Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 359 (2007) 40–45
BBRC
www.elsevier.com/locate/ybbrc

Basic fibroblast growth factor promotes bone marrow stromal cell transplantation-mediated neural regeneration in traumatic brain injury

Suk Ho Bhang [a,b,1], Young Eun Lee [c,1], Seung-Woo Cho [a,b], Jae-Won Shim [b,d], Sang-Hun Lee [d,e], Cha Yong Choi [b], Jin Woo Chang [c,*], Byung-Soo Kim [a,*]

[a] Department of Bioengineering, Hanyang University, Seoul 133-791, Republic of Korea
[b] School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Republic of Korea
[c] Brain Korea 21 Project for Medical Science and Department of Neurosurgery, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[d] Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea
[e] Institute of Mental Health, Hanyang University, Seoul 133-791, Republic of Korea

Received 27 April 2007
Available online 21 May 2007

Abstract

The current study was designed to evaluate the effects of basic fibroblast growth factor (bFGF) on human BMSC (hBMSC) transplantation-mediated neural regeneration in traumatic brain injury (TBI). Fibrin gel was used as a delivery vehicle to release bFGF locally in the TBI sites in a controlled manner. To test this hypothesis, hBMSCs suspended in fibrin gel containing bFGF were transplanted to rat TBI sites. Transplantation of hBMSCs suspended in fibrin gel without bFGF served as a control. hBMSC transplantation and bFGF treatment showed enhanced neural tissue regeneration than that of the control. The infarction volume and apoptotic activity of the transplanted hBMSCs were significantly decreased, and functional outcomes were significantly improved in the hBMSC transplantation and bFGF treatment group than in the control group. This study demonstrates that bFGF significantly enhances histological and functional recovery when used in hBMSC transplantation therapy in TBI.

Keywords: Basic fibroblast growth factor; Bone marrow stromal cell; Brain trauma injury; Fibrin gel

Bone marrow stromal cells (BMSCs) have been reported to have potential for improving the neurological outcome in central nervous system (CNS) disorders such as head stroke and brain trauma [1–5]. BMSCs have the ability to secrete growth factors that are essential for neurogenesis [1,6–8]. It has also been suggested that BMSCs themselves might be able to differentiate into neural cells [9]. These findings suggest a therapeutic potential of BMSCs for human CNS disorders.

To promote the therapeutic efficacy of cell therapy to treat CNS injuries or diseases, the viability of transplanted cells should be maintained at a high level. It is a well-recognized limitation in cell transplantation approaches that survival of the transplanted cells or grafts is often poor [10]. *In vivo* conditions such as a hypoxic environment or an immune reaction can reduce the viability of transplanted cells. Moreover, growth factors are strongly needed for tissue regeneration, and an insufficient amount of growth factors also affects the viability of transplanted cells [10].

Basic fibroblast growth factor (bFGF) has therapeutic potential in CNS regeneration. bFGF is known to induce neurogenesis [11], enhance neural survival, and outgrowth [12], and reduce infarct size following cerebral ischemia in adult rats [13]. In this study, the effects of bFGF on the therapeutic efficacy of human BMSC (hBMSC)

* Corresponding authors. Fax: +82 2 393 9979 (J.W. Chang), +82 2 2298 4101 (B.-S. Kim).
E-mail addresses: jchang@yumc.yonsei.ac.kr (J.W. Chang), bskim@hanyang.ac.kr (B.-S. Kim).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.05.046

transplantation were evaluated in a rat traumatic brain injury (TBI) model. Specifically, the effects of bFGF on the apoptotic activity of transplanted cells and the enhancement of histological and functional recovery in hBMSC transplantation therapy were evaluated. Fibrin gel was used as a bFGF delivery vehicle to overcome the rapid loss of bFGF bioactivity *in vivo* when injected into the body in solution form [14].

Materials and methods

Preparation of the bFGF delivery system. Recombinant bFGF protein was produced using the translation system RTS 500 (Roche Applied Sciences, Mannheim, Germany) as previously described [14]. Fibrin gel kits (Greenplast®) were purchased from GreencrossPD Co. (Yong In, Korea). The fibrinogen was prepared from pooled human plasma and contained factor XIII at approximately 68 U/100 mg fibrinogen. Fibrinogen, aprotinin (66 U/ml), heparin (Sigma, St. Louis, MO), and bFGF were mixed in saline solution. The fibrinogen-bFGF solution was converted to a gel by the addition of calcium chloride (5.9 mg/ml) and thrombin (33.3 U/ml).

