 ± 7.14[b]	40.13 ± 5.76[b]
Folic acid injection	18.57 ± 3.08	33.75 ± 3.81[b]	38.66 ± 4.29b
Combination	16.43 ± 4.39	80.35 ± 8.34[c,d,e]	85.63 ± 6.63[c,d,e]

Note: [a]$P < 0.01$ vs. Normal Group; [b]$P < 0.05$, [c]$P < 0.01$ vs. Model Group; [d]$P < 0.05$ vs. Folacin Injection Group; [e]$P < 0.05$ vs. NSC Transplant Group.

Table 3. The change of BDNF content in spinal cord tissues in different groups (ng/gpr) (n = 4)

Group	1 day	14 days	28 days
Normal	88.56 ± 11.27	78.65 ± 12.12	82.67 ± 10.67
Model	33.29 ± 8.09[a]	53.28 ± 10.07[a]	66.21 ± 12.39[a]
Sham-surgery	79.48 ± 10.21	85.34 ± 12.33	83.09 ± 9.85
NSCs transplantation	34.10 ± 7.65	61.02 ± 10.55[b]	70.81 ± 12.40[b]
Folic acid injection	35.28 ± 6.86	56.73 ± 11.24[b]	63.38 ± 11.77[b]
Combination	32.58 ± 7.27	66.31 ± 12.90[c,d,e]	75.48 ± 13.51[c,d,e]

Note: [a]$P < 0.01$ vs. Normal Group; [b]$P < 0.05$, [c]$P < 0.01$ vs. Model Group; [d]$P < 0.05$ vs. Folacin Injection Group; [e]$P < 0.05$ vs. NSC Transplant Group.

Table 4. The change of NT-3 content in spinal cord tissues in different groups (ng/gpr) (n = 4)

Group	1 day	14 days	28 days
Normal	48.13 ± 8.97	49.61 ± 10.08	47.69 ± 9.35
Model	13.57 ± 5.27[a]	28.17 ± 8.21[a]	35.38 ± 8.36[a]
Sham-surgery	45.39 ± 7.30	48.39 ± 10.23	47.31 ± 9.22
NSCs transplantation	12.29 ± 3.69	33.87 ± 5.87[b]	40.09 ± 7.16[b]
Folic acid injection	13.08 ± 3.11	30.08 ± 4.13[b]	37.34 ± 7.09[b]
Combination	13.25 ± 5.90	35.76 ± 6.79[c,d,e]	44.87 ± 7.94[c,d,e]

Note: [a]$P < 0.01$ vs. Normal Group; [b]$P < 0.05$, [c]$P < 0.01$ vs. Model Group; [d]$P < 0.05$ vs. Folacin Injection Group; [e]$P < 0.05$ vs. NSC Transplant Group.

promoting the restoration of damaged spinal cord.

Folic acid can be reduced to active tetrahydro folic acid in response to folic acid reductase *in vivo*. Tetrahydro folic acid is a main carrier of one carbon unit, involved in purine and pyrimidine synthesis and reciprocal transformation of amino acids [17, 18]. A lack of folic acid *in vivo* could disrupt transmission of one carbon unit, and affect nucleic acid synthesis and amino acid metabolism, which are essential for cell proliferation, tissue growth and development. Thus, folic acid plays a critical role in regulating neurogenesis, proliferation and differentiation of NSCs [19, 20]. The results from the present study show that folic acid treatment for 14 days significantly improved beam balance test scores in SCI rats, up regulated BCL-2 expression, down regulated Bax and caspase-3 expression, inhibited apoptosis, enhanced GDNF, BDNF and NT-3 expression, and nourished nerve cells around the injury focus. Statistical analysis showed that the effects of NSC transplantation alone were better than those of folic acid injection alone, but the difference was not statistically significant ($P > 0.05$). The results also indicated that folic acid may improve the condition of a disease by inducing autologous adult NSC proliferation and differentiation *in vivo*. A combination of NSC transplantation and folic acid injection significantly bettered the above detection indexes compared with the model group at 14 days ($P < 0.01$). Moreover, the therapeutic effects of combination treatment were better than those of NSCs transplantation alone or folic acid injection alone. The results indicate a synergistic effect between NSC transplantation and folic acid injection. *In vitro* supplemented folic acid can induce the proliferation and differentiation of autologous adult NSCs and promote proliferation of transplanted NSCs. Thus, a combination of NSC transplantation and folic acid injection has evident therapeutic effects on SCI. This therapy has some promise for the clinical rehabilitation of SCI patients.

Disclosure of conflict of interest

None.

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