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FIVE YEARS FOLLOW-UP OBSERVATION ON PATIENTS WITH SPINAL CORD INJURY TREATED WITH OLFACTORY ENSHEATHING CELL TRANSPLANTATION

Wang Dong[1], Li Haopeng[1], Xu Siyue[1], Yang Pinglin[1], Wang Guoyu[1], Wu Fei[1], Pei Leilei[2]

[1]No. 2 Orthopedics Department, Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an, Shaanxi Province 710004, China
[2]Statistical Learning and Research Department of Medical School of Xi'an Jiaotong University, Xi'an, Shaanxi Province 710004, China

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ABSTRACT

Olfactory ensheathing cell transplantation (OECT) has been applied to treat patients with spinal cord injury (SCI). This study was to investigate the long-term curative efficacy and safety of OECT by observing the long-term alterations of sensory level (SL), muscle strength, neural function and individual self-evaluations. Methods & Materials: The olfactory ensheathing cells used by this study were derived from allogeneic embryonic olfactory bulb induction. We observed 24 patients with SCI (male/female 20/4; average age 32.4 years, age range 19~45) who were treated with OECT in our hospital from September 2005 to March 2010. Neural function was evaluated based on the ASIA scores. Follow-up time ranged from 0.5 to 5.2 years, averagely was 3.2 years. Statistical analysis was done using ANOVA. Results: After OECT no apparent complications were found in the 24 patients. Among 11 patients with complete paralysis, 10 patients' SL moved downwards by 1-2 spinal segments, and 1 patient's SL no changed. Among 13 patients with incomplete paralysis, 11 patients' SL descended by two spinal segments, 2 patients' SL by three spinal segments and 1 of the 2 showed improvement of flexor function and emergence of thumb long extensor dorsiflexion movement (muscle strength 2 degrees). MRI showed no mass or cavity formation in the sites of OECT. 2 patients gave OECT excellent self-evaluation, 9 good, 12 poor, and 1 very poor. The rate of excellent and good self-evaluation accounted for 50%. The ASIA scores before and after OECT had not obviously difference ($P<0.05$). Conclusion: Though OECT is safe for SCI treatment, its long-term therapeutic effect is not ideal. This therapy still needs further observation and more exploration.

KEYWORDS

spinal cord injury; transplantation; olfactory ensheathing cells; efficacy evaluation.

1. INTRODUCTION

Biological Based on a study, olfactory ensheathing cell transplantation (OECT) is a new therapy for patients with severe spinal cord injury (SCI) [1-5]. Olfactory ensheathing cells have the characteristics of both astrocytes and Schwanns cells. They can improve axon regeneration and myelinization. Therefore they are considered as the optimal cells for transplantation [6-8]. Study showed since they were successfully applied to treat SCI in basic research, the clinical therapeutic effect of OECT has become the research focus [9-11]. This study was to estimate the long-term therapeutic effect of OECT by follow-up investigation.

2. MATERIALS AND METHODS

2.1 Patients

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Medical Collage of Xi'an Jiaotong University. This current study enrolled 24 Chinese patients with SCI (male/female 20/4, average age 32.4 years, age range 19~45) who received OECT in our hospital from Sept 2005 to Mar 2010. The 24 patients included 13 incomplete paralysis and 11 complete paralysis.

2.2 Inclusion Criteria for Oectand Surgical Preparation

Inclusion criteria for OECT contained 1) MRI demonstrated the patients had good spinal stability and no obvious spinal cord compression, 2) the patients had not any change in the level of SCI after rehabilitation and they received other treatments more than six months, we think that the injury was chronical SCI, it was still; 3) the patients were 18~60 years, male or female, and had not obvious surgical contraindication. For those patients who didn't meet the first requirement, surgical preparations before OECT were necessary. The surgeries included spinal stabilization surgery and adhesiolysis of spinal cord surface for relieving spinal cord compression. Postoperative managements contained nerve trophic treatment (GM-1 60mg/QD, 15days), preventive treatment of phlebothrombosis (low-molecular-weight-heparin 20mg BID, 15days), treatment of SCI complications, and rehabilitation care (exercise for power, standing exercise, walk training, etc). All the 24 patients received OECT after they signed the Informed Consent and the Ethics Committee of the Second Affiliated Hospital of Medical Collage of Xi'an Jiaotong University approved the surgery.

