Research on the Impact of Transplantation of Oligodendrocyte Precursor Cells on the Recovery of Spinal Cord Injury in a Rat Model

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Abstract. Objective: To investigate the impact of transplantation of oligodendrocyte precursor cells on the recovery of spinal cord injury in a rat model. Methods: The cerebral cortices of 48-hour neonate rats were harvested for the OPCs primary culture. The growth of OPCs in vitro was observed consecutively under the contrast phase microscope and scanning electron microscope. After the primary culture in vitro, the OPCs were further dissociated by the shaking process and differential adhesion. Seven days after spinal cord injury , the rats of transplantation group received OPCs transplantation, of control group were injected with equivalent saline , and of injury -only group were untreated. The effects of OPCs transplantation on neural functional recovery in spinal cord injured rats and matosensory evoked potentials (SEP) were assessed. Results: the simple morphology of OPCs progressively evolved to more complex forms with profuse outgrowth of elongated processes and extensive secondary branching. The performance of MEP and SEP the normal injured rats were worse than the sham group. Conclusion: The transplantation of OPCs may be helpful to the recovery of neural function of rats.

Object and Method

Main Instruments and Reagents. The main instument and reagents include bovine insulin, transferrin, thyroxine (Sigma Inc.), basic fibroblast growth factor (bFGF), platelet derived growth factor AA (PDGF-AA peprotech company), sodium pyruvate (Ameresco Inc.) and selenite sodium (Shanghai SANGON company). There are also stereo microscope (Germany Leica), desk type thermostatic oscillator (Taicang Jiangsu) and stereo positioning instrument (Japan Narishiger), multi-channel physiological recorder (Powerlab), Australia. Oligodendrocytes medium DMEM and containing 10ng/ml bFGF, 10ng/ml PDGF-AA,% FBS, transferrin 50 ug /ml, 5 ug/ml insulin, sodium selenite concentration was 30 nmol/L, concentration was 30 nmol/L thyroxine, 4mmol/LL glutamine, 5mmol / L sodium pyruvate, 50U / ml penicillin, 50 UG / ml streptomycin. Weigh 2mg hematoxylin dissolved in 100ml95% ethanol, placed in a stoppered bottle placed within one week. The Benjamin alum dissolved in 100ml distilled water until saturated (B liquid). A liquid pulled into the B liquid can be used in a few days to fully mix. 100ml glycerol was added to the 100ml solution of anhydrous acetic acid, mixed with a plug in the bottle and placed in the sun for 2 weeks, and then we take the plug to make anhydrous acetic acid volatile after March (C). We have A, B, C three mixing fluid with hematoxylin.

Cultivation of Oligodendrocyte Precursor Cells. According to Armstrong, this experiment cultivated the OPCs. 96 rats were taken from newborn SD, and the abdominal anesthesia was

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performed with 1% sodium. After anesthesia, the rats were placed on the ice cubes to keep the body temperature. The rats were treated with 70% alcohol disinfection. The following experimental procedures were performed under aseptic conditions. Under the stereo microscope, the cerebral hemispheres were removed. The structure of brain stem and hippocampus was removed, the blood vessel and tissue were completely removed, and the cerebral cortex tissue was transferred into Hanks balanced salt solution. By carefully cut microanatomy of cortical tissue to a size of about 1mm3, repeated pipetting into cell suspension. 74 m screen filter, collecting cells and centrifugal filtrate (1000rpm, 4 degrees C, 10min). With the basic culture re suspended cells and cell counting plate for the regulation of cell concentration, with 2.0 x 106 cells/bottle of cell seeding density on the package is poly lysine culture bottles, added to the basic medium 8-10ml and transferred into the incubator incubation of 5% carbon dioxide 37 degrees C cells. In the initial 3D do not vibrate the culture flask, 3-4d for the first time, after the liquid according to the situation of each 2-3D for liquid. On the basis of the primary culture of 9-10d, the integration of astrocytes and the stratification of the cells in the culture system were carried out. Oscillation in the former, replacement of fresh medium incubated for 24h, the culture bottle transfer to a constant temperature oscillator of the initial oscillation processing (180rpm, 37 degrees C 1 to 2 hours), dump the old medium. Hanks balanced salt solution cleaning culture bottle 3 times, adding fresh medium 8-10ml, into the incubator incubated for 2 h, again culture bottle is arranged in the constant temperature oscillator carried out overnight shaking treatment (200rpm, 37 degrees C, 18-20h). The next day, after the collection of oscillation cell suspension with 74 m screen filter, the filtrate was collected and centrifuged (4 degrees 1000rpm, C, 10min). 2-3ml was used to re suspend the cells in the culture medium, and the cells were inoculated into the glass culture dish which was not coated by poly lysine, and incubated with 1h. Then with medium by gently pipetting bottle of dish surface several times to loose paste attached cells shedding, collecting cell suspension and centrifuged at 1000rpm. 1-2ml OPCs were incubated for 30 min and be transported into a cell incubator continue to grow.