Experimental groups. Rats were divided into four groups: (i) a no injury group ($n = 7$), rats without TBI; (ii) a no-treatment group ($n = 7$), rats with TBI that underwent no treatment; (iii) a TBI group treated with transplantation of hBMSCs suspended in fibrin gel ($n = 7$); and (iv) a TBI group treated with transplantation of hBMSCs suspended in fibrin gel containing bFGF (30 μg per animal, $n = 7$).

Surgical procedures. Sprague–Dawley rats weighing approximately 250 g were anesthetized. The scalps were incised and a craniotomy was performed over the right forelimb motor cortex. Specifically, the rats were put into a stereotactic frame, and the cranium was drilled +4 to −3 mm anterior and posterior from bregma and +1 to +4.5 mm lateral from the midline. The bone was thinned with the drill bit over the forelimb motor cortex. A high-speed microdrill with a diamond point (1 mm) was used to prepare the scalp above the motor cortex, and the last layer of the bone was removed with forceps. Special care was taken to keep the dura intact to prevent bleeding. A cryogenic lesion stamp with a pointed bottom and a circular footprint of 7 in. diameter was prepared and pre-cooled to −70 °C with liquid nitrogen. Cryogenic lesion stamps were used five times for 30 s on the exposed dura. The skin was closed over the lesion. Six days after receiving the cryogenic lesion, the rats received hBMSCs (1×10^7 cells/ml) suspended in the fibrin gel or hBMSCs suspended in fibrin gel containing bFGF (30 μg). The total volume of the cell suspension was identical with the lesion volume (30 μl). To identify the transplanted hBMSCs, the cells were retrovirally pre-labeled with enhanced green fluorescence protein (eGFP). All rats were treated with daily subcutaneous injections of cyclosporine A (10 mg/kg, Chong Kun Dang Pharm., Seoul, Korea) for immunosuppression beginning the day before surgery.

Triphenyltetrazolium chloride (TTC) staining. To identify the morphological differences among TBI brains in each group, the rats were sacrificed 6 days after TBI (day 0) and 14 days after the cell transplantation. The brains were coronally cut into 2-mm slices. The slices were incubated for 30 min in 2% TTC (Sigma) solution and fixed for 30 min in formalin.

Calculation of infarct volume. The brains were cut into 40-μm thick serial sections and stained with hematoxylin and eosin (H&E) for infarct volume measurement. The histological images detected at 10× magnification were gathered for lesion volume measurement. The lesion volumes were determined using Adobe Photoshop software (Adobe Systems Inc., CA). The volume of the lesion was estimated from the slice thickness and the lesion areas of each section. The infarction area of each slide was measured by subtracting the area of the non-lesioned ipsilateral hemisphere from that of the contralateral side [15–17].

Immunohistochemistry. Fourteen days after hBMSC transplantation or hBMSC transplantation and bFGF treatment, the rats were anesthetized with 25% urethane (Sigma) in phosphate-buffered saline (pH 7.4, PBS, Sigma) and intracardially perfused with 250 ml normal saline followed by 250 ml ice-cold 4% paraformaldehyde (PFA) in PBS. The brains of all rats were removed, post-fixed in 4% PFA for an additional 20 min at room temperature, and transferred to 30% sucrose in PBS and immersed for 48 h at 4 °C. The brains were then frozen in O.C.T. compound (Tissue-Tek, Sakura Finetk, Torrance, CA, USA) at −20 °C and sectioned (section thickness 40 μm) using a Cryostat Cryocut Microtome (Leica, CM3050S, Nussloch, Germany). To examine the presence of neural cells, the following primary antibodies were used: Neuronal nuclei monoclonal antibodies (1:100, Chemicon, Temecula, CA, USA) were used to characterize the neurons, and Glial fibrillary acidic protein monoclonal antibodies (1:100, MP Biomedicals, Aurora, OH, USA) were used for astrocytes. To detect the primary antibodies, slides were incubated in PBS containing rhodamine-conjugated secondary antibodies (1:100, Jackson-Immunoresearch, West Grove, PA, USA) for 1 h at room temperature. The slides were mounted with 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Burlingame, CA, USA) for fluorescence and photographed with a confocal microscope. To identify the transplanted hBMSCs, anti-eGFP monoclonal antibodies (1:50, Chemicon) were used with Fluorescein isothiocyanate secondary antibodies (1:100, Jackson-Immunoresearch).