2.3 Oec Preparation

The olfactory ensheathing cells used by this study were acquired from olfactory bulbs of human fetal body (12-16 weeks of pregnancy) under the permission of the Ethics Committee of our hospital. Besides the abortion patients signed the Informed Consent. The aborted fetus was

transported to a lab and placed on a super-clean bench after a quick dip in 75% alcohol (1 minute). T-shaped open of the anterior cranial fossa and bottom-up exposing of the frontal lobe were followed by the removal of the olfactory bulbs. The removed olfactory bulbs were immediately washed in D-Hanks solution (4℃). The cerebral pia mater, the capillary network and the central grey matter were removed with microinstruments under stereomicroscope. The remained white matter of the olfactory bulbs were minced finely, dipped in 0.25% pancreatin solution, and then put into incubator for 15 minutes. Addition of 20% fetal calf serum DMEM/F12 for stopping digestion (10 minutes), centrifugation for 3 minutes (1000 turns/min) and removal of the supernatant were repeated twice for adjusting the cell concentration to 1X106/ml. Based on research, the cells were inoculated in an uncovered culture flask which was then sent into CO2 incubator (37℃, 5% CO2) after purified three times with modified NASH method [12-14]. 7 days latter the OECs were separated with parenzyme, centrifuged and concentrated to 1×109/L. The concentration was identified by P75 cytochemical staining.

2.1 Cell Transplantation

Before transplantation we conducted cell digestion (30 minutes) and then wax-seal of cell suspension. The wax sealed cell suspension was brought into operation room. Conventional skin incision was done to expose laminar or remained laminar after the prior laminar decompression. Subsequently, opening the canal along the both sides of dura from distal to proximal, observing SCI region and releasing the adhesion on the surface of SCI we herein often saw the thickened dura, thinned spinal cord with gray color and hardened texture, disappearance of central blood vessel. The prepared cell suspension was injected into spinal cord at four locations, both posterior horns of spinal cord 0.5 cm distal and proximal to SCI area, under the operating loupe (ø0.5mm pinheaded 10ul scalp acupuncture, needle depth 3mm, injection speed 1ul/min, injection duration per location 10 minutes, the duration of needle stay in spinal cord after injecting 2 minutes) [15]. A total of 40ul cell suspension (about 1×10 4 cells) was injected into spinal cord. The whole process of cell transplantation lasted about one hour. No intra-operative cerebrospinal fluid leak occurred. The dura was sutured with 2/0 suture line.

Postoperative management Routine nursing care was given to the patients after cell transplantation, such as massage with air-cushion couch, venous pump extrusion for lower extremity, etc. Other postoperative treatments included nerve trophic treatment (GM-1 60mg/QD 2 weeks), preventive treatment of phlebothrombosis (low-molecular-weight-heparin 20mg BID, 2 weeks), treatment of dehydration of spinal cord (mannitol 125ml BID and hexadecadrol 10mg QD for 3 days), 3 days of routine antibiotic treatment, and preventive treatment of SCI complications (like bedsore, etc). The immunosuppressive agent was not used for our patients preoperatively and postoperatively. The rehabilitation, like active or passive limb exercising, same as the rehabilitation treatment before OECT, was started gradually from the second day after operation.

2.2 Evaluation of Therapeutic Effects

We evaluated the therapeutic effects by a long-term follow-up investigation. The follow-up was started one month before OECT, conducted once every three months and terminated in March 2010. The mean follow-up period was 3.2 years (rang 0.5～5.2 years). The follow-up approaches included outpatient appointment, home follow-up visit and telephone contact with patient. The follow-up investigations contained 1) the changes of sensory level (SL) and muscular strength;

Table 1: ASIA score of motion, pain and touch in complete and incomplete paraplegia

Paraplegia	*Cases*	*status*	*ASIA scores* *(M±SD)*		
			Motion	Pain	Touch
Complete	11	Preoperative	41.73±9.90	41.27±27.07	40.18±29.15
	11	Postoperative	41.73±9.90 *	48.18±24.89 ▪	47.09±27.03 ◆
	11	Final	41.73±9.90	53.64±26.08	47.09±27.03
Incomplete	13	Preoperative	45.31±11.65	39.85±23.79	39.00±23.14
	13	Postoperative	47.92±12.35 *	46.92±23.39 ▪	45.31±23.25 ◆
	13	Final	48.46±13.45	47.54±22.83	45.77±27.80
		F value	378.582	10.888	84.414

* refers to P<0.05 Incomplete paraplegia compared with Complete paraplegia ; ■ P<0.05 Postoperative compared with Preoperative ; ◆ P<0.05 Postoperative compared with Preoperative. American Spinal Injury Association (ASIA) standards; 3) the neurological function with ASIA scores; and 4) the individual self-evaluation. Among these follow-up investigations the main index was the pre- and post-operation ASIA scores that were used to analyze the influences upon the functional recovery after OECT from age, time of injury, gender, degree of injury and extent of injury.

In individual self-evaluation of patient, "excellent" was defined as the patient was very satisfied to the recovery of some key sensory level or muscular strength, the decrease or disappearance of seizure attacks and the improvement of physical coordination; 2) "good" satisfied to the down-moving sensory level and recovered muscular strength but not satisfied to unrecovered function, no significantly altered seizure attacks and no improved physical coordination; 3) "poor" not satisfied to no obvious alteration in sensory level, muscular strength, seizure attacks and physical coordination; 4) "very poor" not satisfied greatly to the severe complications or postoperative symptoms, the ascended sensory level, the decreased muscular strength and physical coordination, and the increased seizure attacks.

