

Experimental Research

Functional recovery after human umbilical cord blood cells transplantation with brain-derived neurotrophic factor into the spinal cord injured rat

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Summary

There have been many efforts to recover neuronal function from spinal cord injuries, but there are some limitations in the treatment of spinal cord injuries.

The neural stem cell has been noted for its pluripotency to differentiate into various neural cell types. The human umbilical cord blood cells (HUCBs) are more pluripotent and genetically flexible than bone marrow neural stem cells. The HUCBs could be more frequently used for spinal cord injury treatment in the future.

Moderate degree spinal cord injured rats were classified into 3 subgroups, group A: media was injected into the cord injury site, group B: HUCBs were transplanted into the cord injury site, and group C: HUCBs with BDNF (Brain-derived neurotrophic factor) were transplanted into the cord injury site. We checked the BBB scores to evaluate the functional recovery in each group at 8 weeks after transplantation. We then, finally checked the neural cell differentiation with double immunofluorescence staining, and we also analyzed the axonal regeneration with retrograde labelling of brain stem neurons by using fluorogold. The HUCBs transplanted group improved, more than the control group at every week after transplantation, and also, the BDNF enabled an improvement of the BBB locomotion scores since the 1 week after its application ($P < 0.05$). 8 weeks after transplantation, the HUCBs with BDNF transplanted group had more greatly improved BBB scores, than the other groups ($P < 0.001$). The transplanted HUCBs were differentiated into various neural cells, which were confirmed by double immunofluorescence staining of BrdU and GFAP & MAP-2 staining. The HUCBs and BDNF each have individual positive effects on axonal regeneration. The HUCBs can differentiate into neural cells and induce motor function improvement in the cord injured rat models. Especially, the BDNF has effectiveness for neurological function improvement due to axonal regeneration in the early cord injury stage. Thus the HUCBs and BDNF have recovery effects of a moderate degree for cord injured rats.

Keywords: Human umbilical cord blood cells (HUCBs); cell transplantation; spinal cord injury; BDNF.

Introduction

There have been efforts to maximize the therapeutic effects for spinal cord injury (SCI). Many therapeutic strategies for traumatic SCI are currently being studied in order to regain motor functions of the paralyzed limbs through the inhibition of secondary biochemical and pathological changes at the site of the SCI. There are many research attempts for the pharmacological blocking of the excitotoxicity, minimizing of the inflammatory reaction, inhibition of the apoptosis, neural cell and neural tissue transplantation, and testing neurotrophic factors in these management strategies [2, 4, 5, 21, 35].

Neural cell transplantation studies for SCI have been using embryo neural tissue transplantation and various other kinds of stem cell transplantation. It has been reported that the transplanted embryo neural cells synapsed with the recipient neural tissue, and also secreted neural transmitters and so it repaired the damaged neural tissue [9, 27, 30]. Transplanted stem cells would ideally replace injured neurons and glial cells by generating new cells [12]. Many types of stem cells, including embryonic stem cells, neural stem cells (NSCs), bone marrow stem cells and olfactory ensheathing cells have been studied as candidates for cell replacement therapy [12, 16, 24, 36].

It has also been reported that the Neurotrophin-3 (NT-3) and BDNF have effects on neural survival and regeneration in cord injury models [5, 8, 19].

So, the objective of this present study was to evaluate neural cell differentiation of human umbilical cord blood cells (HUCBs) in injured rat spinal cord, the neurological function improvement after HUCBs transplantation, and also the effectiveness of BDNF on cell differentiation, axon regeneration, and the ultimate neurological function improvement.

Materials and methods

Separation and culture of HUCBs

After our protocol had been determined and institutional review board approval obtained, HUCBs were obtained, using sterile syringes, from the umbilical veins immediate after full-term deliveries. All the samples were collected after obtaining written informed consent. The blood sample volume was 100 to 150 ml.

Aspirated blood was diluted 1:1 with Hank's balanced salt solution (HBSS) and centrifuged through a density gradient (Ficoll-Paque Plus; 1.077 g/l; Pharmacia, New York, NY) at $1000 \times g$ for 30 min. The mononuclear cell layer was then recovered from the gradient interface, washed with HBSS, centrifuged at $900 \times g$ for 15 min, and then re-suspended in complete culture medium [Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Carlsbad, CA) supplemented by 20% fetal bovine serum (Gibco BRL, Carlsbad, CA), 100 units/ml penicillin, and 100 μ g/ml streptomycin], with the cells at a concentration of 1×10^6 /ml.