Terminal uridine nick-end labeling (TUNEL) assay. A TUNEL assay was performed to detect apoptotic activity in the transplanted hBMSCs using an ApopTag Red in Situ Apoptosis Detection Kit (Chemicon) according to the manufacturer's instructions. The test was performed 2 days after hBMSC transplantation or hBMSC transplantation and bFGF treatment.

Behavioral tests. Both the rotarod and stepping tests were performed to assess behavior (LE8300 LETICA Panlab, Spain) [18,19]. All training and testing began at 5:00 pm according to the nocturnal habits of the rats. Experimental procedures were followed in accordance with the NIH regulations for animal care and with the approval of the Institution Animal Care and Use Committee of Yonsei University, Seoul, Korea.

Statistical analysis. All quantitative data were expressed as the means ± standard deviation. A one-way analysis of variance (ANOVA) using a Bonferroni test was performed on samples to determine significant differences. The assumptions of ANOVA were found to satisfy Levene's test for homogeneity of variance and to pass tests for normality. A value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

The present study shows that rats receiving hBMSC transplantation with bFGF treatment showed a more significant improvement in both morphological and functional recovery in TBI than rats receiving hBMSC transplantation alone. H&E and TTC staining showed that the brain infarction volume in the hBMSC transplantation group was reduced compared to that of the no-treatment group (Fig. 1). The TBI rats with no treatment had the largest infarction volume and the largest brain volume loss. No treatment resulted in increase in the infarction volume. The TBI rats in other groups showed no further brain volume loss after hBMSC transplantation or hBMSC transplantation and bFGF treatment.

The brain volume loss caused by brain infarction was approximately 20% in the no-treatment group. In the hMSC transplantation group, 10–13% of brain volume was lost. The hBMSC transplantation with bFGF treatment group showed only 5–7% of brain volume lost.

One explanation for this reduction in lost brain volume is that the bFGF released from the fibrin gel enhanced the viability of the transplanted hBMSCs. The apoptotic

