academicJournals

Vol. 8(37), pp. 1812-1817, 4 October, 2013
DOI 10.5897/SRE11.138
ISSN 1992-2248 © 2013 Academic Journals
http://www.academicjournals.org/SRE

Scientific Research and Essays

*Full Length Research Paper*

# Reconditioning effect of oligodendrocyte-like cell transplantation in acute spinal cord injury in rats

**Yongtao Xu[1]*, Anmin Chen[2], Feng Li[2] and Hougeng Lu[1]**

[1]Department of Orthopaedics of Jingzhou Central Hospital, Jingzhou 434020, China.
[2]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China.

Accepted 1 April, 2011

**To observe the reconditioning effect of oligodendrocyte-like cell transplantation in acute spinal cord injury in rats, bone marrow mesenchymal stem cells were differentiated into oligodendrocyte-like cells induced by cytokines including insulin-like growth factor-1(IGF-1). Rats with acute spinal cord injury were treated with the oligodendrocyte-like cells for 8 weeks by local injection. The therapeutic effect was compared with oligodendrocytes transplantation and saline. Regeneration of axon myelin sheath and functional recovery of hind limbs were significantly improved after transplantation with oligodendrocyte-like cells than that with saline. But there was no significant difference between rats treated with oligodendrocyte-like cells and oligodendrocytes. Bone marrow mesenchymal stem cells differentiating into oligodendrocyte-like cells can be induced by cytokines including IGF-1, with similar specific surface markers of oligodendrocytes. These cells can survive in injury sites of the spinal cord for a long duration after transplantation to improve recovery of neural function and reduce cavity area at injury site after acute spinal injury in rats. The possible mechanism may be regeneration of axon myelin sheath, which is helpful for reconstruction of neural reflex function.**

**Key words:** Oligodendrocyte-like cells, differentiation, spinal cord injury, transplantation.

## INTRODUCTION

Treatment and recovery after spinal cord injury (SCI) remain to be tough problems in medical science, among which reconstruction of neural reflex pathway and re-myelinization are crucial. Oligodendrocytes play an important role in protection of axonal function and re-myelinization (Buntinx et al., 2003). But unfortunately, oligodendrocytes are limited in resources. Mesenchymal stem cells are characterized by its potency of multi-directional differentiation and they may be differentiated into neurons and oligodendrocytes (Hassan and El-Sheemy, 2004). Insulin-like growth factor-1 (IGF-1) is an important factor in directional differentiation of oligodendrocyte. It was reported that IGF-1 increased the survival rate of oligodendrocyte during cultivation and promoted the proliferation and differentiation of oligodendrocyte (Zeger et al., 2007). Promoting effect of IGF-1 in the development of oligodendrocytes and re-myelinization was demonstrated in transgenic or genetic knock-out rat models. Over-expression of IGF-1 increased volume of brain and content of myelin sheath, while brain volume decreased, amount of oligodendrocyte and myelin synthesis reduced in IGF-1 knock-out rats (Zeger et al., 2007). IGF-1 induced directional differentiation of neural stem cells into oligodendrocyte was accomplished by Hsieh et al. (2004) in both *in vitro* and *in vivo* study of rats. In the present study, bone marrow mesenchymal stem cells were differentiated into oligodendrocytes under the induction of IGF-1 and some other cytokines. These oligodendrocyte-like cells were transplanted into rats with acute SCI to

*Corresponding author. E-mail: jackjone2008@hotmail.com.

observe the functional recovery of the impaired spinal cord.

