

Fetal Allogeneic Umbilical Cord Cell Transplantation Improves Motor Function in Spinal Cord-Injured Rats

Allojenik Fetal Göbek Kordonu Hücrelerinin Transplantasyonu Omurilik Hasarlı Sıçanlarda Motor Fonksiyonu İyileştirir

ABSTRACT

AIM: The objective of this study was to investigate whether the transplantation of fetal umbilical cord tissue cells as a source of stem cells into the acutely injured spinal cord would produce some regenerations and/or functional recovery in a rat model of spinal cord injury.

MATERIAL and METHODS: Material and Methods: Five pregnant albino Wistar rats of 12 days gestation were used for obtaining an umbilical cord cell graft. At the second stage of the experiment only Th8-Th9 laminectomy was performed in Group A animals while Group B animals underwent spinal cord hemitranssection. The cultured fetal umbilical cord cells coated with Alginate Gel were placed into the lesion cavity immediately after surgery in Group C animals. Group D animals received only Alginate gel sponges into the injured area. All experiment groups were analyzed histologically and immunohistochemically (GFAP, Ki-67, and Pan cadherin) and for motor function after surgery.

RESULTS: Results: The umbilical cord cell transplanted animals showed a significant motor recovery compared to non-transplanted animals at 8 and 21 days after spinal cord injury ($p=0.008$). Significant GFAP and Ki-67 expressions were noted in transplanted animals ($p=0.048$) suggesting astroglial proliferation.

CONCLUSION: Our findings support the possibility of some functional recovery after umbilical cord cell transplantation following spinal cord injury.

KEYWORDS: Allogeneic transplantation, Alginate gel, Function recovery, Mesenchymal stem cells, Spinal cord injury, Umbilical cord cells

ÖZ

AMAÇ: Çalışmanın amacı, sıçan omurilik hasarı modelinde kök hücre kaynağı olarak fetal göbek kordonu dokusu hücrelerinin akut hasarlı omuriliğe transplantasyonunun rejenerasyon ve/veya fonksiyonel iyileşmeye neden olup olmadığını araştırmaktır.

YÖNTEM ve GEREÇ: Yöntem ve Gereç: Beş adet hamile albino Wistar cinsi sıçan gebeliğin 12. gününde göbek kordonu hücrelerini elde etmek için kullanılmıştır. İkinci aşamada, Grup A hayvanlarına T8-9 laminektomi uygulanmıştır. Grup B hayvanlarında sadece omurilik yarı kesimi gerçekleştirilmiştir. Grup C hayvanlarında cerrahinin hemen sonrasında kültüre edilmiş fetal göbek kordonu hücreleri Alginate Jel transplante hücre taşıyıcısına bağlanarak lezyon boşluğuna yerleştirilmiştir. Grup D hayvanlarında hasarlı bölgeye Alginate Jel süngeri yerleştirilmiştir. Tüm deneysel gruplar histolojik ve immunohistokimyasal (GFAP, Ki-67, Pan Cadherin) ve motor davranış olarak cerrahi sonrası değerlendirilmiştir.

BULGULAR: Göbek kordon hücresi transplante edilen hayvanlar transplante edilmeyenlerle karşılaştırıldığında omurilik hasarı sonrası 8. ve 21. günlerde anlamlı motor iyileşme göstermişlerdir ($p=0,008$). Astroglial çoğalmayı destekleyen anlamlı GFAP ve Ki-67 pozitifliği tranplante edilmiş hayvanlarda tespit edilmiştir ($p=0,048$).

SONUÇ: Bulgularımız omurilik hasarı sonrasında göbek kordonu hücreleri transplantasyonunun fonksiyonel iyileşmeye neden olabileceğini desteklemektedir.

ANAHTAR SÖZCÜKLER: Allojenik transplantasyon, Alginate jel, Fonksiyonel iyileşme, Mezenşimal kök hücre, Omurilik hasarı, Göbek kordonu hücresi

Bulent ERDOGAN¹
Murad BAVBEK²
Iffet Feride SAHİN³
Hakan CANER⁴
Ozlem OZEN⁵
Emir Baki DENKBAS⁶
Mehmet Nur ALTINORS⁷

¹ Fatih University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey

² Ministry of Health, Diskapi Educational and Research Hospital, Department of Neurosurgery, Ankara, Turkey

³ Baskent University, Faculty of Medicine, Medical Genetics, Ankara, Turkey

^{4,7} Baskent University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey

⁵ Baskent University, Faculty of Medicine, Department of Pathology, Ankara, Turkey

⁶ Faculty of Chemistry, Hacettepe University, Chemistry; Nanotechnology and Nanomedicine Division, Ankara, Turkey

Received : 25.02.2010

Accepted : 11.03.2010

Correspondence address:

Bulent ERDOGAN

Department of Neurosurgery, Fatih University, Faculty of Medicine, Alparslan Türkeş Cad. No 57.

06510, Emek, Ankara, TURKEY

Phone : +90 312 203 51 32

Fax : +90 312 203 50 28

E-mail : E-mail: uberdogan@yahoo.com

INTRODUCTION

The failure of the spinal cord to regenerate after injury is a major clinical problem (10). However, there is no effective therapeutic option to improve functional outcome. Functional deficits after SCI result from damage to axons, loss of neuron and glia, and demyelination (7). A number of cell types such as olfactory ensheathing cells, genetically modified fibroblasts, Schwann cells, and stem cells have been used to promote axonal regeneration after SCI (14, 26, 31, 33).

