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Influence of Transplantation of Bone Mesenchymal Stem Cells on the Acute Injury Motor Function of the Spinal Cord and Expression of the Nerve Growth Factor of the Rat

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Abstract: Purpose to research the therapeutic affect of the allograft of Bones Mesenchymal Stem Cells (BMSCs) on the acute injury of the spinal nerve of the rat. Method: Take 1 Westar healthy rat, collect the bone marrow, adopt the adherence method to separate BMSCs and culture and mark them, cultivate the BMSCs culture solution with the cell population of about $5 \times 10^4 \mu\text{L}^{-1}$ for transplantation. Establish 40 westar rat models with the acute injury of the spinal cord, which shall be divided as the transplantation group and the control group, 20 pieces for each group. After a week of injury, inject BMSCs slowly to the injury center of the rat's spinal cord, inject the physiological saline to the control group and observe and inspect the rehabilitation efficacy of the hind limb function and the protein expression of the Nerve Growth Factor (NGF) and Brain-derived Neurotrophic Factor (BDNF) of the rats of two groups. Result: The rehabilitation efficacy of the hind limb function of the transplantation group is obvious better than that of the control group after 3-8 weeks of injury and the difference is of significance ($p < 0.05$). Kill two groups of rats after 8 weeks and it is found that the transplantation group is obviously higher than the control group through inspection of the protein expression of NGF and BDNF. The difference is of significance ($p < 0.05$). Conclusion the allograft of BMSCs can remarkably improve the rehabilitation of the lower limb motor function of the rats with acute injury of the spinal nerve, which is possibly related with that the transplantation of BMSCs can promote the regeneration and repair of the rat's spinal nerves. It is proven through the NGF and BDNF protein expression data from the experiment of the transplantation group and the control group that BMSCs transplantation can improve the expression of some NGF of the rats with spinal nerve injury. These nerve factors are beneficial for regeneration, growth and repair of the injured nerve tissue cells, so as to further confirm that the rehabilitation of the lower limb motor function of the rat's with acute injury of the spinal nerve thanks to the induced regeneration, growth and repair of the spinal nerve cells by BMSCs transplantation.

Key words: Mesenchymal stem cells, transplantation, spinal nerve, repair

INTRODUCTION

The acute injury of the spinal cord of the mammal will usually cause limb paralysis and other motor function disorders, which are related with the spinal nerve injury. However, it has been proven through the fundamental research that after spinal nerve injury of the mammal, the newborn nerve cells generated from endogenous self-repair are few and it is difficult to start the functional axon regeneration. Therefore, it is difficult for late rehabilitation. In recent years, the transplantation of the bone mesenchymal stem cells and the development of the nerve tissue engineering have opened up a new path to treat the spinal cord injury. The mesenchymal stem cells are the stem cell which exist in the bone marrow and other tissues and have the multi-directional differentiation capability. It has very strong plasticity and chemo taxis, can enter the tissue and can be differentiated into the

tissue cells from multiple mesoderm's in the suitable conditions, so as to induce cell regeneration and growth and repair the injured tissue (Krause *et al.*, 2001). Along with the rise of stem cell research in recent 20 years, BMSCs have provided the new research direction for treatment of many diseases and show the preliminary efficacy (Harvey and Chopp, 2003). In the aspect of BMSCs transplantation intervention treatment of spinal nerve injury, it is pointed out in the summary by Hao Shuyu and so on that the neural precursor cell with specific differentiation potential can be transplanted to treat the injury of the central nervous system and various neurodegenerative diseases, which can promote the recovery of neurological function of the patient. Through establishing the rat model with acute injury of the spinal nerves, this research conducts the allograft BMSCs transplantation for treatment and observes the transplantation treatment effect.

MATERIAL AND METHOD

Experiment material: Westar healthy rats (purchased from the Animal Breeding Center of the Medical College of Zhengzhou University), clean grade, male, weight (160[±]14) g, 8-10 weeks. Two groups of rat models shall be uniformly fed in different cages and taken care by the special person uniformly. The indoor ventilation is good and the room temperature is controlled at about 24°C. The rats shall be cleaned and disinfected twice in a day, to avoid wound infection. The rats shall be fed once every 8 h and the food amount shall mainly depend on the free food intake of the rat and aided with the fluid food injected from the oral cavity with the needle tube. There is no death in two groups of rats during the care period; the flow cytometry; CO₂ converse; DMEM culture medium; micro-adding sample appliance; fluorescence microscope; fetal calf serum; SP kit; nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) antibody and so on.