Methods. In this study, 96 SD rats were randomly divided into 4 groups, namely (A) OPCs transplantation group, (B) transplantation control group, (C) simple injury group and (D) sham operation group. Each group has 24 rats. OPCS transplantation in the treatment group received SCI surgery +OPCs transplantation; transplantation control group received SCI surgery + medium injected in the simple injury group SCI surgery; sham operation group only received laminectomy. According to the surface location of the spinouts process to determine the surgical incision, surgical field shearing skin preparation processing, 70% ethanol with thorough disinfection of the skin. Take back the median incision, cutting through the skin, subcutaneous tissue, and fascia layer by layer. In addition to the T9-T11 and the whole lamina of the spinal cord was removed, and the corresponding spinal segment was fully exposed. The injury caused by cm SCI in the spinal cord caused by injury to 25g. After successful modeling, the layer by layer close cut. SCI after the operation of the animal management: injury in rats in a cage rearing in the temperature of the environment can be transferred, cannot help but fresh water, observe the rat behavior and give the necessary nursing. Daily artificial micturition three times, until the rat bladder function recovery and be able to self-urination. Penicillin injections (1x105U/kg) were administered daily within one week of SCI to prevent infection.

Results

General Situation of Rats after Operation. 2 to 4h after spinal cord injury, the lower limbs was paralyzed, and the motor function was lost. About 40% rats postoperative hematuria. All rats

were given 3 times a day after the bladder extrusion to promote their urination, and finally to restore the function of autonomic urination. No hemorrhage, infection and necrosis were found in all transplanted rats after cell transplantation. In the whole experiment, 3 rats were killed.

Evaluation Results of Motor Function. All the BBB score were 21 points before and after cell transplantation at different time points. Each group after BBB score at each time point were significantly less than before the operation. The difference was statistically significant (P < 0.05); postoperative at each time point between the differences has statistical significance (P < 0.05). Group A and group B after 1D double lower limbs paralysis, BBB score is approximately 0, postoperative begin with 2D gradually restored, but recovered to varying degrees, 7 days after operation, the difference between the two groups no significant (P > 0.05); after operation, 14 and 28 scores in group A was significantly higher than that of group B, the difference is statistically significant (P < 0.05). After the operation, the motor function recovery of C group was faster, the postoperative 4D recovered to normal, and the time points were higher than B and A group, the difference was statistically significant (P<0.05). The effect of OPCs transplantation on motor function recovery of SCI rats was studied in the experiment. BBB motor function score was used to observe and analyze the recovery of motor function of the hind limbs of the rats before and after OPCs transplantation. The motor function of the hind limbs of rats was divided into 22 (0-21) grades, and the complete paralysis was 0 minutes and the function of the BBB score was 21 points. The basic observation of BBB score included: the number and range of joint activities, weight bearing and walking ability, physical stability, and the coordination of the front and back, and the tail of the fine activities. The study found that the motor function of the hind limbs of rats in the early stage after SCI was a serious obstacle, and the BBB score was 0. With the time prolonging, the motor function of SCI rats showed different degrees of recovery. The results showed that the recovery of motor function of the hind limbs of the OPCs transplantation group was significantly better than that of the control group and the simple injury group. Especially in SCI patients after 35, 42 and 49 days, OPCS transplant group rat hind limb motor function of BBB score and the result was better in transplantation control group of BBB scores (P < 0.05); in SCI postoperative day 56 and 63, OPCS transplantation treatment the hind limb motor function of the rats recovery was significantly superior to that of the transplant control group (P < 0.01). Throughout the study period in the sham operation group rats hind limb motor function basically normal, BBB score results basically to 21 points, and OPCS transplantation treatment of the hind limb motor function of the rats were always compared with sham operated rats hind limb motor function significantly decreased (P < 0.01). In addition, there were no significant differences in the motor function of the hind limbs (p>0.05) between the control group and the rats in the simple injury group.

