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A Comparative Study of Therapeutic Benefits of Intraspinal and Intravenous Bone Marrow Stromal Cell Administration to Spinal Cord Injuries

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ABSTRACT

Background: Recent reports demonstrated that intravenous route as a minimally invasive method, similar to direct injection, is suitable for bone marrow stromal cell (BMSC) transplantation. In this study, we made a comparison of intraspinal and intravenous route of BMSC administration to repair injured spinal cord tissue. **Methods:** Six groups of adult female rats were used in this study. Laminectomy and spinal cord injury (SCI) were carried out at first lumbar vertebra level (L1). Labeled stromal cells were administered intraspinally and intravenously in experimental groups one week after SCI. In control groups, serum was administered in the same way. Another groups consisted of laminectomy alone and SCI. Behavioral testing was performed weekly to 5 weeks post injury. Tissue processing and immune-histochemical studies were performed four weeks after cell transplantation. **Results:** Mean of Basso-Beattie-Bresnehan (BBB) scale scores in intraspinal and intravenous groups were 15.8 ± 0.44 and 15.6 ± 0.54, while in their controls were 10.6 ± 0.33 and 10.6 ± 0.56, respectively. BBB scale in laminectomy and SCI groups were 21 and 10.5 ± 0.36, respectively. Immuno-histochemical staining visualized BMSC in the site of injury. Differentiation of a few implanted cells to neuron and glial cell was detected in intravenous group, while only differentiation to glial cells was detected in intraspinal group. **Conclusion**: The results of this study suggest that intravenous administration of BMSC, such as intraspinal method, provides therapeutic benefits for SCI. *Iran. Biomed. J. 131 (1): 43-48, 2009*

Keywords: Spinal cord injury (SCI), Repair, Bone marrow stromal cell

INTRODUCTION

Since the capacity of nervous system to regenerate after injury is limited, cell transplantation is considered to be the most effective way to repair neural injury [1]. In recent years, bone marrow stromal cells (BMSC) are considered to be a useful cell source for repair of spinal cord injury (SCI) [2]. One of the unsettled problems in cell therapy with BMSC is the method of cell delivery to the injured site [3]. Although experimental studies have shown that direct injection of BMSC to lesion site reduces functional deficits [4, 5], many investigators believe that this route of administration causes an additional damage to the host tissue [6, 7]. Intraventricular and lumbar puncture delivery were reported to be effective routes of administration with minimally damage to the host tissue [6, 7]; although beneficial effects on the tissue repair and behavioral recovery were less than direct injection which reported by others [4, 5].

In previous study, we reported that administrated BMSC into the venous system can be conveyed through the vein, invade the injured region and integrated with host tissue [8]. Moreover, recent studies showed that intravenous administration of BMSC achieves functional recovery after traumatic brain injury [9].

In the present study, we investigated whether intravenously migrated BMSC to the site of injured spinal tissue can provide appropriate therapeutic benefits and compared that with intra-lesional injection route.

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MATERIALS AND METHODS

BMSC preparation. Adult female Sprague-Dawley rats (body weight 250-300 g) were used to isolate the BMSC**,** as described in detail by Rismanchi *et al.* [10]. The cells were identified using sheep anti-human fibronectin antibody (Biozol, Germany) and demonstrated by donkey anti-sheep antibody conjugated with fluorescein isothiocyanate (Chemicon, England) [11]. The cells were labeled with bromodeoxyuridine (BrdU) at a concentration of 3 µg/ml which was added to the incubation medium for 3 days. The cells were checked for labeling using mouse anti- BrdU monoclonal antibody, and then the cover slip was labeled with secondary antibody conjugated with peroxidase [12].

Animals. Six groups of adult female Spargue-Dawley rats were used (bodyweight 250-300g) (Pasteur Institute of Iran, Tehran, Iran) as follow: 1) Laminectomy group (n = 7): in which only a laminectomy was performed; 2) SCI group (n = 7): in which contusion was performed without serum injection; 3 and 4) Intraspinal BMSC injected group (n = 7), and its control group (n = 7): in which serum injected in the same way; 5 and 6) intravenous BMSC injected group (n = 7), and its control group (n = 7): in which serum injected in the same way. Contusive SCI was carried out at L1 vertebra level using the weigh dropping technique [13]. Following the surgery, the recovery of animals was performed [14].

Transplantation and behavioral testing. BMSC was carried out 1 week after the SCI by intraspinal (3×10^5 viable BMSC in 10 µl saline using a micro injection pump) [15] and intravenous (2.5×10^6 viable BMSC in 0.5 ml saline into a tail vein) routes [8]. Basso-Beattie-Bresnehan (BBB) behavioral score test was used weekly to evaluate hind limb motor function five weeks after injury [16].