The cells were next incubated at 37°C for 7 days. To identify and label cells derived from the cord blood, bromodeoxyuridine (BrdU, 3 μ g/ml; Sigma, St. Louis, MO), a thymidine analog and marker of newly synthesized DNA, was added to the medium for 3 days. Thereafter, the cells were subcultured in chambered slides and the BrdU incorporation was verified by BrdU immunocytochemical staining.

Spinal cord injury

15 male Sprague-Dawley rats (Daehan Hiolink, Chungbuk, Korea) weighting 300~350 g at the time of surgery were used. All our animal experiments were approved by the institutional Animal Care and Use Committee of Yonsei University College of Medicine.

Acute SCI was induced using the NYU weight-drop device. Adult male Sprague-Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and a laminectomy was performed at the T8 to T10 level. An opening was then made large enough to accommodate the impactor head (2.5 mm diameter). Leaving the dura mater intact, the dorsal surface of the cord was then subjected to a weight-drop impact. The 10 g weight impact rod was dropped from a height of 25 mm to produce a moderately contused SCI model. During recovery, rectal temperature was maintained at 36~37°C by a heating pad. Postoperative nursing care included bladder expression twice a day. Prophylactic kanamycin (1 mg/kg) was regularly administered for a week.

Transplantation of HUCBs with BDNF

Basso-Beattie-Bresnahan (BBB) locomotor rating scores were obtained before transplantation and every week after SCI. Rats were assigned, without bias, to the control group ($n=5$), HUCBs transplanted group ($n=5$), and HUCBs transplanted with BDNF group ($n=5$). Control group animals were injected 7 days after injury with 20 μ l of PBS into 1.2 mm in depth and just 0.5 mm proximal and 0.5 mm distal to the cord contusion site using a Hamilton syringe. The HUCBs transplanted groups were injected 7 days after injury with 20 μ l, mononuclear cell layer of HUCBs and the group with HUCBs transplanted with

BDNF group were injected 7 days after injury with 10 μ l mononuclear cell layer of HUCBs and 10 μ l of BDNF (8 μ l, R&D Systems, Minneapolis, MN, USA) with fibrin glue (Greenplast, Greencross PBM, Korea) into the same injection site of the control group. Recombinant human BDNF was prepared with Phosphate buffered saline (PBS; GIBCO BRL) including 0.1% bovine serum albumin (BSA; Sigma) at the concentration of 8 μ l/5 μ l. BDNF 8 μ l/5 μ l was then injected into spinal cord contusion site with fibrinogen 2.5 μ l, and then thrombin 2.5 μ l was immediately injected at the same location. Cells were labelled by incubating them in the presence of 2×10^{-6} M Bromodeoxyuridine (BrdU; Sigma, Saint Louis, Missouri, USA) for a week before the transplantation. Cyclosporin A (1 mg/100 gm) was injected daily from 2 days before transplantation to 8 weeks after transplantation.

Study design

The study was designed to find out the functional improvement, neural cell differentiation, and axon regeneration in spinal cord injured rats after HUCBs transplantation with BDNF.

According to a post injury 1st week behavioral test, rats were assigned, without bias to the media-injection group ($n=5$), the HUCBs transplanted group ($n=5$), and HUCBs with BDNF (8 μ l/5 μ l) transplanted group ($n=5$).

Behavioral Assessment after SCI

A behavioral test was performed to measure functional recovery of the hindlimb. The open field testing procedures we used have been described by Basso *et al.* (Table 1). This scale measures hindlimb movements with a score of 0 indicating no spontaneous movement, with an increasing score being given for the use of individual joints, coordinated joint movement, coordinated limb movement, weight-bearing and so on to a maximum score of 21.

Behavioral testing was performed weekly upon each hindlimb from the first postoperative day to 8 weeks after SCI for all animals using the BBB scoring system, by two independent examiners who were kept in ignorance of the rat's treatment status. Experimental rats were disposed of under urethane anesthesia 8 weeks after transplantation [1, 29].