MSCs separation, training and mark: Take 1 Wistar healthy rat, male, weight 168.9 g, 9 weeks. Separate the bilateral femur of the rat under the sterile conditions, collect the bone marrow, separate the BMSCs with the adherence method, inoculate them to 25 cm² plastic bottle, add L-DMEM and the fetal calf serum with the volume fraction of 10%, cultivate them in the 37°C incubator with the CO₂ volume fraction of 5%. Change the solution after 24 h, remove the non-adherent cells, change the solutions 1 time every 3-5 days afterwards, use the 2.5 g L⁻¹ trypsinase for digestion and passage when 80% of the cells are mixed. Change the solution for digestion and so forth, until the 7th generation of cells. Take the seventh generation of cells, add Brdu in the culture solution for mark and the final concentration is 15 µg mL⁻¹. Digest the marked BMSCs with the trypsin (37°C and 5min), to free the cells, add them to the fresh culture medium including serum to suspend the trypsin function, gently blow the cells with the elbow straw, put the cell suspension into the centrifugal tube for centrifugation of 1500 r min⁻¹ and for 5min. Then pull the supernatant, add the non-complete medium of 0.5 mL, re-suspend the cells, take 30 µL to count the trypan blue cells and the cell population is about 5H104 µL.

Establishment of the spinal cord injury model: Take the experimental subject of 50 Wistar rats for backup, clean grade, male, mark the weight and week of each rat. Use the special vertebral lamina clamp to remove the T8 and T9 processes spinouts and vertebral lamina of the experimental animal, to expose the putamen. Use the

aneurysm clamp, to directly clamp the T9 segment spinal cord for about 0.5 sec (operation method: Use the improved aneurysm clamp to remove the T9 segment processes spinouts and vertebral lamina, with the calibrated force of 35g, use the clamp holder to open the aneurysm clamp and then suddenly release the aneurysm clamp, to hit the spinal cord in a sudden (Park *et al.*, 2001). The hitting time shall be about 0.5 sec and the position of the aneurysm clamp shall be accurate during the operation. It is pointed out in the related research that: the method can maintain the integrity of the spinal dura mater and the changes in the anatomical structure and the nerve function after the spinal cord injury is quite similar to the contusion-type spinal cord injury. Meanwhile, it is found when the force is changed from 2-98 g that if the clamping force is higher, the remaining axons in the injured area are less and the functional recovery is less ideal. Therefore, the nominal force in this research is 35 g). For rapid extrusion, causing the acute complete injury of the spinal cord and establish the acute injury model of spinal nerve. The spastic swing of the rat tail and paralysis of the lower limbs shall be taken as the injury standard. Successfully select the model and let in 40 experimental animals, which shall be divided into the control group (n = 20) and BMSCs transplantation group (n = 20) at random. There is no significant difference between the weights, weeks and lower limb paralysis degrees and other basic indicators of 2 groups of rats (p>0.05), with comparability.

BMSCs transplantation: BMSCs transplantation group: Perform the 2nd and 3rd surgical operations on the 7th day and 28th day of spinal cord injury. Expose the injured spinal cord, use the microinjector or to slowly inject the culture solution of 5 µL including BMSCs (about 5H104 µL⁻¹) into the spinal cord injury center, which shall be completed within 3 min. Retain the needle for 5 min and use the medical begum to seal the pinhole, to prevent the cell suspension from overflow and suture wounds layer by layer. The equal amount of physiological saline shall be injected to the control group in accordance with the method of the model group.

Assessment of the hind limb motor function: This research adopts the BBB motor function scoring system to assess the recovery of the hind limb motor function of the rat. The same experimenter shall conduct the single-blind method to the hind limb motor function of the rat for scoring continuously in the 1st, 2nd, 3rd, 4th, 6th and 8th weeks after the 2nd surgical operation respectively in accordance with the detailed rules of BBB scoring method. The BBB scoring standard is a new neurological function rating method formally proposed by

Table 1: BBB Scoring of Hind Limb Motor Function of Rats of 2 Groups (n = 20, $\bar{x}$ sec)

Group	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks
Control group	1.9±0.2	3.6±1.0	4.5±1.1	6.2±1.2	8.6±1.4	9.4±1.3
Transplantation group	2.1±0.3	4.8±1.2	8.6±1.0*	11.1±1.8*	15.5±1.7*	17.2±1.6*
t	0.312	1.946	3.105	3.976	4.163	4.325

the research personnel of U.S. Ohio University in 1995-BBB scoring method. It divides the hind limb movement of the rat into 22 grades. The full paralysis of the hind limb is 0 point and the completely normal is 22 points. See the literature for the specific contents and method.

NGF and BDNF inspection: After 8 weeks, kill the rats in the control group and the transplantation group, open the chest, pour the physiological saline of 200 mL 4°C through the heart to flush away the blood in the blood vessel and then pour 500 mL of 4,-4°C paraformal-dehyde phosphate buffer solution (pH7.2-7.4). Pour the solution and fix it for 30 min and put the T8 and T9 segment spinal cord of the injury area into the 4% paraformal-dehyde phosphate buffer solution for over 24 h. Cut 1cm at the position most close to the crossing at the night before slicing and put it into the 20, -sucrose solution 4°C to stay over. Embed it with the paraffin wax; slice with the cryostat continuously and the slice thickness is 25 μ m. The chemical staining of the immune tissue shall be conducted in strict accordance with the operation instructions on the kit and PBS shall substitute the primary antibody as the negative control. Five slices shall be taken for each rat at random, the image processing system consisting of the image analysis card (produced by Beijing Tiandi Company), IBM586 and Olympus Bx60 microscope shall be applied for observation and SPss13.0 software shall be used to calculate the amount of positive reaction cells. Calculate the average value of the NGF and BDNF positive cells of each group.