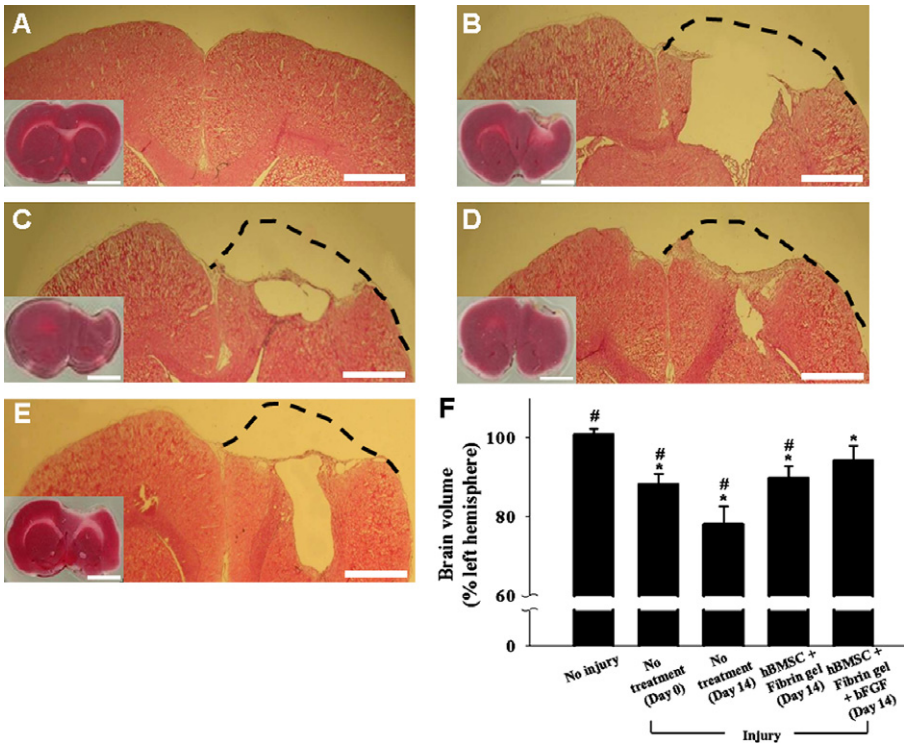

Fig. 1. H&E-stained histological sections of TBI brains 14 days after treatment otherwise specified. (A) No injury. (B) Injury with no treatment. (C) Injury with hBMSC transplantation. (D) Injury with hBMSC transplantation and bFGF treatment. (E) TBI brain before treatment and 6 days after TBI (day 0). The dotted lines show brain tissue size before brain tissue loss. Insets show the TTC staining at a low magnification. (F) Volume of TBI brains ($^{*}p < 0.05$: compared with no injury group, $^{\#}p < 0.05$: compared with hBMSC transplantation and bFGF treatment group). The scale bars indicate 0.5 cm in H&E images and 1 cm in TTC images.

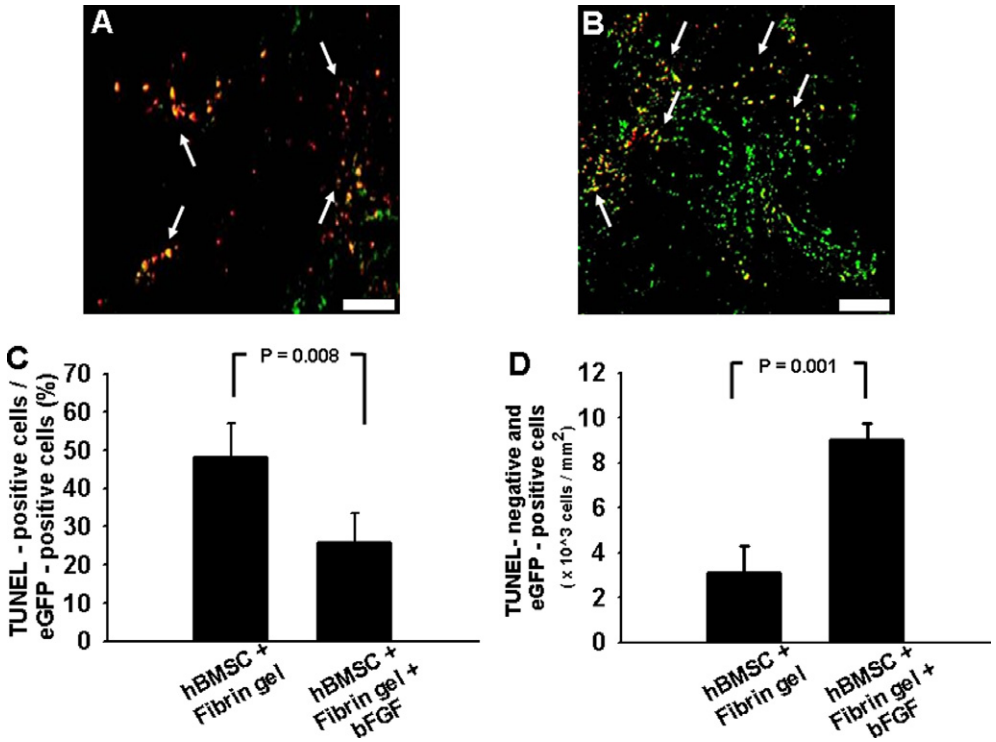

Fig. 2. Apoptotic activity (red, TUNEL-positive cells; green, transplanted hBMSCs (eGFP); yellow, transplanted hBMSCs with apoptotic activity; scale bars indicate 50 μm, 2 days after hBMSC transplantation or hBMSC transplantation and bFGF treatment). (A) Injury with hBMSC transplantation. (B) Injury with hBMSC transplantation and bFGF treatment. (C) Quantitative results of the apoptotic activity. (D) The number of viable hBMSCs in unit graft area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

activity of the transplanted hBMSCs was reduced in the hBMSC transplantation with bFGF treatment group to almost half the level of that of the hBMSC transplantation group (Fig. 2). This result suggests that the bFGF release system could make a better environment for hBMSC survival in TBI. Previously, it has been reported that bFGF can play a critical role in cell proliferation, differentiation, and neuroprotection in CNS [20].

