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Effects of Mesenchymal Stem Cells Transplantation on Repair of Rat Spinal Cord Injury

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Abstract:
To investigate the effects of mesenchymal stem cells (MSCs) transplantation on promoting the repair of spinal cord injury (SCI). A modified Allen method was used to establish the rat model of SCI. The rats were randomly divided into a control group (Group A) that was intraspinally microinjected with 10 μl of normal saline 5d after SCI, and a MSCs transplantation group (Group B) that was intraspinally microinjected with 10 μl of MSCs suspension 5 d after SCI. Oblique board test was performed respectively on days 10, 20 and 30 after transplantation, the recovery of rat motor function was observed using the BBB scale method of spinal cord motor function, the recovery of nerve function was observed using spinal cord evoked potential, the changes of the empty area of SCI were observed by hematoxylin-eosin (HE) staining, and the survival and differentiation of transplanted MSCs as well as the regeneration of nerve fibers in the injured site were discerned using the immunohistochemical method. The differences in the incline angle of oblique board, BBB score, MEP incubation period, SEP incubation period and nerve axon counting 30 d after transplantation were statistically significant between the two groups ($P<0.05$). In the experimental group, astrocytes and nerve fiber regeneration could be obviously observed and the empty area of the injured site of spinal cord was significantly reduced. MSCs can be differentiated into neurons and neurogliocytes in the injured site of spinal cord, thus reducing the empty area of the injured site and facilitating the regeneration of axon and motor function recovery.

Keywords: spinal cord injury; mesenchymal stem cell; transplantation; functional recovery

Introduction

Bone-mesenchymatic stem (BMSCs), present in the bone marrow, may differentiate into a directional or multi-directional differentiation (1), osteocytes, chondrocytes or adipocytes. BMSC is an important part of the hematopoietic bone-marrow micromedia by supporting and regulating hemato-poiesis both in-vitro and in-vitro (2,3), as another type of cell population with high-level plasticity excepting HPSC. A variety of possible clinical applications for culture, induction and differentiation in vitro, gene treatment, cell therapy or tissue engineering etc. are available at BMSC, capable of differentiating into osteocytes, adipocytes, myocytes, neurocytes and other tissual cells under specific conditions of induction (4.5).

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BMSCs have been very attracted to its rich sources, easy sampling, easy isolation and culture, multi-directional differentiation and mild rejection (6). Furthermore, the BMSC not only provides mechanical HSC support for bone marrow, but also secretes a number of factors of growth in support of hematopoiesis (e.g. IL, IL11, LIF, M-CSF and SCF). The damaged neurons or axons cannot be regenerated following injuries to the central nervous system but can be resolved by cell transplantation. In this study, BMSCs are transplanted to the injured spinal cord of rats to see their functional rehabilitation.

Materials and Methods

Materials: materials

The Department of Laboratory Animal Science of Capital Medical University has purchased forty (220 ± 20) g, adult or adult rats of body weight. Below are the main reactives and devices. DMEM / F12 (1:1): Shanghai Jiaanglai Bio-tech Co., Ltd.; BSA / F12 and tripsin: Shanghai Boyan Bio-tech Co., Ltd.;

Mouse of monoclonal anti-human neurofilament (NF) antibody anti-human glial fibrillal acidic protein (GFAP) anti-human proteins monoclonal: AMRESCO; microscope: Tohnichi, Japan; clean bench:

MSC preparation

5-week-old rats were chosen for cervical dislocation to be killed. In order to extract the muscle tissue from the bone surface, the bilateral femurs and tibias have been rinsed with phosphate-buffered salines (PBS) and have the epiphysical end cut to expose the marrow cavity, then have a needle of sterile syringe washing in the cavity with DMEM medium containing 10% calf serum and collecting prepa cell bone marrow cells After 2 d, nonadherent cells have been discarded. The adhering cells were confirmed to be BMSC by means of a preliminary study of the surface markers of adherent cells by flow cytometry. The BMSC was cultivated by amplification to the third passage. For transplantation the 3rd passage BMSC, before washing cells three times with PBS and digesting them 3 ml with 0.5 percent trypsin, centrifuging them three times with low speed to get cell debris out of the suspension, washing cells with PBS and adjusting them to the ultimate mass content of 1105 / ml.

Model establishment

A modified Allen method was used to establish Spinal Cord Injury (SCI) model. Intraperitoneally, the rats were injected with a sodium pentobarbital volume fraction 0.5 percent (50 mg / kg), fixed to the test table after back shave, in a prone position, and disinfected with 5 per cent povidone-jodine volume fraction. After the hole sheet was paved on the back, the center was selected for the midline of the back for incision with a length of approximately three centimetres, and skin and subcutaneous tissue were opened to expose and to remove T8 spiny and total spine plate through special forceps to expose the spinal dura mater. According to the theory of Allen's method of heavy hammer dropping hit, a 10 g homemade hammer was used to hit the spinal cord with the gravity intensity of 2 cm × 10 g under the guidance of glass catheter, and the extreme contraction of both hind limbs was considered as successful impact, causing moderate injury of spinal cord, that was incomplete paraplegia. At last, the incision layer by layer was sutured.