Several experimental studies have indicated that several stem or progenitor cell types including embryonic stem cells (ESCs) neural stem cells, bone marrow stromal cells (BMSCs) and glial-restricted precursor cells have the ability to induce neurogenesis and motor recovery following transplantation into the injured spinal cord (3, 9,18, 20).

In particular, ESCs are derived from the blastocyst in the developing embryo prior to implantation and are valuable sources for cell transplantation (31). However, the ethical and technical difficulties associated with ESCs have promoted a search for alternatives (8). Today, adult bone marrow derived mesenchymal stem cells (MSCs) have mostly been utilized as a source of non-ESCs to treat a variety of disorders including cardiovascular, hepatic, ophthalmic, orthopaedic, neurological and endocrine diseases (8).

Stem cells derived from the umbilical cord blood (UCB), umbilical cord tissue, amniotic fluid and placenta, have been used in different clinical and experimental studies (6, 23). Another potential source of mesenchymal cells is Wharton's jelly (WJ) of the human umbilical cord (28). WJ is the connective tissue surrounding the two arteries and one vein of the umbilical cord including fibroblast-like cells. It has been suggested that these mesenchymal cells are multipotent with the ability to proliferate and differentiate (28).

Autologous patches and heart valves engineered from human umbilical cord cells represent a promising concept in pediatric cardiovascular surgery for patients diagnosed with congenital heart lesions (2). Stem cell therapy is currently being tested in spinal cord injured patients in some clinical trials (16).

In this experimental study, we investigated whether the transplantation of allogeneic umbilical cord tissue cells into the injured spinal cord would produce regeneration and/or functional benefits in a rat model of spinal cord injury.

MATERIAL and METHODS

All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee of the Baskent University in accordance with the US National Institute of Health guidelines for the care and use of laboratory animals (principles of laboratory animal care, NIH, publication No. 80-23, revised 1996).

Tissue culture

Five pregnant albino Wistar rats at 12 days of gestation were used for obtaining the umbilical cord cell graft in the first stage of the study. The pregnant rats were anesthetized with intraperitoneal xylazine at a dose of 10 mg/kg (Rompun, Bayer, İstanbul, Turkey) and ketamine sulphate at 50 mg/kg (Ketalar, Parke Davis, Eczacıbasi, İstanbul, Turkey). The rats were placed in the supine position. Fetuses were extracted from the uterus through a suprapubic incision. Only the umbilical cord was dissected and separated carefully with the use of operating microscope.

Tissue samples were taken under sterile conditions during the operation and put into transport medium (Dulbecco's modified Eagle's medium (DMEM) containing penicillin and streptomycin). As soon as the samples arrived at the laboratory, they were minced mechanically and tissue cultures were set up in DMEM containing %10 fetal calf serum, 200µM L-glutamine and penicillin/streptomycin in a 37° C CO2 incubator. Cells were observed and the culture medium was refreshed every 2-3 days until the cultures were harvested on the 10th day. Cultured cells are shown in Figure 1. Cultured cells were isolated by trypsin and they were washed with culture medium three times before incubation with alginate.

Preparation of alginate sponges

Since the majority of transplanted cells are easily lost after implantation into a spinal cord lesion, we used Alginate gel as a carrier of grafted cells in this study. Alginate is a well-established natural bioabsorbable acidic polysaccharide extracted from

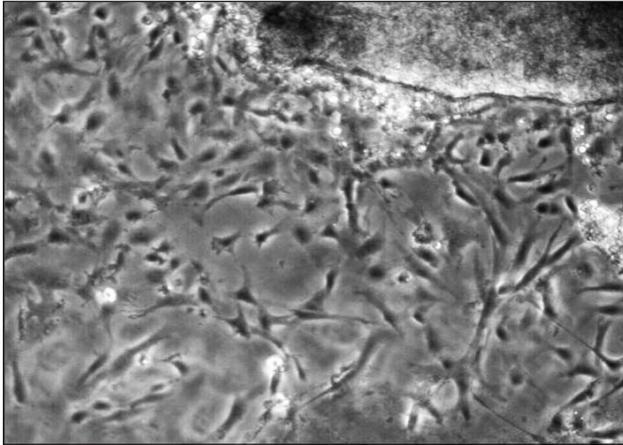


Figure 1: Dividing umbilical cord cells originating from tissues colonized on the flask surface before trypsinization (X200).

brown seaweed (12). It is suggested that alginate gel has a favorable biocompatibility for regenerating axon extension in the injured spinal cord with the characteristics of low cytotoxicity, small foreign-body reaction and low scar tissue formation (12). Sodium alginate or alginic acid sodium salt (Fluka, Switzerland) was used to prepare the alginate sponges. Alginate sponges were prepared by precipitation of the polymer in non-solvent (i.e. CaCl_2 solutions) in the form of a spongy type cylindrical rod. In a typical procedure, a certain amount of sodium alginate (i.e. 250 mg) was dissolved in aqueous solution (i.e. 5 % v/v) in an ultrasound bath for 6 hours. All the obtained gelatinous sodium alginate was filled into an injector (10 ml injector without needle). Sodium alginate solution was injected into the beaker filled with CaCl_2 solution. The concentration of CaCl_2 solution was varied in the range of 1 to 20% to change the cross-linking densities of the alginate sponges. Precipitated alginate rods were cured in the same medium for 30 minutes. Afterwards alginate rods were taken out and lyophilized overnight, then stored in a desiccator at 4°C for further analysis and applications.