## MATERIALS AND METHODS

Clean-grade Sprague Dawley (SD) rats without restriction on gender (weighing 250±20 g) were provided by the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All animal experiments were in accordance with Standards of Animal Ethics. Reagents used in this study were listed as follows: DMEM-F12 (1:1) culture medium (Hyclone Co. Ltd.); high-quality fetal bovine serum (GIBCO Co. Ltd.); N2 Supplement, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), IGF-1 (Invitrogen Co. Ltd.); rabbit anti-myelin basic protein (MBP) monoclonal antibody (Sigma Co. Ltd.); rabbit anti-mouse galactocerebroside (Galc) (Chemicon Co. Ltd.); rabbit anti-glial fibrillary acidic protein (GFAP), mouse-anti microtubule-associated protein-2 (Boster Co. Ltd., Wuhan, China); fluorescein isothiocyanate (FITC), rhodamine-labeled goat anti-mouse, goat anti-rabbit IgG secondary antibody (Zhongshan Bio-tech Co. Ltd., Beijing, China).

### Preparation of bone marrow mesenchymal stem cells

Rats of 4 weeks old were sacrificed by cervical dislocation. Bilateral tibias and femurs were dissected under sterile condition. After exposing, the marrow cavity was washed with 10% DMEM-F12 medium several times and the wash solution was collected in a 25 $cm^2$ culture bottle. About 3 to 5 ml DMEM medium was added to re-suspend the cells and then cells were planted into another 25 $cm^2$ culture bottle. The cultural system contained Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), 2 mmol/L glutaminate, 100 U/ml penicillin and 100 mg/L streptomycin. Conditions of cell incubator included 37°C, 5% $CO_2$ and saturated humidity. Culture medium was changed and inadherent cells were removed 3 days later.

Then the culture medium was changed every 3 days until adherent cells covered more than 90% of bottom of the culture bottle. The adherent cells were digested with 2.5 g/L trypsin till the cells turned round, intracellular space increased and cytoplasm retracted. Then cell suspension was transferred to sterile centrifuge tube and centrifuged at 1000 rpm for 5 min. After removing the supernatant, cells were re-suspended and passed 1:2. With the culture medium changed and passage cells purified, cells at passage 4 were used for experiment.

### Separation and purification of oligodendrocytes

Oligodendrocytes were acquired by separation and purification through agitation and chemical definition serum-free medium according to McCarthy's method (McCarthy and DeVellis, 1980). SD rats within 48 h after birth were sacrificed and the brain was collected under sterile condition. Cerebral cortex was separated, sheared and blown in PBS. After being filtered, solution was centrifuged at 1500 rpm for 5 min and the supernatant was removed. Then the cells were added into high-glucose DMEM containing 15% Fetal Calf Serum (FCS) to make the cells thoroughly isolated and inoculated with a density of $I\times10^6$. Conditions of cell incubator included 37°C and 5% $CO_2$, and culture medium was changed every 3 days. The cells were divided into 2 layers 7 to 8 days later. With the bottle beak sealed, culture bottle was agitated at 150 rpm in concentrating table of 37°C for 2 h.

Then, the supernatant was aspirated and microglia was removed. High-glucose DMEM with 15% FCS was added and the remaining cells were agitated at 250 rpm for 17 to 19 h. Exfoliated cells were collected and centrifuged at 1500 rpm for 10 min. The cells were then re-suspended with high-glucose DMEM containing 15% FCS and inoculated in 24-well culture plate with a density of $3\times10^4$/L. After incubation for 6 h, the culture medium was replaced with DMEM/F12 culture medium A for further cultivation. Then 02 A cells were obtained. Three days after cultivation, the medium was replaced with DMEM/F12 culture medium B (Givogri et al., 2006) for differentiation induction. Finally, mature oligodendrocytes were obtained.

### Differentiation induction of bone marrow mesenchymal stem cells into oligodendrocytes

Bone marrow mesenchymal stem cells at passage 4 were incubated with induction solution (Neurobasal A, 1% N2 Supplemented, 20 µg/L bFGF, 20 µg/L EGF and 20 mmol/L L-glutamine). Then, cells were transferred into culture dish with a coverslip at a density of $1\times10^4/cm^2$. After incubation in 5% $CO_2$ for 48 h, medium was replaced with culture medium containing 500 µg/L IGF-1, neurobasal A and 1% N2 Supplemented. Three days later, the cells were prepared for transplantation.