Tissue processing and immunohistochemistry. Five weeks after injury, the animals were perfused with 4% paraformaldehyde. The spinal cords were removed at the region of T13, L1 and L2 vertebra level, and transferred in 30% sucrose solution at 4°C overnight, embedded in OCT medium and cut in a cryostat at 8 µm. Implanted cells were identified according to the following procedure [17]: the sections were incubated in 50% formamide (Merck, Germany)/2× SSC (standard sodium citrate:0.3M NaCl and 0.03 M sodium citrate) at 65°C for 2 hours, washed for 10 minutes with 2× SSC at room temperature and incubated in 2N HCL (Merck, Germany) at 37°C for 30 minutes. Then, they were rinsed in 0.1 M boric acid (pH 8.5, Merck, Germany) for 10 minutes, washed in phosphate buffer saline and incubated with mouse anti-BrdU monoclonal antibody (Sigma, Germany) at 4°C over night, incubated with secondary antibody conjugated with horseradish peroxidase (goat anti-mouse IgG-peroxidase, Sigma, Germany) for 2 hours, demonstrated with diaminobenzidine tetrahydro-chloride for 5 minutes and then mounted.

The numerical density per area was used to evaluate the longitudinal distribution of the labeled BMSC in the injured spinal cord. The sampling was carried out randomly from the end and the middle of T13, between T13 and L1, the middle of L1, between L1 and L2, and the middle and the end of L2 region. Immuno-fluorescence double-labeling method was performed to detect of cellular specific protein expression of rat insulin promoter, glial fibrillary acidic protein (GFAP), neurofilament-68 kDa and neurofilament-200 kDa in transplanted cells [17].

Statistical analysis. Significance between each of two groups was examined by using a student's *t*-test. A value of $P<0.05$ was considered significant.

RESULTS

Behavioral recovery. The BBB locomotor rating scores in the end point of the experiment are shown in Figure 1.

A clear and progressive recovery observed in experimental groups that received intraspinal and intravenous administration of BMSC, compared to control groups. Five weeks after injury, the mean (± standard deviation) of BBB scale score were 15.8 ± 0.44 in intraspinal group, 15.6 ± 0.54 in intravenous group, 10.6 ± 0.33 in first control group and 10.6 ± 0.56 in the second control group (the maximum score in this scale, corresponding to an animal without motor deficits, is 21). In functional terms, the rats of experimental group could walk with consistent weight-supported plantar steps. In contrast, the rats of control groups exhibited obvious motor function deficits. Moreover, BBB scale in the laminectomy and spinal cord injury groups were 21(normal) and 10.5 ± 0.36, respectively. The statistical differences between the experimental

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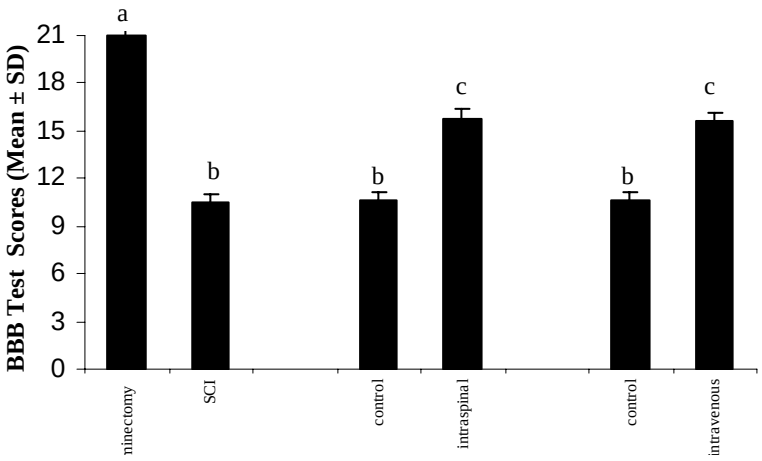

Fig. 1. The difference in BBB scores between the test groups at 4 weeks after cell transplantation. The values of BBB scores were significantly different between a and b, a and c, b and c (P<0.05), while between b and b, c and c were not significant (P>0.05).

groups, between control groups ,and between the control and SCI groups, were not significant (P>0.05), while the differences between the experimental groups and their controls were significant (P<0.05).

Immunohistochemical findings. Figure 2A shows intraspinal transplanted BMSC which survived and distributed throughout the damaged tissue. Figure 2B indicates that BMSC were delivered to spinal cord via an intravenous route, selectively migrated and distributed into the injured tissue. Double-staining immuno-histochemistry of spinal cord sections revealed that some BrdU-positive cells were reactive for the neural and glial markers. Figure 3 shows co-localization of immuno-fluorescent labels both for BrdU and rat insulin promoter (A), GFAP (B), neurofilament-68 kDa (C) and neurofilament-200 kDa (D) in intravenously administrated cells. In contrast, intra-spinal transplanted cells expressed rat insulin promoter and GFAP only (not shown).