Binding cells with alginate

Alginate rods were sterilized with ethylene oxide and sterile 3D alginate scaffolds that were minced into 1-2 mm sized fragments suitable for the created lesions were each put in the bottom of a 1.5 ml sterile Eppendorf tube. 1 ml cell suspension containing 6×10^6 cells was added to the tube and the tube was centrifuged at 100g for 15 minutes. Supernatant was

discarded and 200 μl fresh culture medium was added to the tube. Alginate and cell mixtures were incubated in a 37°C CO_2 incubator overnight and the next morning, the bound complexes were transplanted to the rats.

Experiments

Eighty adult male Wistar rats, aged 4-6 months, each weighing 268-342g. were used in these experiments. The animals were anesthetized with 10 mg/kg intraperitoneal xylazine (Rompun, Bayer, Turkey) and 50 mg/kg ketamine (Ketalar, Pfizer, Turkey). Twenty rats formed the laminectomy only (sham) group (Group A). Th8-Th9 laminectomy was performed by the posterior midline approach. Care was taken not to damage the spinal cord in Group A. In the remaining 60 rats, the thoracic spinal cord (T8-T9) was hemitranssected on the left side after laminectomy. The spinal cord was hemitranssected with microscissors under an operating microscope. The microscissors were inserted into the spinal cord with the tip touching the midline until the left side of the cord was completely divided. During the surgery, the rats were kept on a heating pad to maintain the body temperature at $37.0 \pm 0.5^\circ\text{C}$. The hemitranssected animals were divided into three equal groups. Group B (n=20) animals underwent only spinal cord injury, and received no treatment (injured animals). In Group C animals (n=20), the cultured fetal umbilical cord cells coated with Alginate Gel as a carrier for transplanted cells as previously described (12) were grafted into the cavity of injured spinal cord immediately after the operation (injured+transplanted animals). In Group D animals (n=20), only alginate sponges without umbilical cord cells were placed into the injury cavity. The wound was closed in layers. The 20 rats in each group were further subdivided into four subgroups of 5 rats each for assessment of motor behavior and histological and immunohistochemical analysis of the spinal cord at 1, 3, 8, 21 days after surgery. At the end of each experimental period, the rats were sacrificed with an overdose of pentobarbital and then perfused transcordially with 50 ml saline followed by 200 ml 4% paraformaldehyde in 0.1 M phosphate buffer.

Animal care

Rats were housed with free access to tap water and rat chow with a dark/light cycle of 12/12 h.

They were examined neurologically and their bladder were emptied by the Cr de manoeuvre three times daily until reflex bladder emptying was established, and antibiotic therapy (Baytril-K, Bayer, Istanbul; Enrofloxacin 2.5 mg/kg, s.c) was given for 3 days. All rats in each group received Cyclosporine (10 mg/kg, subcutaneously) daily to prevent rejection beginning on the day of any surgery and/or transplantation. No significant immune responses were detected in response to the transplanted material.

Histological and Immunohistochemical Analysis

The spinal cord segment from each animal corresponding to the injured area was embedded in paraffin and processed for histological and immunohistochemical studies. These sections were centered at the middle of the core including the lesion cavity and boundary zone. Sections were stained with hematoxylin & eosin, cresyl violet and toluidin blue for general morphology.

Formaldehyde-fixed and paraffin-embedded spinal cord tissues were sectioned. After deparaffinization and rehydration, each section was immunostained using monoclonal antibodies for Ki-67 (MIB-1 clone, rabbit polyclonal, Neomarkers), Pan Cadherin (C-terminal, rabbit polyclonal, Neomarkers) and GFAP Glial Fibrillary Acidic Protein (rabbit polyclonal, Neomarkers). Astrocytes were identified by their morphology and GFAP-positivity; ependymal cells and neurons were identified by their morphology. Cell proliferation was assessed immunohistochemically using Ki-67. Cadherin expression was detected by immunohistochemistry using the polyclonal antibody Pan Cadherin. Immunohistochemistry procedures were carried out using the standard avidin-biotin complex method according to the following procedures:

For Ki-67 and Pan Cadherin, antigen retrieval was performed in a microwave oven in 10mM citrate buffer (pH 6.0) at 700 W for 15 min. Endogenous peroxidase activity was then blocked with 0.3% H₂O₂ for 30 min. After incubation with 5% bovine serum albumin in Tris-buffered solution (TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.4) for blocking of nonspecific binding for 10 min., sections were incubated with primary antibodies for Ki-67 and GFAP for 1 hour in a humidified chamber at room

temperature. Sections were then incubated with peroxidase-labeled polymer for 30 minutes followed by DAB+ substrate-chromogen solution. Next, they were counterstained with hematoxylin and mounted. Between steps, the slides were washed twice in TBS. For negative controls, the primary antibodies were omitted and non-immune serum was used instead. For positive controls, we stained sections of tissues that were considered suitable according to the manufacturer's protocol.