Grouping of animals

The rats were divided randomly into a group (Group A) and group BMSC (Group B). The incision inserted at the original stage was 10 μl of regular saline and BMSC 5d suspension after SCI, and after 1 minute of the injector, the needle was held. Finally, layer by layer the incision was sutured. Paraplegic rats have been pushed 3 times every day to avoid injuries on the bladder surface for manual urination. After therapy 20 rats were chosen for testing 10, 20 and 30d activity of the spinal cord.

Observation of product law

There were also muscle powers, motions and synchronization of the rat's hind leg.

Inclined test for aircraft

In the vertical direction of the coronary axis, rat was positioned on a 50 cm/ 60 cm flat plank in a supine posture, while the longitudinal axis was progressively raised on the head-side, with a steady gradation of 5 at each point. It considered its operative value to be the maximum angle of the rat's 5S, and the level of a platter inclined (9).

Level of BBB engine

Similar to the size of the locomotive BBB, the rat was positioned in a fixed location to study its shoulder, knee, elbow, harness, trunk activity, and agility, as well as its hind limb action, trunk posture and stability, gait, balance, paw posture, the toe clearances, and tail position. Two non-experts from this trial who knew BBB score criteria at 8:30 a.m. conducted blinded score assessments. Since transplantation of 10, 20 and 30 d , respectively (10).

Detection of future spinal cord

Sodium pentobarbital was injected for anesthesia intraperitoneally with the rats for detection. The skin of the skull was cut open and the periosteum was removed to drill the blind hole 5 mm behind the coronal suture that corresponds to the center of the gyroscopic area of the right part of the cortex, the skin in the rear skull was cut open and the suture was cut out and exposed to the left leg sciatic nerve. Skulls of the skull were cut and left leg stitched. The evocative potential technique has been used to measure the somatosensory (SEP) and engine evocative potential (MEP) microwave-shaped curves.

Sight and immunohistochemical therapy of the sample

On days 10, 20 and 30, after transplanting the rats got 3 percent volume fraction of paraformaldehyde (pH 7.2), therefore the damage to the spinal cord was sliced by 2~3 cm in thickness, dropped in 10 percent sucrose and sink and then

put in the microtoma of cryostate to -30 ° to slicing stage. The samples had been cut along the horizontal axis of the backbone and six slices with a thickness of 3 μm, three of which had been coated with hematoxiline-Eosin (HE), with immunohistochemical staining by utilizing an ABC process on the remaining three slices. Astrocytes and neurofilament antibody (NF) neuronal axons were used for Glial Fibrillar Acid Protein Antimatter (GFAP).

Injured regions nerve fiber recording

The blood chamber matrix counting size (3 μm2 per column) has consecutively calculated the numbers of neuronal axons in five neighboring damaged areas for average values.

Analyzing data

SPSS 13.0 evaluated all details. The data was usually distributed and interpreted as a mean ± standard derivative (x (-) ±s) and subjected to separate t-test samples.

Results

Observation in animal comportement

Following the Study, a common paraplegia syndrome existed in all rats who established two hind extremity paralysis, lower stress muscle, 0 ° muscle intensity and symptoms, and rats started crawling twelve hours after wounded foot dragging on two hind limbs. No big variations were identified between the behavior of the two groups 10d after transplants though hind limbs were moving in Group B marginally more vigorously on day 20 than in Group A with well-grounded paws and hind limbs were moving more flexibly with Group B rats on the day 30 after the transplant.

Reclined flight outcomes for aircraft

In typical rats the critical angle of inclination was on average 70 ° C. The vital angle of inclination in both groups was steadily increased after transplantation. The crucial angle suggested an apparent development trend for Group B. The disparity between both was statistically relevant on the day 30 after the transplant and was comparatively stable in group A (P<0.05, Table1).

Table 1. **Inclined plane test results**

Group	10 d after transplantation	20 d after transplantation	30 d after transplantation
Group A	41.3±8.6	48.4±9.0	55.7±9.2
Group B	42.7±8.3	52.9±9.4	60.1±9.5*

Compared with Group A, *P<0.05.

BBB scale results

The BBB scale of normal rats was 20 points. The BBB score for the hind limb motor function of the rats in Group B 30d after transplantation was significantly higher than that of Group A with statistically significant difference (P<0.05, Table 2).

Table 2. **BBB scale results**

Group	10 d after transplantation	20 d after transplantation	30 d after transplantation
Group A	3.2±0.6	4.4±0.8	7.2±1.3*
Group B	3.4±0.7	5.7±1.1	11.5±2.1

Compared with Group A, *P<0.05.

Evoked potential results

With time extension after transplantation, the MEP and SEP latent periods of both groups were gradually decreased, between which the differences were statistically significant on days 20 and 30 after transplantation respectively (P<0.05, Table 3 and 4).

Table 3. **MEP latent period**

Group	10 d after transplantation	20 d after transplantation	30 d after transplantation
Group A	8.16±0.92	6.97±0.84*	5.62±0.81*
Group B	8.17±0.91	5.94±0.88	4.12±0.79

Compared with Group A, *P<0.05.