Immunohistological analysis showed the highest expression of astrocyte and neuronal marker expression in the hBMSC transplantation with bFGF treatment group. Although most of the cells in the TBI area were astrocytes in both the hBMSC transplantation and hBMSC transplantation with bFGF treatment groups, the hBMSC transplantation with bFGF treatment group showed a larger amount of neurons than did the hBMSC transplantation-alone group (Fig. 3). In addition, bFGF affected astrocyte penetration into the TBI area in hBMSC transplantation. The hBMSC transplantation with bFGF treatment group showed a greater amount of astrocyte penetration into the TBI area than did the hBMSC transplantation group (Fig. 3), and this penetration compensated for the TBI cavity caused by brain infarction. The no-treatment group did not show any astrocyte penetration into the TBI cavity and showed little neuronal marker expression around the TBI.

bFGF also significantly enhanced the functional recovery in hBMSC transplantation for TBI. The hBMSC transplantation with bFGF treatment group rats showed more improvement in both the stepping and rotarod tests than the hBMSC transplantation group rats (Fig. 4). The rats with TBI injury and no treatment served as a negative control and showed nearly no improvement.

In the present study, the major contribution of bFGF in the hBMSC transplantation therapy for TBI could be the enhancement of the viability of transplanted hBMSCs. An increased viability of transplanted stem cells would be indispensable for tissue regeneration. bFGF treatment enhanced morphological and functional recovery in the hBMSC transplantation therapy after TBI. The results of poor tissue regeneration and functional recovery in the hBMSC transplantation group could be due to the high apoptotic activity of the transplanted cells. However, no evidence was found indicating the presence of transplanted hBMSCs 14 days after cell transplantation, despite staining for eGFP, human nuclei, and human mitochondria to detect the transplanted hBMSCs. A previous study reported that no or a very low percent of neurons differen-