Immunohistochemistry

The rats from each of the 3 groups were sacrificed 8 weeks after SCI by a transcardial perfusion using PBS containing 4% paraformaldehyde. Spinal cords were immediately removed and the injured region dissected. Segments 20 mm rostral and caudal to the injury site were then embedded in paraffin.

For the immunological studies, deparaffinized spinal cord sections were boiled in citrate buffer (pH 6) for 10 minutes in a microwave oven. Following blocking in normal serum, the sections were incubated with monoclonal antibodies (mAb) against human nuclear proteins (mAb 1281, dilution 1:50 in PBS, Chemicon, Temecula, CA) or bromodeoxyuridine (anti-BrdU, dilution 1:100 in PBS, Chemicon, Temecula, CA). The sections were then incubated with the biotinylated secondary antibody (dilution 1:100, Zymed, CA) for 10 min at 37°C. After an extensive washing in 20 mM PBS, the slides were incubated for 10 minutes in streptavidin conjugated to horseradish peroxidase in Tris-HCl buffer. A substrate chromogen solution, prepared by using diaminobenzidine chromogen tablets, was then applied to the slides for 5 minutes.

To identify cells co-expressing BrdU antibody with the neuronal marker (GFAP, MAP-2), we made use of the fluorescence immunohistochemical staining techniques in all 3 groups ($n=5$, control; $n=5$, HUCBs; $n=5$, HUCBs with BDNF). After being blocked in 10% fetal bovine serum in PBS, the sections were placed in a medium containing anti-BrdU antibody (sheep poly-Ab, dilution 1:100; Biotodesign, Saco,

Table 1. *Basso, Beattie, and Bresnahan locomotor rating scale*

0	No observable hindlimb (HL) movement
1	Slight movement of one or two joints, usually the hip and/or knee
2	Extensive movement of one joint or extensive movement of one joint and slight movement of one other joint
3	Extensive movement of two joints
4	Slight movement of all three joints of the HL
5	Slight movement of two joints and extensive movement of the third
6	Extensive movement of two joints and slight movement of the third
7	Extensive movement of all three joints of the HL
8	Sweeping with no weight support or plantar placement of the paw with no weight support
9	Plantar placement of the paw with weight support in stance only (i.e. when stationary) or occasional, frequent, or consistent weight-supported dorsal stepping and no plantar stepping
10	Occasional weight-supported plantar steps; no FL–HL coordination
11	Frequent to consistent weight-supported plantar steps and no FL–HL coordination
12	Frequent to consistent weight-supported plantar steps and occasional FL–HL coordination
13	Frequent to consistent weight-supported plantar steps and frequent FL–HL coordination
14	Consistent weight-supported plantar steps, consistent FL–HL coordination, and predominant paw position during locomotion is rotated (internally or externally) when it makes initial contact with the surface as well as just before it is lifted off at the end of stance; or frequent plantar stepping, consistent FL–HL coordination, and occasional dorsal stepping
15	Consistent plantar stepping and consistent FL–HL coordination and no toe clearance or occasional toe clearance during forward limb advancement; predominant paw position is parallel to the body at initial contact
16	Consistent plantar stepping and consistent FL–HL coordination during gait and toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off
17	Consistent plantar stepping and consistent FL–HL coordination during gait and toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact and lift off
18	Consistent plantar stepping and consistent FL–HL coordination during gait and toe clearance occurs consistently during forward limb advancement; predominant paw position is parallel at initial contact and rotated at lift off
19	Consistent plantar stepping and consistent FL–HL coordination during gait, toe clearance occurs consistently during forward limb advancement, predominant paw position is parallel at initial contact and lift off, and tail is down part or all of the time
20	Consistent plantar stepping and consistent coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift off, and trunk instability; tail consistently up
21	Consistent plantar stepping and coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, and consistent trunk stability; tail consistently up