For MIB-1 evaluation, immunoreactivity was defined as intense, diffuse or granular nuclear staining. The level of MIB-1 immunoreactivity in each section was assessed by counting the stained nuclei in approximately 1000 cells in the regions of maximal staining. The MIB-1 LI was calculated as a percentage based on the number of positive cells per total cells counted.

Pan Cadherin expression was semiquantitatively evaluated. The staining degree was graded as follows: 3, stronger positive than control; 2, positive as strong as control; 1, positive but weaker than control; 0, no staining.

Both the injury and peri-lesional zones were investigated for Ki-67 positivity, GFAP and Pan Cadherin expression. These recordings were conducted by two investigators with no knowledge of the experimental group from which each sample had been obtained. The mean recorded by these investigators were regarded as final values.

Behavioral analysis

Functional tests were performed before the injury and transplantation. When neurological function was assessed, lower limb monoplegia was evident in all spinal cord hemitransected animals. Although many scales have been developed since Tarlov (BBB, AOB, and so on), we have evaluated each rat's motor functions with the modified Tarlov's motor grading scale at 1, 3, 8 and 21 days after spinal cord injury until they are sacrificed (1,25). This scale measures hindlimb motor functions ranging from 1 to 5, as follows: Grade 1= shows no voluntary hindlimb movement; Grade 2= minimal voluntary hindlimb movement, but unable to stand; Grade 3= able to stand, but unable to walk, Grade 4 = able to walk with incoordination of the hindlimb; Grade 5= able to walk normally (25).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences for Windows (version 15.0; SPSS, Inc., Chicago, Illinois, USA). Data were expressed as mean \pm standard deviation (SD). Kruskal-Wallis test was used to compare the differences between groups. For the motor paralysis data, differences in motor scores were compared using the Kruskal-Wallis nonparametric analysis of variance (ANOVA) and Mann-Whitney U-tests. The Wilcoxon-signed rank test was used to compare the changes among the individual groups. We used the unpaired Student's t-test for a comparison of the number of immunopositive cells in the experimental groups. Differences were considered to be statistically significant when the probability value was less than 0.05.

RESULTS

Assessment of motor behavior

The measurement of modified Tarlov's motor scores for each animal was performed beginning with the first day after SCI. These observations continued at each period of the experiment (at 1, 3, 8 and 21 days after surgery). Control animals (Group A) were neurologically intact and were given Tarlov scores of 5 at each period of the experiment (data not shown). During the first day after SCI, the difference between the mean scores of SCI group (Group B) (1.2 ± 0.45) and umbilical cord cells transplanted group (Group C) (1.2 ± 0.45) was not significant ($p > 0.05$). On day 3 after spinal cord injury, there was also no significant difference between the mean scores of the SCI group (1.2 ± 0.45) and transplanted group (1.4 ± 0.55) ($p > 0.05$). However, on day 8 post-injury, the motor scores in the transplanted group were significantly higher (3.6 ± 0.55) than those in the SCI group (1.2 ± 0.45) ($p=0.008$). On day 21 after SCI, motor scores were higher (4.2 ± 0.45) than those in the injured group and (1.2 ± 0.45). These are significantly different ($p=0.008$). (Figure 2) In addition, a statistically significant difference was also not found between Group B and Group D after surgery at all intervals ($p > 0.05$). (Data is not shown in Figure 2)

Immunohistochemistry and histopathology:

For Ki-67 and GFAP; there was no significant difference between groups in immunostaining on days 1, 3 and 8 after spinal cord injury. Although at

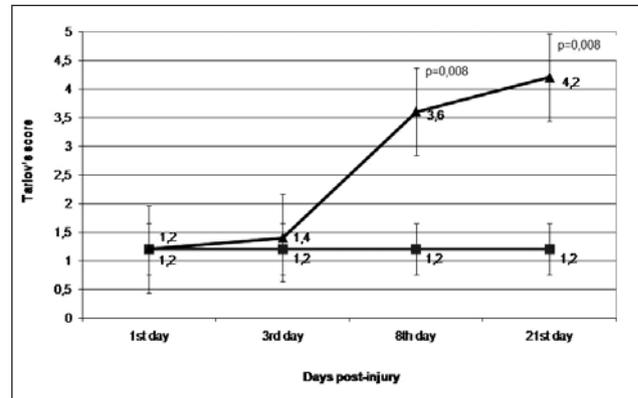


Figure 2: Recovery from motor paralysis assessed by modified Tarlov's scoring. During the first day after SCI, the difference between the mean scores of only spinal cord injury group (Group B) (1.2 ± 0.45) and umbilical cord cells transplanted group (Group C) (1.2 ± 0.45) was not significant ($p > 0.05$). On day 3 after spinal cord injury, there was also no significant difference between the mean scores of Group B (1.2 ± 0.45) and Group C (1.4 ± 0.55) ($p > 0.05$) animals. However, on day 8 post-injury, the motor scores in the Group C were significantly higher (3.6 ± 0.55) than those in the Group B (1.2 ± 0.45) ($p=0.008$). On day 21 after spinal cord injury, motor scores were 4.2 ± 0.45 in the Group B and 1.2 ± 0.45 in the Group B ($p=0.008$). (Numeric values represent the means and the error bars are \pm standard deviation: triangles (\blacktriangle), umbilical cord cells transplanted group; squares (\blacksquare), spinal cord injured ad non-transplanted group.)