3. STATISTICAL ANALYSIS

All data were processed with the statistical software package SPSS 13.0. Data of ASIA scores are expressed as mean ± standard deviation (x ±s) and analyzed with ANOVA. P<0.05 was considered as statistically significant.

4. RESULTS

The OECT operations of all the 24 patients were smooth (operation time 1～3 hours, average operation time 1.5 hours, mean intra-operative blood loss 400ml). The mean postoperative hospital stay was 12.6 days (range 10～21 days). All the 24 OECT patients were followed-up. Among the 11 paraplegia patients, the postoperative sensory level of 9 patients with complete paralysis descended by 1-2 spinal segments compared to the SCI level, but the motor no changed; 1 patient was significantly improved in seizure attack; 1 patient did not have any change in sensory level and motor. Among the 13 cases of quadriplegia, the postoperative sensory level of 10 patients descended by 2 spinal segments; that of 2 patients descended by 3 spinal segments; and finger flexor function was improved in 1 of the 2 patients, presenting with the emergence of dorsiflexion movement in long extensor muscle of thumb with level-2 muscular strength. Besides, 1 case of quadriplegia appeared frequent limb spasticity but was not detected having organic abnormality on MRI and no significant improvement after positive treatment.

Operation complications were not found in all the 24 patients, and partial mass or cavity formation at transplantation site were not detected by postoperative MRI. Individual self-evaluations of the patients were: "excellent" 2 cases, "good" 9 cases, "poor" 12 cases, and "very poor" 1 case (excellent and good rate accounting for 50%).

Table 1 lists the ASIA scores before and after OECT. ANOVA results

revealed that there were differences in incomplete paraplegia between the preoperative and the postoperative 3-month ASIA scores (P<0.05). No obvious difference consisted in motion scores of complete and incomplete paraplegia patients between the pre-operation, the postoperative 3-month and the last time ASIA scores (P > 0.05). There was a significant difference between pre-operation and postoperative 3-month in pain and touch scores of all cases. But the postoperative 3-month motion, pain and touch scores are not different from those of the last time follow-up (P > 0.05).

5. DISCUSSION

Clinical studies have reported the application of OECT in treating SCI. Though the reported therapeutic effects are different in these studies, it is unanimous that the procedure and outcome of OECT are safe and no apparent complications are found [16-18]. Our follow-up data are also supportive to this. Meanwhile, our data reveal that part of OECT patients' clinical symptoms are to some extent improved; some patients' OECT is ineffective; one patient presents with worse symptoms after OECT. We think the following factors are related to the treatment ineffectiveness and symptom worsening. 1) based on a study, there are less nerve fibers and more scar tissue in SCI area, which makes the number and length of regenerated axons decreased [19-22]. 2) Nerve fibers and neurons might be injured in the process of injecting [23]. 3) Embryo age, the death time of embryo, vigor and amount of OECs may also exert some negative impacts on OECT [24, 25]. 4) Compression could originate from bleeding at the OECT site during and after injection [26]. 5) Immunoreaction could occur in CNS [27, 28]. Because we did not perform the matching analysis of OECs in this study, whether OECT will cause immunoreaction in CNS needs a further study.

We use ASIA scores in combination with subjective satisfaction survey to analyze the improvement of neurologic symptoms. We found if ASIA scores are higher the patients' subjective satisfaction degree is obviously improved, and vice versa. But a part of patients are not in line with this. Though their ASIA scores are relatively low, their satisfaction degree is higher. We consider this situation could be resulted from two reasons. The one is that ASIA score can not reflect the changes in the frequency and intensity of spasm. Thus despite a low ASIA scores, some patients feel better due to the decreased spasm attacks. The other is that although the patients with cervical SCI caused by low cervical vertebra trauma have not a higher ASIA scores, the recovery of muscular strength in part key muscles which leads to a large improvement to daily life can help them have a higher subjective satisfaction degree. So we suggest that the assessment to the SCI recovery should be a combination of ASIA score, the frequency and intensity of spasm and subjective satisfaction survey. We think it's necessary to set a control group using sham-operation in the assessment of OECT, and adhesiolysis of spinal cord surface can be considered as the sham-operation [29-31]. Besides, we think that whether SCI is new or old should also be taken into consideration when making the inclusion criteria for OECT. Because the status of the patients with old SCI is relatively stable, these patients' functional improvement resulting from OECT can more realistically reflect the therapeutic effects of OECT. We considered further research have to be done because the results were interfered by several clinical factors, such as the type and duration of injury, the patient's psychology, and so on, these factors did not included in this study.

6. CONCLUSION

The transplantation of olfactory ensheathing cells derived from allogeneic embryonic olfactory bulb induction is a safe therapy for spinal cord injury. But it can not bring about a significant functional restoration except that only a small part of SCI patients treated with OECT may improve clinical symptoms and have a long-term functional restoration.

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