Maine), Nestin (mAb, concentration 1:100; Sigma, St. Louis, MO), GFAP (mAb, concentration 1:100; Sigma, St. Louis, MO), and MAP-2 (mAb, concentration 1:100; Sigma, St. Louis, MO) antibodies overnight at 4°C. On the following day, the sections were rinsed and incubated in a solution containing a secondary antibody to sheep antibodies conjugated with Texas red (anti-sheep poly-Ab, 1:200; Vector Laboratories, Burlingame, CA) and a secondary antibody to mouse antibodies conjugated with fluorescein isothiocyanate (FITC) (anti-mouse mAb, 1:200; Vector Laboratories, Burlingame, CA). The sections were then washed, mounted and examined under a laser scanning confocal microscope equipped with a Bio-Rad MRC 1024 (argon and krypton) laser scanning confocal imaging system mounted on a NIKON microscope (Bio-Rad). For immunofluorescence, double-labelled green (FITC for HUCBs) and red (Texas red for Nestin, GFAP, and MAP-2) fluorochromes on the sections were excited using a laser beam at 488 and 647 nm; the emissions were sequentially acquired using 2 separate photomultiplier tubes through 522- and 680-nm emission filters, respectively.

Retrograde labelling of descending axons

A retrograde tract tracing study was performed to determine the extent of the supraspinal neurons with axons projecting from the spinal cord across the injured site. Eight weeks after the transplantation, the rats were anesthetized as described above. The spine (L1) was exposed and micro-injections of the retrograde tracer, fluorogold (FG; Fluorochrome, Denver, CO, USA), were made into the spinal cord caudal to the injury

site using a glass needle attached to a Hamilton syringe in all groups. Two injections ($2 \times 0.5 \mu\text{l}$, FG) were bilaterally made at 0.5 mm lateral to the postero-median vein and 1.2 mm in depth. One week after the injection of the tracer, the rats were perfused and the fixed brains were removed and then sectioned. Every second section was mounted onto gelatin coated glass slides for the quantification of the number of FG-labelled cells. The retrograde FG label was visualized under UV excitation with a $10\times$ objective on a fluorescence microscope.

The count of FG labelled axon was checked by two independent examiners who were kept in ignorance of the slide status.

The sections were pictured and modified using Meta Morph computer software (Meta Morph version 46r5, Downingtown, PA, USA). For the quantification of the slides, the labelled cells in the raphe nucleus and red nucleus were manually counted for each group.

Statistical analysis

Comparisons of BBB scores between each group were made using an ANOVA test. Significance was accepted for P values of <0.05 .

Results

BBB locomotion scores

The BBB scores of each group had an average of 6.2 points at post SCI week one. There were no differences

Table 2. BBB locomotion scores of each group

	Group A			Group B			Group C		
	Left	Right	Average	Left	Right	Average	Left	Right	Average
Injection	6	6.4	6.2	6.2	6.2	6.2	6.2	6.2	6.2
1 week	6.8	7	6.9	7.4	7.4	7.4	8	8.2	8.1
2 weeks	7.6	7.6	7.6	8	8	8	9.2	9.2	9.2
3 weeks	8	8.4	8.2	9	9	9	9.8	10	9.9
4 weeks	8.4	8.6	8.5	9.2	9.2	9.2	10.6	10.6	10.6
5 weeks	8.6	9.4	9.0	9.8	9.8	9.8	11.4	11.4	11.4
6 weeks	9.4	10	9.7	10.4	10.6	10.5	12	12.2	12.1
7 weeks	10.4	10.8	10.6	11.6	11.4	11.5	12.8	13.6	13.2
8 weeks	11	11	11	11.8	11.8	11.8	13.2	13.8	13.5

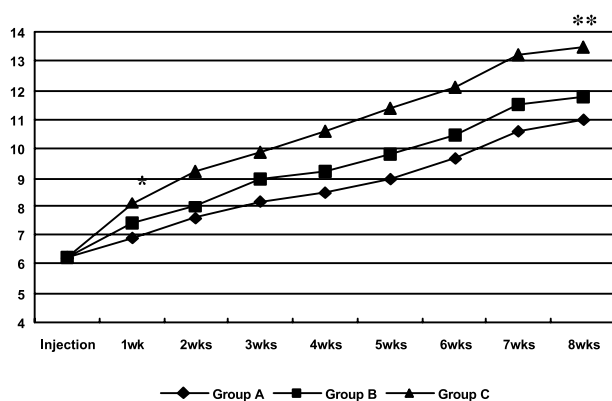


Fig. 1. BBB scores of each group: The transplanted group with HUCBs and BDNF was more greatly improved on their BBB locomotion scores as compared with the other groups 1 week after transplantation* ($P < 0.05$). 8 weeks after transplantation, the transplanted group with HUCBs and BDNF was more greatly improved on their BBB locomotion scores as compared with the other groups** ($P < 0.001$)