the 21st day there was no expression of Ki-67 and GFAP in spinal cord injured animals, expression was evident in 80% of umbilical cord cells transplanted animals (4 of 5 animals). The difference was statistically significant ($p=0.048$ for both Ki-67 and GFAP) (Figure 3A, B; Figure 4A, B). Astrocytic proliferation presumably leading to a glial scar was demonstrated by GFAP expression. Activated and proliferated glial cells showed significant Ki-67 expression especially in these areas. However, when the number of cells that showed Ki-67 positivity in ependymal cells and neurons was analyzed, there was no significant difference between non-transplanted and transplanted animals. When we compared Pan cadherin expressions in the experiment groups, we could not find any significant differences. Besides that, none of the tested variables were found to be statistically significant in sections for histopathological analysis between the groups.

DISCUSSION

While the non-transplanted spinal cord injured rats showed no improved motor recovery in this study, the rats subjected to intralesional

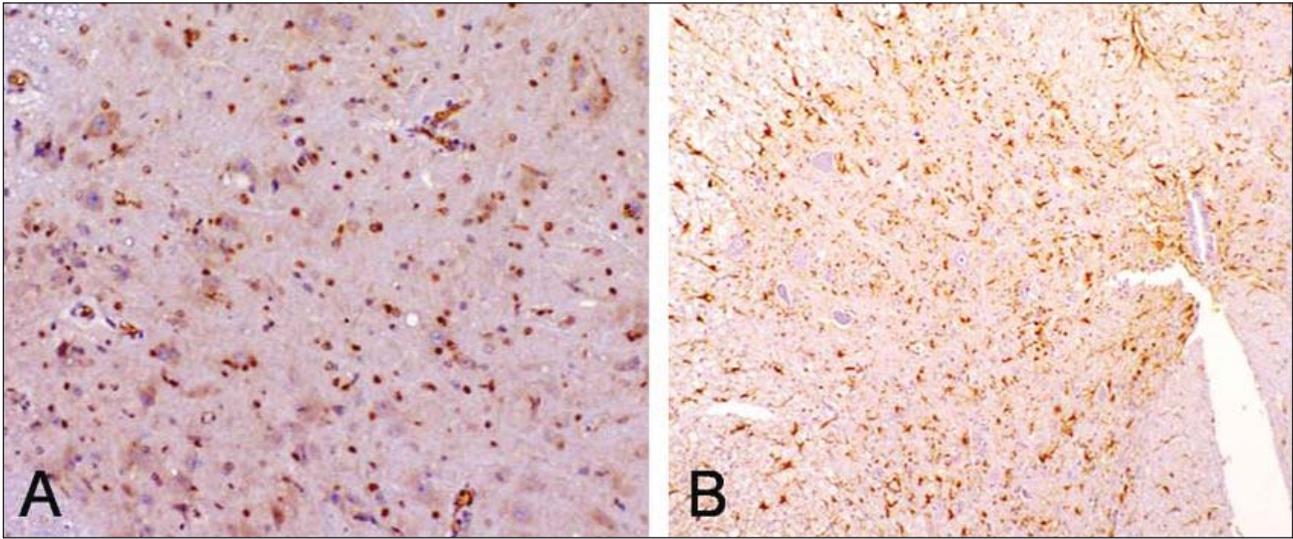


Figure 3: (A) Spinal cord sections with Ki-67 proliferation index 45% and (B) GFAP expression at days 21 in umbilical cord cells transplanted animals. (Immunoperoxidase x 20, x10).