Statistical treatment: Apply PSS13.0 software for the statistical treatment. The measurement data shall be expressed in ($\bar{x}$ sec), the comparison among groups adopt the t inspection and $p < 0.05$ represents the discrepancy has the statistical significance.

RESULTS

BBB scoring results: The BBB score of each group before injury is 21 points. The rats of each group after injury are fully paralyzed and the score is 0 point. Pinprick the hind leg 5 days later and there is the retraction reaction but there is no difference significance between two groups. Conduct the comparison therapy of both groups after transplantation 1 week later (on the 7th day

Table 2: Counting of the positive motor nerves of chemical staining of the NGF and BDNF immune tissue of the control group and the transplantation group (piece, n = 20, $\bar{x}$ sec)

Group	NGF	BDNF
Control group	35.26±4.71	112.34±5.74
Transplantation group	97.14±7.52	265.82±6.15
t	3.575	3.015

Comparison between the transplantation group and the control group, $p < 0.05$

of damage), the hind limb activities appear after 2 weeks, the obvious hind limb activities appears after 3 weeks and the hind limb activities are coordinated after 6 weeks. The scoring difference between 2 groups after 3-8 weeks of the injury has the statistical significance ($p < 0.05$). Table 1.

NGF and BDNF protein inspection result. After BMSCs transplantation treatment, inspect the influence of BMSCs transplantation on the expression effect of NGF and BDNF through comparison of the expression of NGF and BDNF in the transplantation group and control group After 8 weeks, kill the rats in the control group and transplantation group and inspect the NGF and BDNF protein expression. It is found that the NGF and BDNF protein expression amount is obviously higher than that of the control group and the difference is of the statistical significance ($p < 0.05$) Table 2.

DISCUSSION

For a long time, it is considered in the medical circle that the nerve cell is a kind of permanent cell, which lacks the regeneration capacity. Therefore, it is considered in the traditional view that: If the central nervous system is injured, causing loss of a great amount of neurons, the injured function is difficult to recover due to that the new neurons cannot be generated and the new synaptic connection cannot be established. Therefore, the disease of the central nervous system has always been a worldwide difficulty in clinical treatment.

Along with the improvement of the medical treatment, people find the enormous potential of the stem cell in treatment of the central nervous system injury. The discovery of the stem cell changes the knowledge that the neuron of the nerves centralism of the adult mammals cannot be regenerated. For example, Park and so on put the genetically modified bone marrow MSC into the healthy female body and observe the protection function of the bone marrow MSC to the substantial Ingra cells by

establishing the Parkinson's disease animal model. It is found that the positive substantial Ingra neurons of immunological competence tyrosine hydroxyls in the treatment group is obviously higher than that of the control group, which indicates that MSC transplantation can improve the neurological symptoms of the Parkinson's patients and reach the treatment effect. Harvey and so on think that BMSCs can be induced and divided into the neurons and neuralgia cells and move to the diseased region, generating many kinds of neurotro

Through BMSCs transplantation to the rat with acute injury of the spinal cord in this research, they observe the influence on the treatment effect of the spinal nerve injury of the rat. It is found in the research that after the treatment, the rehabilitation efficacy of the hind limb motor function of the rat in the transplantation group is obviously higher than that in that control group and the NGF and BDNF protein expressions of the rats in the transplantation group is obviously higher than that in the control group. NGF is a kind of special protein which is proven that it can promote and maintain the nerve growth, survival and executive function and save the injured neurons. Through gene transfer NGF research, it is proven that after spinal cord injury of the adult rat, NGF can induce the axon elongation of the sensory neuron and nor epinephrine neuron, so that the local motion neurofibrillary in the nusus can sprout. The exogenous NGF is injected into the local of the transverse spinal cord injury, whi.

This experiment successfully establishes the in vitro culture system of the allograft BMSCs of rats. After BMSCs transplantation experiment, the rehabilitation efficacy of the hind limb motor function of the rats of 2 groups shall be inspected. The rehabilitation efficacy of

the transplantation group is remarkably better than that of the control group, which is due to that BMSCs transplantation can induce the NGF and BDNF expression. Because the leg motor function of the rat model with spinal nerve injury is related with the spinal nerve injury, the spinal nerve repair directly influences the motor function of the leg. However, NGF and BDNF are the important substances intervening in the neural repair mechanism. Their full expression can promote the survival and regeneration of the neuron of the rats with spinal nerve injury and regeneration and repair of the spinal nerve and has an important influence and restrictive function in good growth and repair of the nerves. Through comparison of the NGF and BDNF pr

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