in each group (P -value 1.00). At week 1 after transplantation, the BBB scores were 6.9, 7.4, and 8.1 points for the A, B, and C groups, respectively. Thus, the HUCBs transplantation group showed an early improvement compared with the control group, especially in the HUCBs with BDNF transplantation group, which was dramatically improved in neurological function ($P < 0.05$). At 8 weeks after transplantation, the BBB scores were 11.0, 11.8, and 13.5 points in the A, B, and C groups, respectively (P -value < 0.001) (Table 2) (Fig. 1).

Immunohistochemical results

The amount of cells which had settled and survived after the transplantation was determined by BrdU immunoreactivity in the longitudinal sections, including the epicenter of the spinal cord, 8 weeks after the transplantation. Double immunostaining with BrdU and nestin antibodies showed that cells with BrdU and nestin

immunoreactivity were scattered in the spinal cord. Double immunostaining with GFAP and MAP-2 antibodies demonstrated that the scattered BrdU-reactive cells expressed the astrocyte marker, GFAP and the neural cells marker, MAP-2 (Fig. 2).

Retrograde labelling of descending axons

The labelled cells in the raphe nucleus were 18, 27 and 32 for the A, B, and C groups, respectively, and in the red nucleus the labelled cells were 21, 31, and 35 for the A, B, and C groups, respectively. The HUCBs transplanted with BDNF group, the 10 μ l, mononuclear cell layer of HUCBs was transplanted, but in HUCBs transplanted without BDNF group, the 20 μ l, mononuclear cell layer of HUCBs was transplanted.

But, the fluorogold labelled cells in the HUCBs with BDNF transplanted group were much more than that of the HUCBs without BDNF transplanted group.

Discussion

The neural stem cell has pluripotency to differentiate into various neural cell types and the human umbilical cord blood cells (HUCBs) are more pluripotent and genetically flexible than bone marrow neural stem cells and they can also be more easily obtained. The cell therapy treatment of cord injury is a cell substitution for the destroyed spinal cord, and axon regeneration and/or applying a neurotrophic factor to recover the neural tissue. It has been reported that neural stem cells transplanted into the injured lesion were able to differentiate into oligodendrocytes and astrocytes and then integrate into axonal pathways and regenerate and remyelinate the injured axons [9, 11, 20, 23, 25, 26, 33, 34].

It has also been reported that neural stem cells can be differentiated into hematological cells and bone marrow stem cells, and that HUCBs can be replicated and