DISCUSSION

Our results show that 4 weeks after intraspinal and intravenous administration of BMSC, functional outcome significantly was improved compared to non-treated rats. These data indicate that intravenous administration, similar to intraspinal injection in this study and those published by others [4, 5, 14, 15] can effectively promote the behavioral recovery. Moreover, the BBB locomotor rating score, which is achieved following intravenous route in the present study, significantly is superior to the other reported minimally invasive methods [6, 7]. To achieve these improvements, several mechanisms have

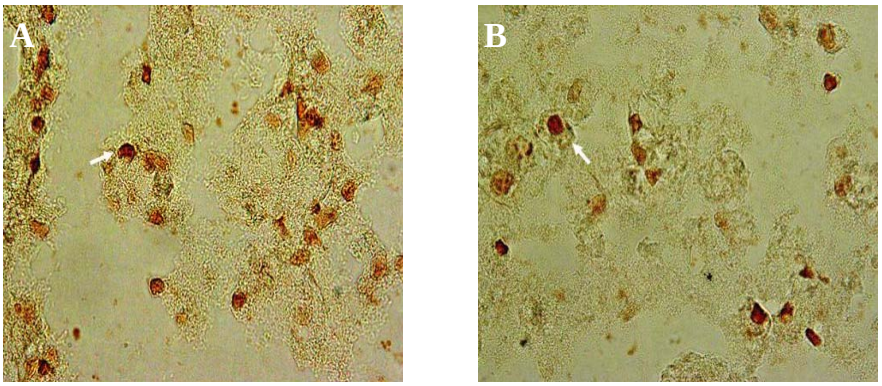

Fig. 2. Immunohistochemical findings of the spinal cord 4 weeks after BMSC transplantation, coronal section. Brdu-reactive bone marrow stromal cells (dark brown) are present in the injured spinal tissue after intraspinal **(A)** and intravenous **(B)** transplantation (arrows), ×800.