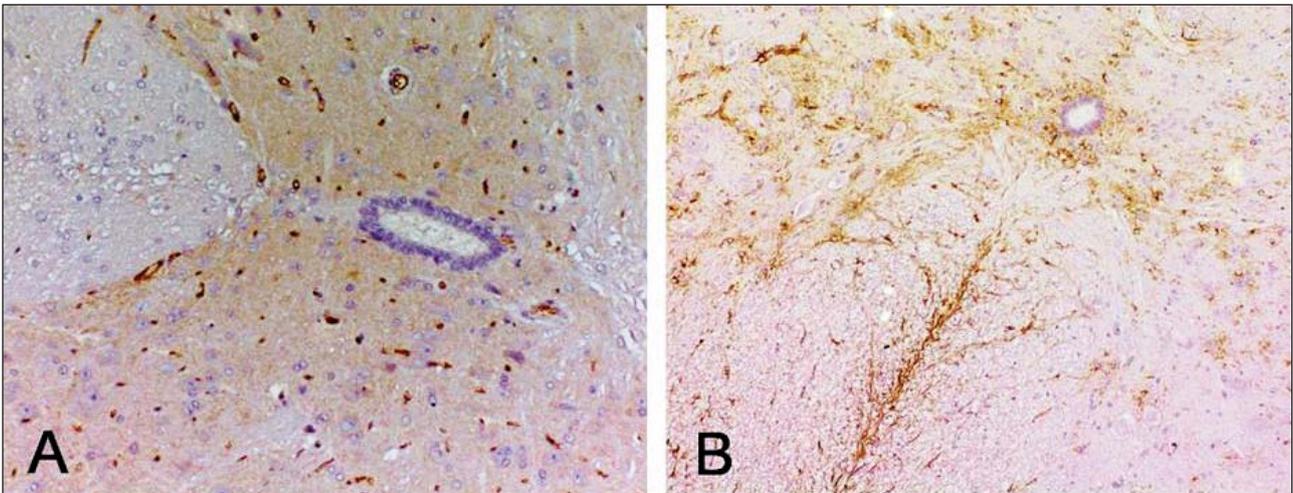


Figure 4: Spinal cord sections without (A) Ki-67 proliferation and (B) and GFAP expression at days 21 in spinal cord injured animals. (Immunoperoxidase x 20, x10).

transplantation of umbilical cord cells immediately after injury showed a progressive recovery of motor functions, starting at 8th day after the surgical procedure. As demonstrated in some studies related to stem cells therapy, hindlimb functional deficits were maximal at day 3 and then showed partial recovery over the next several days with a plateau seen 8 days after SCI (3, 9, 31). Cho et al. demonstrated that stem cells transplanted animals also showed somatosensory-evoked potentials recovery with an improvement in locomotor rating scales (4).

Following transplantation on day 21 post-injury, immunohistochemical staining of GFAP and Ki67

demonstrated an increased activity in glial cells. Astrocytic proliferation presumably leading to a glial scar was demonstrated by GFAP expression. Especially, in these areas, activated and proliferated glial cells showed a significant expression of Ki-67. In our study, although the Ki-67 expression was not detected in neurons and ependymal cells, motor scores in the umbilical cord cells transplanted animals were significantly higher than those in the non-transplanted animals.

Following spinal cord injury, astrocytes become reactive, usually called reactive gliosis or glial scarring and express high levels of the intermediate filament proteins such as glial fibrillary acidic

protein (GFAP), vimentin, and nestin (22). Pekny et al. have shown that GFAP and vimentin are required for proper glial scar formation in the injured central nervous system (22). In addition to physical or molecular barriers presented by glial scarring at the lesion site, it has been suggested that the growth inhibitors such as Nogo, myelin-associated glycoprotein and chondroitin sulfate proteoglycans or lack of neurotrophic factors are the main factors contributing to the failure of axonal regeneration (1, 13, 19, 30). Thus, the evolution of the scar following SCI is a complex phenomenon where different mechanisms may be contributed.

In the adult mammalian brain, it is considered that neurogenesis persists in limited regions such as the hippocampal dentate gyrus and the subventricular zone of the lateral ventricle. These multipotential progenitor cells also exist in the surrounding central canal of the spinal cord. In the spinal cord, the main function of these cells to migrate to the site of injury and produce new neurons and glial cells (9,11). It has been suggested that, after spinal cord injury, nestin-expressing periventricular cells start to migrate into the injury site and start to express GFAP (11). In our study, significant expression of GFAP in umbilical cord cells transplanted animals with high Tarlov's motor scores may be explained by this mechanism.

The timing of the transplantation is important for successful functional recovery of the damaged CNS (9,31). We transplanted the stem cells immediately after SCI. Others believe that 7 to 14 days after injury is the optimal time to transplant neural stem cells into the spinal cord (9,21). Because the immediately post-traumatic microenvironment of the spinal cord is in an acute inflammatory stage, it is not favorable for the survival and differentiation of stem cell transplants. On the other hand, in the chronic stage after injury, glial scars form in the injured site that inhibits the regeneration of neuronal axons (9,21). If we performed the transplantation between 7 to 14 days after injury it might have been possible to produce more favorable results. Regardless, further studies are needed to clarify how, when or in which cases a stem cell transplant should be used.

Cadherins are calcium-dependent cell adhesion molecules, which play an important role in the growth and development of cells via mechanisms of control of tissue architecture and the maintenance of

tissue integrity. It has been suggested that Cadherin activity is essential for axon-dendritic spine contact, synaptic plasticity and rearrangement (1). Atalay et al. showed an increased cadherin expression in spinal cord injured rats that had improved motor function. Unfortunately, in the presented study, cadherin expression was not significantly different between umbilical cord cells transplanted and non-transplanted animal. This discordance may be related with the extent of spinal cord injury and timing of transplantation. Furthermore, the effect of stem cell transplants on the tissue architecture may be seen many weeks after the transplantation.

There is no consensus yet on what kind of stem/progenitor cells is an ideal source for cellular grafts. In the presented study, we used the umbilical cord cells as a source of mesenchymal stem cells and immediately grafted into the injured spinal cord. Different parts of the umbilical cord, which include cord blood, subendothelial layer of cord vein, cord vein endothelial lining and the cord Wharton's jelly contain stem cells with the ability of proliferating and differentiating into various tissues (6,23). Umbilical cord blood stem cells have demonstrated efficacy in reducing lesion sizes and enhancing behavioral recovery in animal models of ischemic and traumatic central nervous system injury (15). Weiss et al. treated Parkinson's disease with human umbilical cord mesenchymal cells (hUCMSCs) transplantation in a rodent model (29). In their study, the transplanted tissues showed significant regenerative signs with behavioral improvement. Wang et al. transplanted hUCMSCs into spinal cord hemisection in a murine model and noted that transplanted hUCMSCs survived and migrated to the injured site (27).