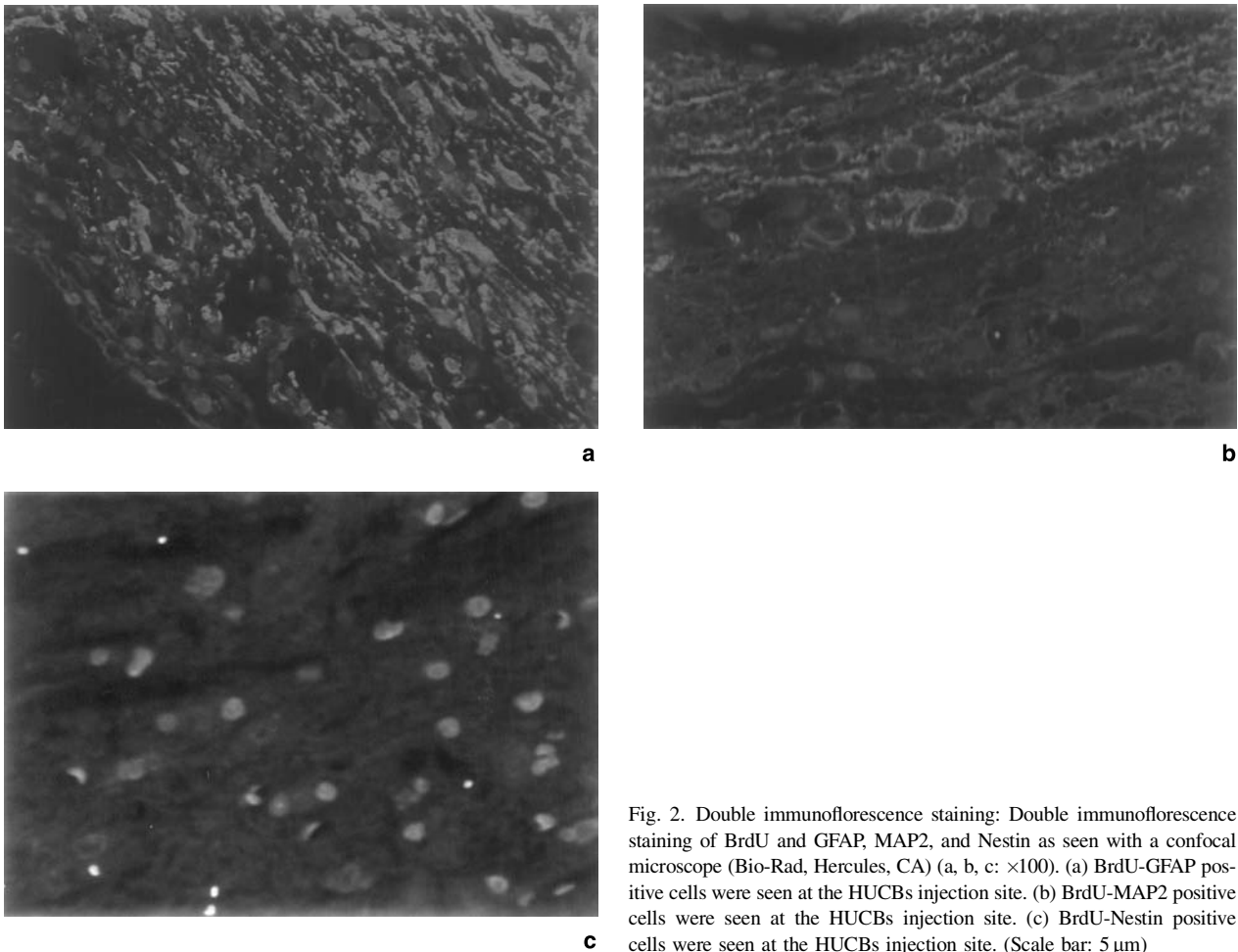


Fig. 2. Double immunofluorescence staining: Double immunofluorescence staining of BrdU and GFAP, MAP2, and Nestin as seen with a confocal microscope (Bio-Rad, Hercules, CA) (a, b, c: $\times 100$). (a) BrdU-GFAP positive cells were seen at the HUCBs injection site. (b) BrdU-MAP2 positive cells were seen at the HUCBs injection site. (c) BrdU-Nestin positive cells were seen at the HUCBs injection site. (Scale bar: 5 μm)

differentiated into muscle, myocardium, skeletal cells, hepatocytes, oligocytes, and neurons [13, 28, 31, 33, 37]. For *in vitro* cultures of bone marrow stem cells and HUCBs, there was differentiation into cells positive for the markers of NeuN, Neurofilament, MAP-2, GFAP, beta-tubulin III, and Gal-C [3, 6, 13, 15, 37].

There have been many efforts to recover neuronal function in SCI, but there are some limitations in SCI treatment. Neural stem cells are able to repair the destroyed neural tissue and also secrete the nerve growth factor (NGF), BDNF, and glial cell line-derived neurotrophic factor (GDNF); it also was reported that neurotrophic factors such as NF-3, BDNF, FGF, and NGF enhanced the regeneration of damaged axons and so helped to recover neurological functions [1, 5, 10, 14, 18, 22, 33, 36].

The transplanted embryonic neural stem cells were noted to survive and integrate with the host spinal cord 1 month after transplantation. And so the intraspinal treatment with NSCs and GDNF synergistically reduced lesion size and improved functional outcome after a compressive SCI in adult rats [32].

The intracerebral grafting with a combination of bone marrow and BDNF into the ischemic boundary zone of middle cerebral artery occluded rat brain enhanced differentiation of bone marrow cells and significantly improved motor recovery. This suggests that bone marrow cells along with neurotrophic factors may provide a powerful autoplasmic therapy for human neurological injury and degenerative disorders [7].