### Animal experiments

After weighing, the rats were anesthetized by 5 ml/kg 8% hydral intraperitoneally. Hair around the surgical site was sheared and animals were fixed at operating table. Medial incision of back centered at T10 spinous process was made, with a length of about 4 cm. Skin and superficial fascia were incised and a round region centered at spinal cord with a diameter of 3 mm was exposed. A plastic mattress with a camber similar to spinal cord surface was place at the surface of dura mater. A cylinder metal stick weighed 10 g and was dropped vertically at a height of 5 cm in the guidance of fine glass tube. The mattress was punched to induce a moderate contusion at T10 level. The strength was about 5 cm ×10 g. The model was regarded as successful if bilateral hindlimbs contracted fiercely. After successful modeling, muscle, fascia and skin were sutured layer by layer. Medical drape was used for external fixation.

### Grouping

Rats were randomly allocated into 3 groups: oligodendrolyte-like cell group (A), oligodendrolyte group (B) and saline group (C). Nine days after spinal cord injury, 5 µl $4\times10^5$/ml cells or saline was injected along the previous incision. Food and drinking was not restricted after treatment. Artificial bladder urination was done 3 times per day until urination function recovered.

### Slope test

Slope test was performed using the Rivlin method (Martin et al., 2002). A rubber pad was placed on a rectangle board. Rats were placed on the board with head forwards and body longitudinal axis vertical to board longitudinal axis. The angle between board and horizontal plane was gradually increased until rats could maintain their position for only 5 s. This angle was called critical angel of tilting plane. The critical angle was measured 2, 4 and 8 weeks after transplantation, respectively.

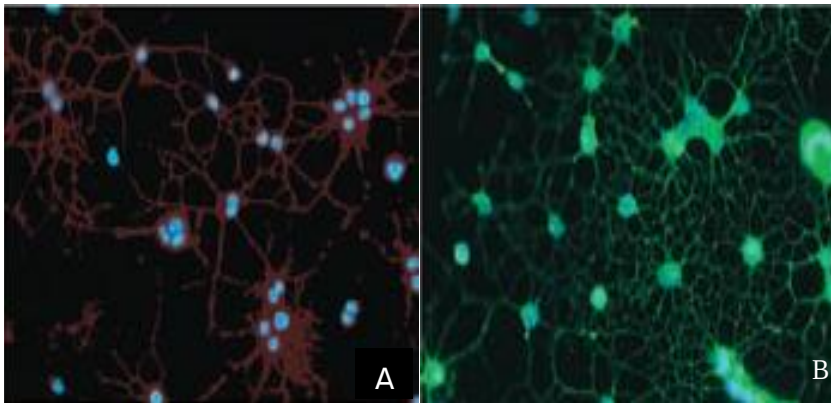


**Figure 1.** Immunocytochemical staining of specific oligodendrocyte marker 72 h after differentiation induction with 500 µg/L IGF-1 of different concentrations. A) Galc fluorescent staining (blue: nuclei; red: Galc); B) MBP fluorescent staining (blue: nuclei; green: Galc).

#### Basso Beattie Bresnahan scoring

The scores were measured 2, 4 and 8 weeks after transplantation. The Basso Beattie Bresnahan scoring was performed by a staff other than the experimental persons but very familiar with the scoring criteria (Barros and Molina, 2008).

#### Determination of spinal evoked potential

Rats were anesthetized with intraperitoneal pentobarbital. Skin at back of skull was dissected and periosteum was stripped. A hole was drilled in skull bone, 3 mm posterior to coronal suture and 1 mm right to sagittal suture, which corresponding to the right sensory region of postcentral gyrus of cortex. Left tibial nerve was also exposed. Microwaves of sensory and motor evoked potentials (SEP and MEP) were noted.