Table 4. **SEP latent period**

Group	10 d after transplantation	20 d after transplantation	30 d after transplantation
Group A	7.82±0.85	6.32±0.74*	5.11±0.72*
Group B	7.64±0.81	5.01±0.72	3.41±0.65

Compared with Group A, *P<0.05.

The findings of light and immunohistochemistry microscopes

On the 30th day after transplantation, several cavities were seen in Group A. After staining, there were comparatively more spinal cord cavities and fewer residual light microscope white matter. Microcapsules of nerve fiber and cell necriticism emerged in several different sizes in White Matter. In the proximal injury region, some astrocytes and regenerated axons have been identified. In Group B, the spinal cord was coated with a vast amount of hyper plastic tissues, no apparent weakness in the spinal cord and few, thin, comparatively light vacuoles. In the proximal injury region, there were comparatively more astrocytes and axons regenerated than were scattered throughout this field (Table 5).

Table 5. **Axon counting in injured areas (strip/mm^2)**

Group	10 d after transplantation	20 d after transplantation	30 d after transplantation
Group A	53.9±9.4	45.1±8.7	37.6±7.3*
Group B	56.8±9.3	48.8±8.2	44.3±7.6

Compared with Group A, *P<0.05.

Discussion

In order to facilitate functional restart, the BMSCs will survival, production and differentiation of host spinal cord and establish neural connections. The mechanisms available are: BMSCs would fill the harm area like the bridge of the spinal cord and provide chemical or mécanical instructions for the development of the vertebrae and recovery of the damaged nerves in the guild to the injury location; BMSCs may provide neurons for compensatory harm to the missing cell structure; BMSCs may establish a number of factors beneficial to the spinal cord in the host region (11-13); Main and secondary spinal cord damage are caused by neural impairment following SCI. The destruction of primary damage spinal nervous cells is permanent, so successful therapeutic steps aimed at minimizing secondary harm are essential to the recuperation of spinal cord activity and to enhancing patient prognosis (14,15). Axonal and efficient recovery during SCI has always become a daunting challenge to overcome. The spinal cord is mainly responsible for the sound processing and signal transduction of the central nervous system of the target person. Axon fracturing and myelin disintegration, along with necrosis and apoptosis of cells in the nerves, are pathological forms of SCI. For neuronal transplantation in the management of SCI, then how easily axonal regeneration and myelination can be encouraged (16,17). In the study of central nervous system damage, the SCI therapy with BMSC transplantation has a number of advantages, including convenient screening, quick amplification of the spinal cord and multidirectional separation and simple incorporation (18,19). SCI therapy This thesis developed a SCI model in Allen rats, the clinical procedure of which was frequé.

The most accurate assessment of regeneration after SCI was the engine function value, and the regeneration of SCI depends essentially on the regenerative role (20). The discrepancy between Groups B and A on day 30, after transplantation (P < 0.05) in the latent SEP and MEP time ranges was statistically important in this analysis.

MEP latent intervals and the total amplitude instability pattern were in line with motor score shifts. A comparatively higher degree of cavities was finding in Group A 30d after donation. After staining there were significantly higher concentrations of light microscope cavities in spinal cord tissue; several microcapsules were present in white matter with dark chromosome and nerve fiber necrosis and comparatively more vacuums developed near to the transaction. The spinal cord was covered with several hyperplastic tissues with group B. An apparent spinal cavity was observed in comparatively light-colored spinal cord tissue following bleeding and multiple nerve fibers and astrocytes were not identified. For pathomorphological studies, the sum of neurons and morphological shifts is of great importance. The the number of stained neurons was a result of enhancing the motor activity of laboratory rats. The discrepancy between groups B and A was statistically important in the number of regenerated axons per unit region on day 30 after transplantation (P<0.05). The improvement of limb motor activity in this time frame was also important. These findings demonstrate that the BMSC transplanted matrices forming in damaged spinal cord will substantially reduce glial scar formation after SCI and encourage neuronal survival, axonic regeneration, and myelin growth. The findings from this analysis reveal that, while oblique-plan test and group-BBB at every stage after transflation is higher than those of group A, the discrepancy was considered statistically meaningful only when the group-B motor function

value was greater than 10 points at day-30 of transplants (P<0.05). This indicates that group-U is statistically important on day 30 after transplantation (P<0.05). This may be attributable to the fact that BMSC may migrate in the damaged spinal cord, differentiating into neuronic cells that are propitious to central nervous system functional recovery. The variations in SEP and MEP reflect a degree in spinal cord physiological function. The shorter the latent time and the larger the acceleration, the smoother the conduction of the spinal cord and the engine.

After transplantation, the latent time latency of group B, SEP and MEP started to emerge, and the amplitude value steadily improved, thereby signaling the incremental restore of backbone activity and major improvement on day 30 after transplantation.

In brief, transplanted BMSC can live in vivo and separate between neurons and astrocytes. It may also partly restorate the engine role of rats with an impaired backbone.

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Conflict of interest. The authors declare no conflict of interest.

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