In this study, we transplanted HUCBs into an injured spinal cord *in situ* to evaluate the role of transplanted HUCBs in a SCI model. Functional improvements and neural differentiation were then studied.

It has been reported that the Neurotrophin-3 (NT-3) and BDNF have effects on neural survival and regeneration in cord injury models, but the neurotrophic factors have short half life times, so to achieve the ideal effects, a continuous release of these factors is required. Nowadays, two specific methods are used: fibroblasts containing the neurotrophic factor gene by using gene engineering, and the micro-infusion pump for the continuous release of neurotrophic factors, but there are the

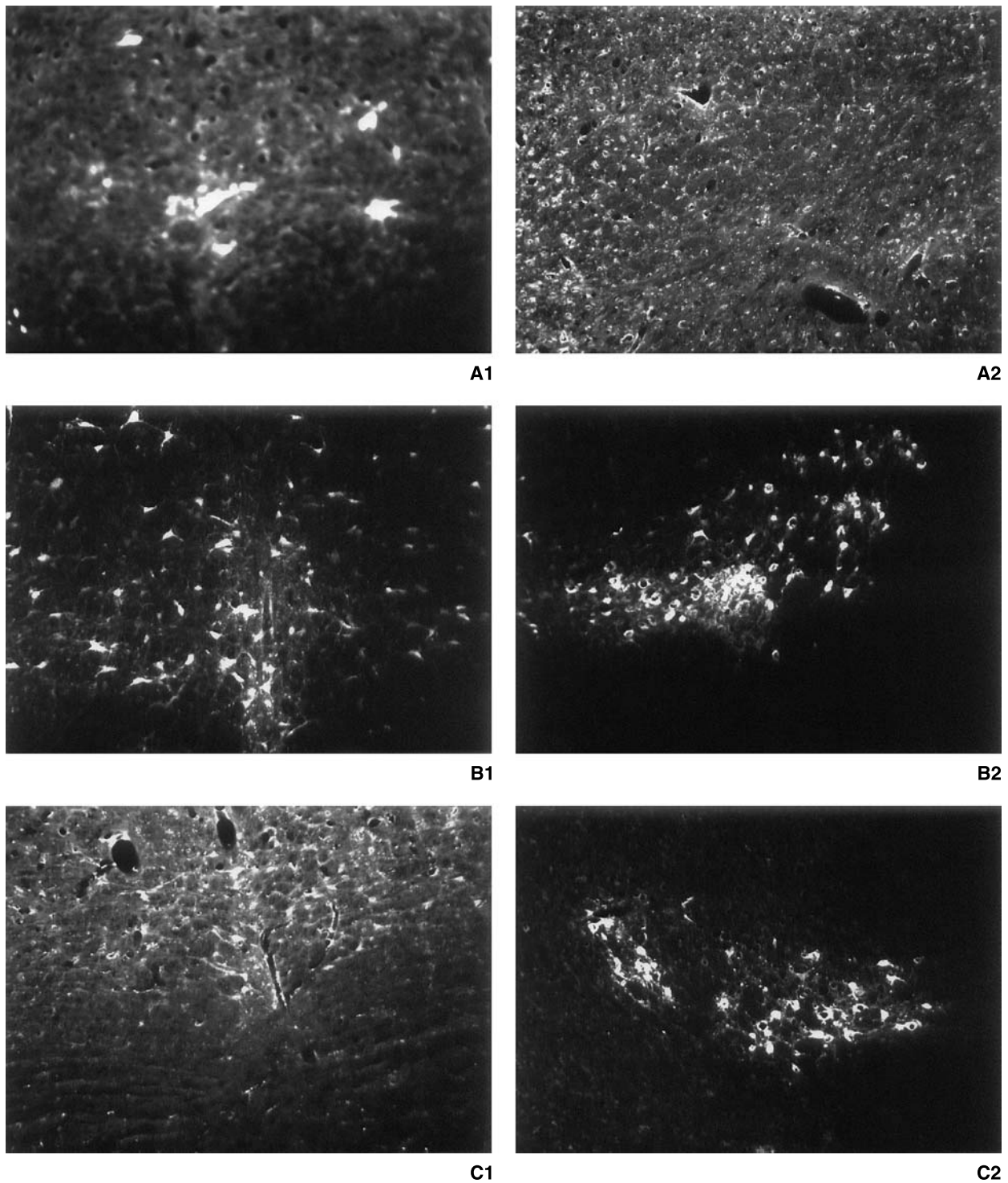


Fig. 3. Retrograde labelling of brain stem neurons: (A1, B1, C1) Fluorogold-axon staining of Group A, B, C in the raphe nucleus. (A2, B2, C2) Fluorogold-axon staining of Group A, B, C in the red nucleus. HUCBs transplantation promoted axonal regeneration in the raphe and red nucleus. The increased amount of FG-positive neurons was especially seen in the HUCBs with BDNF injection group rather than the HUCBs without BDNF group

risks of tumor cell differentiation and infection. So, we applied the BDNF using the tissue bio-engineering technique with fibrin glue.

The various neurotrophic factors were secreted automatically at the time of the HUCBs transplantation, and this improved neurological function due to axon

regeneration. Especially with the addition of BDNF, a more powerful recovery effect was noted for the remyelination and regeneration in injured axons.

8 weeks after transplantation, neural differentiation from HUCBs was found with the use of double immunohistochemistry, and the regeneration of injured axons was found with the use of retrograde labelling by fluorogold staining. The retrograde labelling by fluorogold staining is mainly performed via intact axons skirting around the injury, rather than via regenerated axons through the site of injury, but there are marked differences among the 3 groups on the retrograde labelling in the brain stem. So, the stem cells and BDNF have a good effect on the axon regeneration and recovery to some extent.

The BBB scores of HUCBs with BDNF transplantation group were improved more than the other groups, and the HUCBs without BDNF transplantation group was improved more than the control group. The BBB scores were improved continuously after 1 week, and this may have been due to continuous axon regeneration effects of the various neurotrophic factors that were secreted automatically in combination with HUCBs transplantation and BDNF. The neurological motor function of spinal cord injured rats was improved due to remyelination and regeneration effects of BDNF on the injured axons, and the neural differentiation of the transplanted HUCBs.