#### Light microscopy and immunohistochemistry test

Rats were perfused with 4% paraformaldehyde 4 and 8 weeks after transplantation. Impaired spinal cord was dissected and slices were made for immunohistochemistry test. Transplanted cells were visualized using Galc.

#### Statistical analysis

All data was expressed in $\bar{X} \pm s$ and statistical analysis was performed in SPSS 10.0. Difference between 2 groups was compared using t test. $P<0.05$ was considered statistically significant.

## RESULTS

### Morphological changes of bone marrow mesenchymal stem cells during differentiation

After differentiation induction, most bone marrow mesenchymal stem cells showed morphological characteristics of oligodendrocytes. Cytoplasma was retracted towards nucleus and cell process was extended. Light refraction was strengthened and cell processes communicated with each other to form a classic reticular structure. Specific bands of Galc (A) and MBP (B) mRNA were detected after differentiation induction. Immunocytochemical staining showed that, positive rate of Galc was 65% and that of MBP was 45%, indicating they processed the characteristic of oligodendrocytes (Figure 1)

### Behavior observation

Typical paraplegia was present after spinal cord injury, including paralysis of 2 hindlimbs, low muscular tension and 0-grade muscle strength. Rat behavior was similar 2 weeks after transplantation. Motor function recovered significantly 2 to 3 weeks after surgery and the recovery became slow 4 weeks after surgery.

### Slope test

The critical angles of rats in the 3 groups 2, 4 and 8 weeks after transplantation are shown in Table 1 and Figure 1. The critical angle increased 2 weeks after spinal injury but no significant difference was present among the groups ($P>0.05$). The angle increased more significantly in group A and B 4 and 8 weeks after injury than in group C ($P<0.05$).

### BBB scoring

BBB Scores of the 3 groups are shown in Table 2. The

**Table 1.** Changes of critical angles in slope test within 8 weeks after cell transplantation in rats with spinal cord injury ( $\bar{x}$ ±s).

| Group | 2 week | 4 week | 8 week |
|---|---|---|---|
| A | 21.47±2.46 | 38.36±2.34* | 46.23±3.89* |
| B | 21.36±3.08 | 39.59±3.63* | 47.65±3.56* |
| C | 21.56±2.72 | 25.86±3.02 | 29.09±2.78 |

* P<0.05 vs. group C.

**Table 2.** BBB scores of rats with spinal cord injury within 8 weeks ( $\bar{x}$ ±s).

| Group | 2 week | 4 week | 8 week |
|---|---|---|---|
| A | 2.45±0.28 | 5.86±0.21* | 6.96±0.25* |
| B | 2.36±0.21 | 5.97±0.19* | 6.87±0.27* |
| C | 2.40±0.18 | 3.52±0.16 | 3.89±0.19 |

*Compared with group C (P<0.05).

**Table 3.** MEP latency (ms, $\bar{x}$ ±s).

| Group | 2 week | 4 week | 8 week |
|---|---|---|---|
| A | 6.67±1.32 | 4.31±1.16* | 2.16±1.35* |
| B | 6.36±1.15 | 4.66±0.56* | 2.45±1.52* |
| C | 6.86±1.13 | 6.05±1.26 | 4.85±1.08 |

* Compared with group C, P<0.05

**Table 4.** SEP latency (ms, $\bar{x}$ ±s).

| Group | 2 week | 4 week | 8 week |
|---|---|---|---|
| A | 6.26±1.03 | 4.87±0.85* | 3.16±0.69* |
| B | 6.16±0.78 | 4.69±0.94* | 3.68±0.74* |
| C | 6.09±0.98 | 5.76±0.77 | 5.23±0.81 |

*Compared with group C (P<0.05).

BBB scores were increased greatly 2 weeks after spinal injury, but no significant difference was observed among the 3 groups (P>0.05). BBB scores were increased more significantly in group A and B than in group C 4 and 8 weeks after injury (P<0.05).