The mechanism of the effect of stem cells on injured spinal cord is unknown at this time. It has suggested that are a reliable, easily accessible, noncontroversial source of mesenchymal stem cells. It has been believed that hUCMSCs is a rich source of autologous stem and progenitor cells. Additionally, these stem cells have a potential to be differentiated into neuron-like cells in vitro and in vivo. However, both the nature of the cells responsible within the umbilical cord stroma and the mechanisms underlying the beneficial effect observed were unclear (15). The lack of evidence of substantial lesion site engraftment, survival, and neural

differentiation suggests that the beneficial effects may be largely derived from a "bystander" function of these cells, either through secretion of neurotrophic/survival factors or through suppression of inflammatory death (17).

Before the study begins, we hypothesized that neurogenesis may be attributed not only to the transplanted stem cells, but also to an increased cadherin expression in transplanted rats with high motor scores, but this expectation was not fulfilled. Although previous reports showed that stem cells had a higher ratio to differentiate into neuron (3, 8,15), in this study no verification that umbilical cord cells produced substitute therapy effect through differentiating various neural cells was observed. The main limitation of the present study is that neuronal differentiation and incorporation into the neural circuitry could not be demonstrated in umbilical cord cell transplanted rats with high motor scores. In the presented study, we only found improved motor function and increased GFAP and Ki67 expressions in umbilical cord cells transplanted animals. The elevation of GFAP in transplanted rats may be related to the endogenous neurogenesis originating from multi potential progenitor cells in the surrounding central canal of the spinal cord as previously reported (9,11). Hence, further studies are needed to better understand the effects of stem cells on injured spinal cord using cellular immunocytochemical and immunohistochemical methods combined with confocal and electron microscopy (32).

For SCI, stem cell therapy is currently being tested in some clinical trials (5,16). In 2008, Mc-Kay and co-workers reported the outcomes of a Phase I/IIa clinical study in which six patients had spinal cord injury (16). They concluded that autologous transplantation of olfactory ensheathing cells into the injured spinal cord is feasible and safe for up to three years after implantation without any adverse effects. This study suggests that the window of opportunity for treating spinal cord injury with stem cells may be wider than previously thought.

Both umbilical cord and umbilical cord blood can be collected and stored at birth with no harm to mother or baby and cryopreserved in a cell bank. These stem cells have a strong potential to form various tissues and many advantages because of the immaturity of newborn cells compared with adult

cells (24). From ethical and technical view, umbilical cord cells can be used as an alternative source mesenchymal stem cells for experimental and clinical applications.

CONCLUSION

In summary, the present study suggests that the possibility of using cell therapy with umbilical cord cells for the treatment of spinal cord injury. It is obvious that diverse mechanisms of recovery may play a role after transplantation, including expression of neurotrophic factors, or activation of endogenous mechanisms able to restore neurological functions previously suppressed. A combination of stem cells transplantation with other repair strategies that might have synergistic effects may be needed. Finally, some important points regarding this issue such as timing of transplantation, survival of the stem cells, stability of differentiation, method of administration, risk of tumor formation, etc. should be extensively investigated before stem cells transplantation will become a safe therapy in spinal cord injured patients.

REFERENCES

1. Atalay B, Bavbek M, Cekinmez M, Ozen O, Nacar A, Karabay G, Gulsen S: Antibodies neutralizing Nogo-A increase pan-cadherin expression and motor recovery following spinal cord injury in rats. *Spinal Cord* 45: 780-786, 2007
2. Breymann C, Schmidt D, Hoerstrup SP: Umbilical cord cells as a source of cardiovascular tissue engineering. *Stem Cell Rev* 2: 87-92, 2006
3. Cao Q, Xu XM, Devries WH, Enzmann GU, Ping P, Tsoulfas P, Wood PM, Bunge MB, Whittemore SR: Functional recovery in traumatic spinal cord injury after transplantation of multilineurotrophin-expressing glial-restricted precursor cells. *J Neurosci* 25: 6947-6957, 2005
4. Cho SR, Yang MS, Yim SH, Park JH, Lee JE, Eom YW, Jang IK, Kim HE, Park JS, Kim HO, Lee BH, Park CI, Kim YJ: Neurally induced umbilical cord blood cells modestly repair injured spinal cords. *Neuroreport* 19: 1259-1263, 2008
5. Deda H, Inci MC, Kürekçi AE, Kayihan K, Özgün E, Ustünsoy GE, Kocabay S: Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up. *Cytotherapy* 10: 565-574, 2008
6. Gucciardo L, Lories R, Ochsenbein-Kölble N, Done E, Zwijsen A, Deprest J: Fetal mesenchymal stem cells: Isolation, properties and potential use in perinatology and regenerative medicine. 116: 166-172, 2009
7. Guest JD, Hiester ED, Bunge RP: Demyelination and Schwann cell responses adjacent to injury epicenter cavities following chronic human spinal cord injury. *Exp Neurol* 192: 384-393, 2005