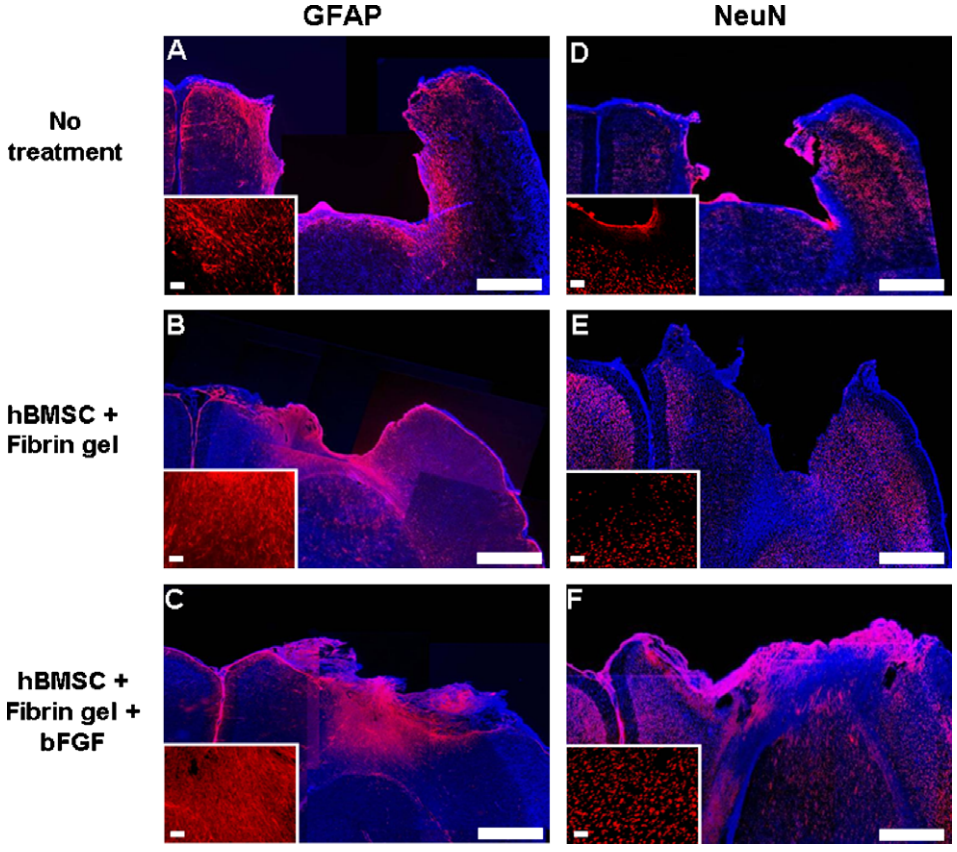

Fig. 3. Immunohistochemistry results showing astrocytes (red) stained with anti-GFAP antibodies: (A) injury with no treatment, (B) injury with hBMSC transplantation, (C) injury with hBMSC transplantation and bFGF treatment and neurons (red) stained with anti-NeuN antibodies, (D) injury with no treatment, (E) injury with hBMSC transplantation, (F) injury with hBMSC transplantation and bFGF treatment. Brain sections were also stained with DAPI (blue). The scale bars indicate 100 μm. (The images in the left corners show astrocytes in (A–C) and neurons in (D–F) in TBI, 14 days after hBMSC transplantation or hBMSC transplantation and bFGF treatment. The scale bars indicate 100 μm.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

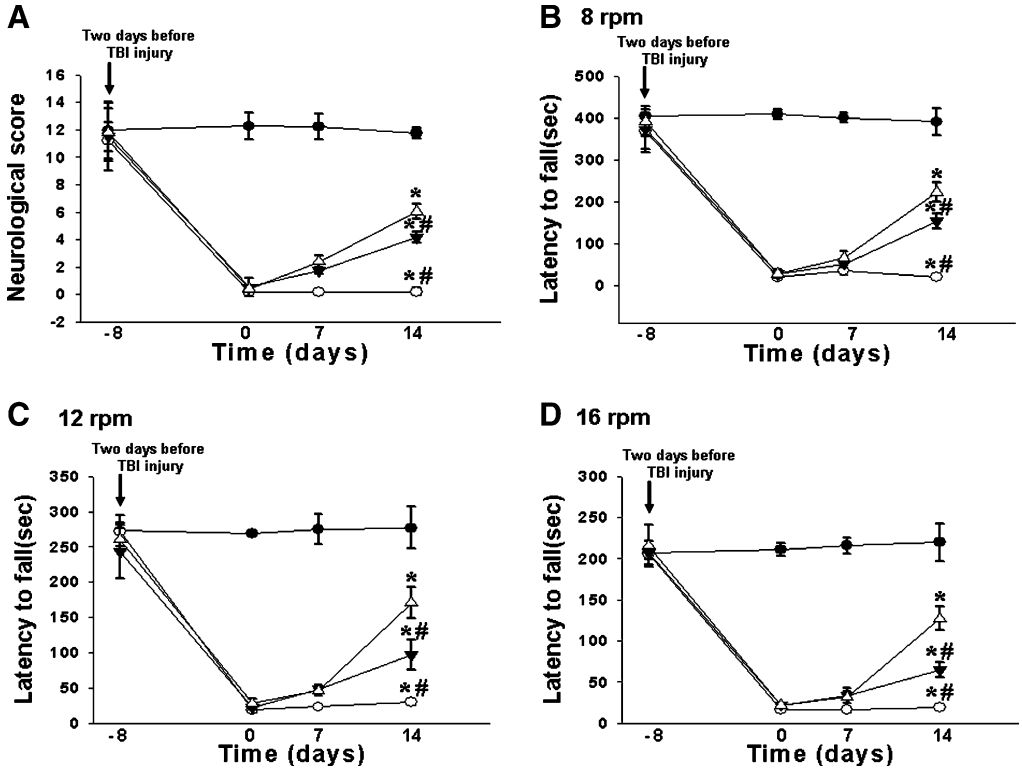

Fig. 4. The stepping and rotarod tests (●, no injury; ○, injury with no treatment; ▼, injury with hBMSC transplantation; △, injury with hBMSC transplantation and bFGF treatment). (A) Stepping tests ($^{*}p < 0.05$: compared with no injury group, $^{\#}p < 0.05$: compared with hBMSC transplantation and bFGF treatment group). (B–D) Rotarod tests at various rotation speeds ($^{*}p < 0.05$: compared with no injury group, $^{\#}p < 0.05$: compared with hBMSC transplantation and bFGF treatment group).

tiated from transplanted cells were found after transplanting embryonic or fetal stem cells into TBI models [21]. Therefore, it is possible that the functional and morphological improvement in the present study could be due to the paracrine effect of the transplanted hBMSCs or bFGF itself rather than neuronal differentiation of the transplanted stem cells.