Testing Results of MEP and SEP. During the experiment, the MEPN1 wave crest potential of sham operated group was 7.79 ~ 8.26ms, and the MEPN1 wave peak latency in the other three groups was significantly longer than that in sham operation group (P<0.01). MEPN1 wave crest latency of spinal cord injury in rats showed a certain degree of recovery after the operation, and the transplantation group was significantly better than the control group. At fourth weeks and eighth weeks after transplantation, the MEPN1 wave crest potential was significantly shorter in the transplanted group than in the control group. During the whole observation period, there was no significant difference between the control group and the MEPN1 wave peak latency of the simple injury group, the MEPN1 wave crest of the transplanted group was significantly longer than that of the sham operation group (P<0.01). During the experiment, the SEPN1 wave crest of the sham operation group was in 15.16 ~ 15.42ms, and the other three groups of spinal cord injury rats were significantly longer than those of sham operation group SEPN1. SEPN1 peak latency of spinal cord

injury in rats showed different degrees of recovery after the operation, and the recovery of the transplantation group was significantly better than that of the control group. At eighth weeks after transplantation, the SEPN1 wave crest latency was significantly shorter in the transplanted group than in the control group. During the whole experiment, no significant difference was observed between the control group and the SEPN1 wave peak latency of the simple injury group. The SEPN1 wave crest of the transplanted group was significantly longer than that of the sham operation group ($P<0.01$).

Discussion

The cell plays an important role in maintaining normal function of axon myelin. At present, it is believed that oligodendrocyte precursor cells' death is the result of an important reason to the demyelination of axons, and demyelination. Promoting axonal myelin and improving the conduction function of the axon can help the recovery of the nerve function of spinal cord injury. In recent years, studies have shown that nerve cells, olfactory unsheathing cells, embryonic and neural stem cells transplantation therapy can improve the spinal cord injury axonal myelination and promote neurological function recovery in this experiment in 8 weeks after the transplantation of OPCS observation was studied in spinal cord tissue pathological changes. The destruction and loss of myelin sheath after spinal cord injury hindered the electrical conduction of the central nervous system, which led to the loss of motor function, and the degree of damage was also an important factor to determine the degree of functional impairment. MBP is in the central nervous system of vertebrates of oligodendrocyte precursor cells in the synthesis of a hydrophilic. MBP plays an important role in maintaining the integrity and function of myelin sheath, and as a specific marker, it is widely used in the research of myelin sheath and related diseases. Studies have shown that content of MBP level can quantitatively assess myelination level, MBP expression increased myelination and regeneration, reduce that demyelination or bad. In this study, we found that the content of MBP in group A was significantly higher than that in group B, which was beneficial to the axon of spinal cord injury, which was beneficial to the recovery of nerve function.

Myelin sheath is a kind of special structure in the nervous system, which is the basis of the rapid transmission of nerve impulse. OPCs were transplanted into the treatment of SCI to improve axon myelin, playing the role of residual nerve fiber, and promoting the recovery of injured spinal cord nerve function. Implications for understanding the OPCS transplantation treatment on the recovery of spinal cord nerve conduction function of, the further by neural electrophysiological detection method evaluated the OPCS after treatment of injured spinal cord, sensory nerve conduction system function recovery. With the development of electrophysiology, evoked potentials in curative effect evaluation and function evaluation is the important role is gradually recognized, as an objective and sensitive detection method has more and more application in evaluation of the nerve function. Nerve electrophysiological examination is an objective and reliable evaluation method, especially in the experiment, the effect of animal's sense, muscle strength and reflex is not easy to measure. The commonly used neural electrophysiological examination indexes include MEPs and SSEPs, which have the advantages of objectivity, sensitivity and good reproducibility. MEPs are an electrical signal that stimulates the central nervous system. They were recorded in the distal end of the spinal cord, which can directly reflect the functional state of the spinal cord and the peripheral motor nerve.

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