8. Harris DT, Rogers I: Umbilical cord blood: A unique source of pluripotent stem cells for regenerative medicine. *Curr Stem Cell Res Ther* 2: 301-309, 2007
9. Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L: Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci USA* 99: 2199-2204, 2002
10. Hulsebosch CE: Recent advances in pathophysiology and treatment of spinal cord injury. *Adv Physiol Educ* 26: 238-255, 2002
11. Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisén J: Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* 96: 25-34, 1999
12. Kataoka K, Suzuki Y, Kitada M, Ohnishi K, Suzuki K, Tanihara M, Ide C, Endo K, Nishimura Y: Alginate, a bioresorbable material derived from brown seaweed, enhances elongation of amputated axons of spinal cord in infant rats. *J Biomed Mater Res* 54: 373-384, 2001
13. Lemons ML, Howland DR, Anderson DK: Chondroitin sulfate proteoglycan immunoreactivity increases following spinal cord injury and transplantation. *Exp Neurol* 160: 51-65, 1999
14. Li Y, Field PM, Raisman G: Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* 277: 2000-2002, 1997
15. Low CB, LiouYC, Tang BL: Neural differentiation and potential use of stem cells from the human umbilical cord for central nervous system transplantation therapy *J Neurosci Res* 86: 1670-1976, 2008
16. Mackay-Sim A, Féron F, Cochrane J, Bassingthwaighe L, Bayliss C, Davies W, Fronck P, Gray C, Kerr G, Licina P, Nowitzke A, Perry C, Silburn PA, Urquhart S, Geraghty T: Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. *Brain* 131: 2376-2386, 2008
17. Martino G, Pluchino S: The therapeutic potential of neural stem cells. *Nat Rev Neurosci* 7: 395-406, 2006
18. McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, Gottlieb DI, Choi DW: Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* 5: 1410-1412, 1999
19. McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE: Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* 13: 805-811, 1994
20. Ogawa Y, Sawamoto K, Miyata T, Miyao S, Watanabe M, Nakamura M, Bregman BS, Koike M, Uchiyama Y, Toyama Y, Okano H: Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. *J Neurosci Res* 69: 925-933, 2002
21. Okano H, Ogawa Y, Nakamura M, Kaneko S, Iwanami A, Toyama Y: Transplantation of neural stem cells into the spinal cord after injury. *Semin Cell Develop Biol* 14: 191-198, 2003
22. Pekny M, Johansson CB, Eliasson C, Stakeberg J, Wallén A, Perlmann T, Lendahl U, Betsholtz C, Berthold CH, Frisén J: Abnormal reaction to central nervous system injury in mice lacking glial fibrillary acidic protein and vimentin. *J Cell Biol* 145: 503-514, 1999
23. Romanov YA, Svintsitskaya VA, Smirnov VN: Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate MSC-like cells from umbilical cord. *Stem Cells* 21: 105-110, 2003
24. Sessarego N, Parodi A, Podestà M, Benvenuto F, Moggi M, Raviolo V, Lituanica M, Kunkl A, Ferlazzo G, Bricarelli FD, Uccelli A, Frassoni F: Multipotent mesenchymal stromal cells from amniotic fluid: Solid perspectives for clinical application. *Hematologica* 93: 339-346, 2008
25. Soy O, Aslan O, Uzun H, Barut S, İğdem AA, Belce A, Colak A: Time-level relationship for nitric oxide and the protective effects of aminoguanidine in experimental spinal cord injury. *Acta Neurochir (Wien)* 146: 1329-1335, 2004
26. Tuszynski MH, Peterson DA, Ray J, Baird A, Nakahara Y, Gage FH: Fibroblasts genetically modified to produce nerve growth factor induce robust neuritic ingrowth after grafting to the spinal cord. *Exp Neurol* 126: 1-14, 1994
27. Wang GS, Zhang QZ, Han ZC: Evaluation of neurological function recovery following human umbilical cord mesenchymal stem cells transplantation to injured spinal cord in rats. *Chin J Neurosurg (Chin)* 22: 18-21, 2006
28. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC: Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 22: 1330-1337, 2004
29. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G, Troyer D: Human umbilical cord matrix stem cells: Preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* 24: 781-792, 2006
30. Widenfalk J, Lundströmer K, Jubran M, Brene S, Olson L: Neurotrophic factors and receptors in the immature and adult spinal cord after mechanical injury or kainic acid. *J Neurosci* 21: 3457-3475, 2001
31. Willerth SM, Sakiyama-Elbert SE: Cell therapy for spinal cord regeneration. *Adv Drug Deliv Rev* 60: 263-276, 2008
32. Vitellaro-Zuccarello L, Mazzetti S, Madaschi L, Bosisio P, Fontana E, Gorio A, De Biasi S: Chronic erythropoietin-mediated effects on the expression of astrocyte markers in a rat model of contusive spinal cord injury. *Neurosci* 24: (151): 452-466, 2008
33. Xu XM, Guenard V, Kleitman N, Aebischer P, Bunge MB: A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Exp Neurol* 134: 261-272, 1995