When the brain is damaged, major changes occurring in the surrounding brain tissue are due to reactions of neurotrophic factors [22,23]. Brain injuries such as TBI are believed to be associated with alterations in gene expression and the up-regulation and release of a number of both potentially damaging and restorative neurochemical factors that interact in a complex network, leading to both delayed cellular dysfunction and death or tissue regeneration [24]. Therefore, the extended nature of these cascades might offer the possibility for improving neural tissue regeneration after TBI [25,26]. Given this background, it is reasonable to speculate that neurotrophic factors released from transplanted hBMSCs play an important role in TBI recovery. Neurotrophic factors released from transplanted hBMSCs may underlie their beneficial effect, which could protect host neurons and facilitate the host regeneration found in other stem cells such as neural stem cells [27,28]. The enhanced TBI recovery resulting from hBMSC transplantation in the present study could be caused by a prolonged paracrine effect of the transplanted hBMSCs that had a lower apoptotic activity than cells in the hBMSC transplantation group.

In the present study, fibrin gel was used as a bFGF delivery vehicle to enhance the efficacy of bFGF. bFGF undergoes rapid degradation in soluble form, and a delivery system that can release bFGF for an appropriate period in a controlled manner could enhance the efficacy of bFGF for nerve regeneration. Fibrin gel has been used as a vehicle to deliver various growth factors and can release them over a long time period by slowing their diffusion [29]. In addition, fibrin gel can be easily transplanted *in vivo* by injection through a needle without invasive surgery. Fibrin gel is a biocompatible material that has received approval from the Food and Drug Administration of United States for human use as sealant [30,31].

Acknowledgment

This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No. 03-PJ1-PG1-CH07-0004).

References

[1] Y. Li, J. Chen, XJ. Chen, Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery, Neurology 59 (2002) 514–523.