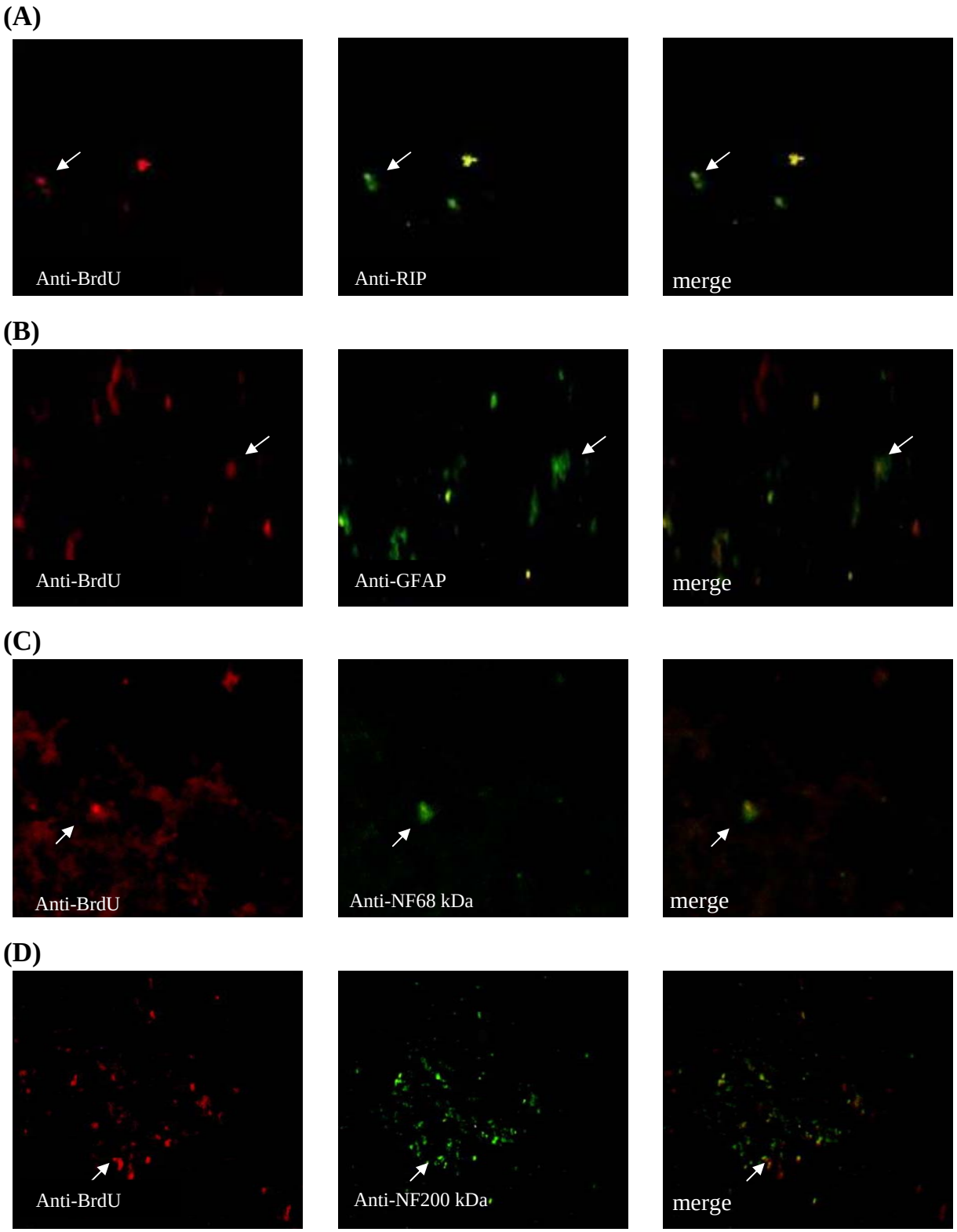

Fig. 3. Photomicrographs that show the double immunostaining on the spinal cord slides 4 weeks after intravenous administration of BMSC. Arrows indicate BrdU-positive cells that expressing rat insulin promoter (RIP) **(A)**, glial fibrillary acidic protein (GFAP) **(B)**, neurofilament-68 kDa **(C)** and neurofilament-200 kDa (NF200) **(D)**. A-C (×400) and D (×200).

been proposed. Some investigators believe that transplanted BMSC facilitate recovery of spinal cord lesions by secretion of many factors including neurotrophic factors promoting tissue plasticity [14], brain natriuretic peptide and other vasoactive factors [18] as well as protective factors that prevent neuronal apoptosis [19]. In contrast, some of the investigators believe that transplanted BMSC regenerate the injured spinal tissue by forming bundles and supportive matrix on guide regenerative neuropil through the spinal cord lesion [15, 20] and by differentiating into neurons and glial cells which are replacing destroyed endogenous cells [21].

Several reports demonstrated that BMSC are multi-potent cells which can be induced *in vitro* to differentiate into glial cells and neuron under specific experimental condition [22], and *in vivo* after transplantation [23]. Our data indicate that a small percentage of intravenous transplanted BMSC in injury site express protein phenotypic of neuron and glial cells. Also, Mahmood *et al.* [9] reported that intravenously transplanted BMSC preferentially were entered and migrated into parenchyma of the injured brain and expressed the neuronal and glial markers. On the other hand, the investigation of cell fate after BMSC transplantation with intraventricular route [6] did not reveal any differentiation. Moreover, the intraspinal injected BMSC displayed only the glial markers, which is supported with the findings of other reports employing similar procedures [20, 23].

Chopp *et al.* [14] reported both neuronal and glial differentiation of intraspinal transplanted BMSC. These differences in cell fate after transplantation, may be related to subtle differences in culture condision before grafting or the local environment into which the BMSC were introduced [20], or may require activation by specific exogenous factors [24].

Differentiation of transplanted BMSC to neuron and glial cells suggests that the cells participate probably in the partial repair of injured spinal cord and behavioral recovery. However, no irrefutable data exist regarding the physiological function of this cellular graft [23]. Moreover, some investigators believe that these relatively few differentiated cells are insufficient to provide adequate replacement of tissue [1].

In addition, because of functional benefits are detected a few days after treatment and just a small proportion of transplanted cells expressed proteins phenotypic of parenchymall cells, the believe that the enhanced recovery from neurological deficits is mediated in part by growth factors secreted by BMSC *in situ* is supported.

On the other hand, Ohta *et al.* [6] reported that although injected BMSC through intraventricular route did not differentiate into neurons and glial cell, they achieved the behavioral improvement; these investigators suggested that BMSC can exert effects by producing some trophic factors into the cerebrospinal fluid.

Chopp *et al.* [25] believe that homing in BMSC to site of cerebral injury via intravenous route, is reminiscent of the response of inflammatory cells to injured tissue. Following SCI, the vascular permeability was revealed to increase around the area of injury [26], which may contribute to the migration of BMSC into the injured spinal cord [27]. In summary, our findings suggest that intravenous infusion of BMSC, similar to intraspinal injection, provide therapeutic benefits after spinal cord injury; while it can achieve the benefits without sever surgical infliction; and this method can be employed as a renewable source for replacing lost cells for the treatment of SCI [28].

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