### Spinal evoked potential

With the duration increased after cell transplantation, the latency of motion evoked potential (MEP) and sensory evoked potential (SEP) decreased gradually. The difference between different groups was statistically significant 4 and 8 weeks after transplantation (Tables 3 and 4).

### Staining test of the spinal cord

Brdu and Galc immunofluorescence double staining showed double staining was present in some cells 4 weeks after transplantation (Figure 2), which was also confirmed by the immunochemistry staining (Figure 3).

## DISCUSSION

Recent studies showed MSCs can differentiate into not only mesodermal cell but ectodermal and endodermal cells, which are the characteristics of stem cell plasticity (Myckatyn et al., 2004; Nagy et al., 2005; Reynolds and Weiss, 1992; Eriksson et al., 1998; Carlo-Stella and Gianni, 2005; Rydén et al., 2003). In addition, the cytokines secreted by cells can also affect the differentiation of surrounding cells. Direkze et al. (2003) revealed the neural stem cells can induce the differentiation of mesenchymal stem cells into neural stem cell-like cells via paracrine and interactions between intercellular receptors, and the later cells could differentiate into neuron cells and glial cells *in vitro*. In the *in vitro* study of Hassan and El-Sheemy (2004), the mesenchymal stem cells were induced to differentiate into Schwann cells which were used to repair rat sciatic nerve achieving favorable outcome. Oligodendrocytes are closely related to the injury of the central nervous system including spinal cord injury. Following spinal cord injury, oligodendrocytes then die and the demyelination of nerves occurs, which affects the recovery of spinal cord function. Therefore, the number of viable oligodendrocytes is a contributing factor of myelination following axonal regeneration. Study shows oligodendrocytes play important roles in the myelination (Emery, 2005), and can provide growth factors and neurotrophic factors for the central nervous system.

The mechanism underlying the *in vitro* differentiation of stem cells is still poorly understood (Karlsson et al., 2007; Kim et al., 2006; Bai et al., 2007). In the present study, mesenchymal stem cells were isolated from the rat bone marrow and then induced with bFGF, EGF, and IGF-13. These cells differentiated into a variety of cells with expressions of markers (Galc and MBP) (Buntinx et al., 2003; Paez et al., 2006) of oligodendrocytes and some cells with expressions of markers of neurons and astrocytes (MAP-2 and GFAP) (Kuhn et al., 2005; Pillai et al., 2006) in the serum free N2 supplement medium. In the differentiation, bFGF and EGF are the factors promoting the differentiation, and involve in the initiation of differentiation of mesenchymal stem cells into neurons. Study reveals members of fibroblast growth factor family, play a critical role in the differentiation of stem cells into neurons (Jo et al., 2005).

In the present study, the bone marrow mesenchymal stem cells were induced to differentiate into oligodendrocyte-like cells with expressions of markers of