Houweling *et al.* have reported on a local application of BDNF on functional recovery after dorsal spinal cord transection in the adult rat. The performance of the rats on local application of BDNF group was significantly increased as compared with controls, but histological examination of the spared spinal cord tissue at the lesion 4 weeks after the initial injury showed no significant difference between the control and BDNF-treated animals [19].

McDonald and Howard have reported that embryonic stem cells differentiate to oligodendrocytes, and they then regenerate and remyelinate the injured axons in SCI [23].

Demyelination contributes to the loss of function consequent to central nervous system (CNS) injury. Optimizing remyelination through the transplantation of myelin-producing cells may offer a pragmatic approach to restoring meaningful neurological function. ES cell-derived oligodendrocytes are capable of rapid differentiation and myelination in mixed neuron/glia cell cultures. When transplanted into the injured spinal cord of adult rodents, the neural-induced precursor cells were capable of differentiating into oligodendrocytes and myelinating the host axons [20, 23].

The best time for neural stem cells transplantation is 2–4 weeks after cord injury for maximizing axon regeneration and functional recovery because of 2–4 weeks is the proper time for cell survival and cell differentiation due to the micro-environment in SCI [8, 17, 21, 26, 33]. However, we transplanted HUCBs 7 days after SCI, and we confirmed neural cell differentiation and functional improvement.

There is repair of injured axons, transplantation of neural precursor cells, and application of neurotrophic factors in cell therapy after SCI. After stem cell transplantation, neural stem cells are integrated into axonal pathways for regeneration and remyelination of axons and also differentiated into oligodendrocytes and astrocytes [9, 11, 20, 23, 25, 26, 33, 34].

Conclusions

We have shown that the HUCBs and BDNF reduced the neurological function deficit to a moderate degree for spinal cord injured rats. The HUCBs have survived and differentiated into neural cells after transplantation, and BDNF enhanced the axonal regeneration in spinal cord injured rats.

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Comment

The authors describe an animal model for spinal cord repair in the rat, using transplantation of human umbilical cord cells (HUCBs) and BDNF. Functional and histological improvements were seen up to eight weeks after HUCB transplantation, which was further improved with the addition of BDNF. The concept is novel and the experimental model appropriate.

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