[2] D. Lu, Y. Li, A. Mahmood, L. Wang, T. Rafiq, M. Chopp, Neural and marrow derived stromal cell sphere transplantation in a rat model of traumatic brain injury, J. Neurosurg. 97 (2002) 935–940.
[3] D. Lu, A. Mahmood, Y. Li, M. Chopp, Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome, Neuroreport 12 (2001) 559–563.
[4] A. Mahmood, D. Lu, Y. Li, JL. Chen, M. Chopp, Intracranial bone marrow transplantation after traumatic brain injury improves functional outcome in adult rats, J. Neurosurg. 94 (2001) 589–595.
[5] A. Mahmood, D. Lu, L. Wang, M. Chopp, Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells, Neurosurgery 49 (2001) 1196–1204.
[6] X. Chen, M. Katakowski, Y. Li, Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production, J. Neurosci. Res. 69 (2002) 687–691.
[7] D. Lu, A. Mahmood, M. Chopp, Biological transplantation and neurotrophic induced neuroplasticity after traumatic brain injury, J. Head Trauma Rehab. 18 (2003) 357–376.
[8] A. Mahmood, D. Lu, M. Chopp, Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury, J. Neurotrauma 21 (2004) 33–39.
[9] J. Sanchez-Ramos, S. Song, F. Cardozo-Pelaez, C. Hazzi, T. Stedeford, A. Willing, T.B. Freeman, S. Saporta, W. Janssen, N. Patel, D.R. Cooper, P.R. Sanberg, Adult bone marrow stromal cells differentiate into neural cells in vitro, Exp. Neurol. 164 (2000) 247–256.
[10] P. Brundin, J. Karlsson, M. Emgard, G.S. Schierle, O. Hansson, A. Petersen, R.F. Castilho, Improving the survival of grafted dopaminergic neurons: a review over current approaches, Cell Transplant. 9 (2000) 179–195.
[11] J.P. Wagner, I.B. Black, E. DiCicco-Bloom, Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor, J. Neurosci. 19 (1999) 6006–6016.
[12] J.J. Ramirez, S.P. Finklestein, J. Keller, W. Abrams, M.N. George, T. Parakh, Basic fibroblast growth factor enhances axonal sprouting after cortical injury in rats, Neuroreport 10 (1999) 1201–1204.
[13] Q. Li, D. Stephenson, Postischemic administration of basic fibroblast growth factor improves sensorimotor function and reduces infarct size following permanent focal cerebral ischemia in the rat, Exp. Neurol. 177 (2002) 531–537.
[14] O. Jeon, S.H. Ryu, J.H. Chung, B.S. Kim, Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin, J. Control. Release 105 (2002) 249–259.
[15] B. Yulug, U. Kilic, E. Kilic, M. Bahr, Rifampicin attenuates brain damage in focal ischemia, Brain Res. 996 (2004) 76–80.
[16] E. Kilic, GP. Dietz, D.M. Hermann, M. Bahr, Intravenous TAT-Bcl-Xl is protective after middle cerebral artery occlusion in mice, Ann. Neurol. 52 (2002) 617–622.
[17] E. Kilic, J. Weishaupt, U. Kilic, G. Rohde, B. Yulug, K. Peters, D.M. Hermann, M. Bähr, The superoxide dismutase1 (sod1) g93a mutation does not promote neuronal injury after transient focal brain ischemia and optic nerve transection in mice, Neuroscience 128 (2004) 359–364.
[18] M. Olsson, G. Nikkhah, C. Bentlage, A. Bjorklund, Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test, J. Neurosci. 15 (1995) 3863–3875.
[19] G. Rozas, M.J. Guerra, J.L. Labandeira-Garcia, An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism, Brain Res. 2 (1997) 75–84.
[20] S. Yoshimura, T. Teramoto, M.J. Whalen, M.C. Irizarry, Y. Takagi, J. Qiu, J. Harada, C. Waeber, X.O. Breakefield, M.A. Moskowitz, FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice, J. Clin. Invest. 112 (2003) 1202–1210.
[21] A. Wennersten, X. Meier, S. Holmin, L. Wahlberg, T. Mathiesen, Proliferation, migration, and differentiation of human neural stem/progenitor cells after transplantation into a rat model of traumatic brain injury, J. Neurosurg. 100 (2004) 88–96.
[22] C.D. Winter, A.K. Pringle, G.F. Clough, M.K. Church, Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury, Brain 127 (2004) 315–320.
[23] M. Bondanelli, M.R. Ambrosio, A. Margutti, P. Boldrini, N. Basaglia, P. Franceschetti, M.C. Zatelli, E.C. Degli Uberti, Evidence for integrity of the growth hormone/insulin-like growth factor-1 axis in patients with severe head trauma during rehabilitation, Metabolism 51 (2002) 1363–1369.
[24] T.K. McIntosh, K.E. Saatman, R. Raghupathi, D.I. Graham, D.H. Smith, V.M. Lee, The molecular and cellular sequelae of experimental traumatic brain injury: pathogenetic mechanisms, Neuropathol. Appl. Neurobiol. 24 (1998) 251–267.
[25] T.K. McIntosh, M. Juhler, T. Wieloch, Novel pharmacologic strategies in the treatment of experimental traumatic brain injury, J. Neurotrauma 15 (1998) 731–769.
[26] N.C. Royo, S. Shimizu, J.W. Schouten, J.F. Stover, T.K. McIntosh, Pharmacology of traumatic brain injury, Curr. Opin. Pharmacol. 3 (2003) 27–32.
[27] R. McKay, Stem cells in the central nervous system, Science 276 (1997) 66–71.
[28] J. Ourednik, V. Ourednik, Graft-induced plasticity in the mammalian host CNS, Cell Transplant. 13 (2004) 307–318.
[29] S.E. Sakiyama-Elbert, J.A. Hubbell, Development of fibrin derivatives for controlled release of heparin-binding growth factors, J. Control. Release 65 (2000) 389–402.
[30] Y. Tabata, K. Yamada, S. Miyamoto, I. Nagata, H. Kikuchi, I. Aoyama, M. Tamura, Y. Ikada, Bone regeneration by basic fibroblast growth factor complexed with biodegradable hydrogels, Biomaterials 19 (1998) 807–815.
[31] D.D. Hile, M.L. Amirpour, A. Akgerman, M.V. Pishko, Active growth factor delivery from poly(D,L-lactide-co-glycolide) foams prepared in supercritical CO_2, J. Control. Release 66 (2000) 177–185.