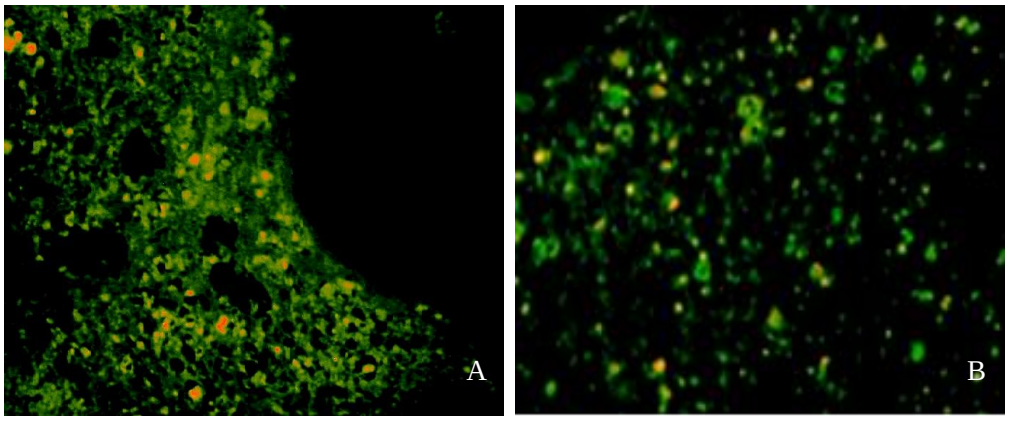


**Figure 2.** Brdu and Galc immunofluorescence double staining. Double staining was present in some cells 4 weeks after transplantation, ×200 (groups A and B).

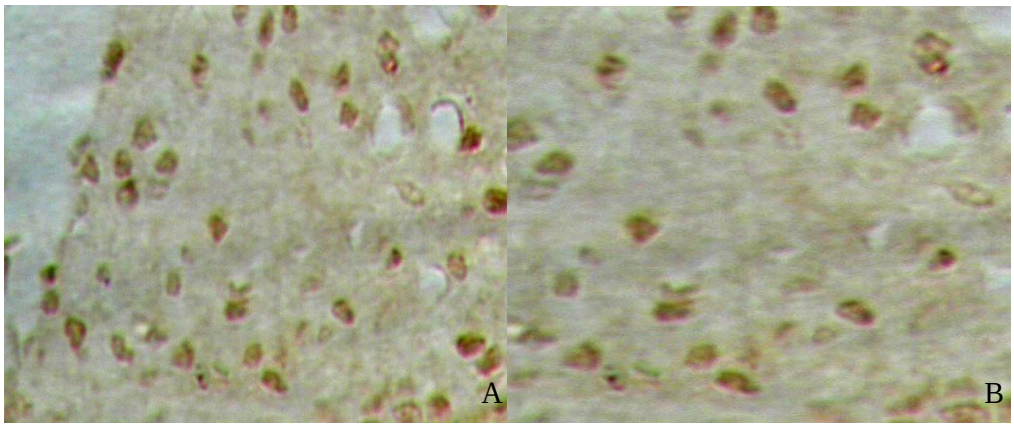


**Figure 3.** Distribution and survival conditions of oligodendrocyte-like cells in spinal cord 4 weeks after transplantation, ×200(group A and B).

oligodendrocytes. The later cells were then transplanted into rats with SCI. Rats treated with normal oligodendrocytes and normal saline served as controls. Our results showed that, there was no significant difference in the improvement of spinal cord function between oligodendrocyte-like cell group and oligodendrocyte group. Two weeks after transplantation, the critical angle of tilting plane increased but there were no significant differences between different groups ($P>0.05$). At 4 and 8 weeks after transplantation, the critical angle of tilting plane in oligodendrocyte-like cell group and oligodendrocyte group was markedly increased when compared with the normal saline group ($P<0.05$).

In addition, BBB score is a sensitive and reliable method and can be used to predict the recovery of behaviors. At 4 weeks after transplantation, there were marked differences in the BBB score between oligodendrocyte-like cell group, oligodendrocyte group and normal saline group and the differences could also be noted at 8 weeks after transplantation. In the detection of spinal cord evoked potential, the latency reflects the speed of nerve impulses. At 4 and 8 weeks after transplantation, the latency decreased gradually and there were remarkable differences in the latency between oligodendrocyte-like cell group, oligodendrocyte group and normal saline group.

Our results show bone marrow mesenchymal stem cells can be induced to differentiate into oligodendrocyte-like cells, and the later cells can survive in the spinal cord after transplantation into the injured spinal cord which promotes the recovery of spinal cord function. In this process, oligodendrocyte-like cells exert effects similar to those of oligodendrocytes and can form myelin, secret growth factors and neurotrophic factors, facilitating the survival of neurons, which finally promotes the recovery of neurological function.

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