

Original Article

Evaluation of Mesenchymal Stem Cells and Granulocyte Colony Stimulating Factor in Treatment of Complete Spinal Cord Injury

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Abstract

Objective: Spinal cord injury (SCI) mostly affects the young and has no definite treatment. There is a growing body of literature showing the efficacy of stem cells or growth factors in the treatment of partial SCI, but a few studies demonstrated complete SCI recovery. Thus, the aim of the present study was to assess the efficacy of Mesenchymal Stem Cells (MSCs) or Granulocyte Colony Stimulating Factor (GCSF) therapy in the complete SCI recovery.

Material and Methods: 45 Rats were divided into 5 groups (GCSF, MSCs, GCSF + MSCs, control, and sham) and underwent SCI (except the sham group) using aneurysmal clip compression. Each group received its relevant therapy.

Results: After induction of SCI, there was a non-significant increasing trend in (Louisville Swim Scale) (LSS) score in all the treated groups, notwithstanding a plateau in the recovery in the sham group. No significant difference was found in the locomotor recovery among the groups.

Conclusion: The evidence from the present study indicates that GCSF and MSCs therapy fail to recover SCI.

Keywords: Spinal cord injury, Mesenchymal stem cells, Granulocyte colony stimulating factor

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Introduction

Spinal cord injury (SCI), is a disabling condition mostly affecting the young. There is no definite cure for SCI yet. Despite the fact that SCI is fatal, some degrees of spontaneous recovery after SCI have recently been reported [1]. SCI has two steps: the primary injury is caused by a mechanical force to the nervous tissue resulting in direct damage and a secondary insult which aggravates the condition and is mediated by inflammation. The secondary damage stems from various factors, including glutamate release, ion imbalance, lipid peroxidation, disturbance of pulsatile hydrodynamics, inflammatory processes, and ischemia [2]. More recently, attentions have been scattered on neuroprotection and spinal cord repair in the treatment of SCI. Administration of high-dose MPSS (methyl prednisolone) has been shown to be a standard treatment for acute SCI; yet, its

side effect in the respiratory and digestive systems is a major concern [3]. Stem cells and many growth factors such as neurotrophins have shown different rates of success in the treatment of SCI [4].

It has been postulated that stem cells can recreate the spinal cord leading to a stable functioning in neural tissues. Transplantation of Schwann cells [5], embryonic stem cells [6], macrophages [7], neural stem cells [8], olfactory ensheathing cells [9], choroid plexus [10], epidermal neural crest stem cells [11], and bone marrow stromal cells (BMSCs) has thus far been investigated for the treatment of SCI [12]. In addition, transforming growth factor (TGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), upregulated expression, restricts excitotoxicity in central nervous system (CNS) injury [13].

Moreover, in experimental models, transplantation of human Mesenchymal Stem Cells (MSCs) has been found to improve functional recovery after SCI [14]. MSC transplantation may be an alternative pathway of macrophage activation and functional recovery after SCI [15]. Intravenous administration of MSCs derived from bone marrow after contusive SCI improved functional outcome [16]. Granulocyte Colony Stimulating Factor (G-CSF) is one of the few clinically approved growth factors. *In vivo* and *in vitro* experimental studies demonstrated neuroprotective role of G-CSF [17]. G-CSF, which is a 19.6 kDa glycoprotein, is commonly utilized for treatment of neutropenia [18] and is present in mesothelial cells, monocytes, fibroblasts, and endothelial cells. Its receptors exist on precursor and mature neutrophilic granulocytes, monocytes, platelets, and endothelial cells. G-CSF stimulates neutrophilic granulocyte precursors growth at the myeloid progenitor cell level [19] and regulates mature neutrophils survival through inhibition of apoptosis [20]. It also possesses a strong anti-apoptotic property in mature neurons activating cell survival pathways. Expression of G-CSF and its receptors occurs in the CNS and is induced by ischemia indicating an autocrine protective signaling mechanism. Furthermore, adult neuronal stem cells express G-CSF receptors, and G-CSF induces neuronal differentiation *in vitro*. G-CSF can cause a long-term behavioral outcome cortical ischemia, which is due to stimulating neural progenitor response *in vivo* [21]. G-CSF also causes sensory and motor recovery in ICH (intracerebral hemorrhage) by preventing brain edema, inflammation reaction, and perihematoma cell death [22]. Moreover, G-CSF exerts angiogenic effects. G-CSF accelerates angiogenesis in animal models of limb, myocardial [23] and cerebral ischemia [24]. Vascular surface area, vascular length, vascular branch points, number of bromodeoxyuridine-labeled endothelial cells, and endothelial nitric oxide synthase and angiopoietin 2 (ANGPT2) expression grow in the focal cerebral ischemia model in G-CSF-treated rats [25]. G-CSF augments plasma levels of vascular endothelial growth factor (VEGF) from neutrophils *in vivo*, in parallel with a growth in the number of circulating neutrophils [26]. VEGF pathway blockade prevents G-CSF-induced angiogenesis, indicating that VEGF helps G-CSF-induced angiogenesis in the murine ischemic hind limb model [26]. SCI treatments have been investigated by different animal models of SCI including weight drop model, aneurysmal clip compression, feeding artery ligation and balloon compression [27]. Three different methods of application of stem cells and growth factors, i.e. intrathecal [28], intravenous [16] and intraspinal [29] transplantations, have far been developed in animal models of SCI. Intraleisional model transplants most cells in injury site; however, it is invasive and requires one more anesthesia and surgery in the lab animal. Despite the fact that intravenous model is the easiest method in this regard, the least cells are transplanted in the injury site. Intrathecal injection is intermediately invasive with an intermediate number of the cells being transplanted in the injury site. Stem cell therapy has been studied on partial SCI; however, little is known about its efficacy on complete SCI. Hence, in present study, we evaluated intrathecal MSCs and G-CSF in the treatment of complete SCI induced by compressing the cord with aneurysmal clips in rats.

Materials and Methods

Forty-five female Wistar rats (250 to 300 grams) were divided into 5 groups (15 animals each) of GCSF, MSCs, GCSF + MSCs, control, and sham. To assess the treatments, Louisville Swim Scale (LSS) score was calculated [30]. LSS score uses five modalities including fore limb dependency, hind limb movement, hind limb alteration, body angle, and trunk instability. The total score illustrates the locomotor in the animal. In order to decrease distress, all rats underwent training in a maze special for swimming for 1 week before the surgery so that all learned to reach to the end. On the same day and before the surgery, the LSS score was recorded as a base-line score (LSS0) for each rat. Surgery was done under general anesthesia using ketamine (60 mg/kg) and xylazine (5 mg/kg) cocktail. The hairs on thoracic area of the animals were shaved and prepped by povidone iodine and draped. A longitudinal incision was made on the lower thoracic vertebrae with a length of nearly 2 cm. The 10th thoracic vertebra was found and the paraspinous muscle was dissected to expose the laminae. Laminectomy was performed using small rongeurs bilaterally and the cord was thoroughly exposed. This was all done for the sham group as well, and after washing the wound, it was closed in separate layers. The rats in the other groups underwent injury to their cords by means of compression with aneurysmal clips. The clip was advanced in lateral to medial direction to stand the blades adjacent to the anterior and posterior surfaces of the cord. Thus, closing the clip compressed all the cross-sectional area of the cord. The clips leaved closed for three minutes after which they were removed and the wounds were washed and closed. To prevent chewing of the wounds by the other rats in cages, saturated picric acid was used around the wounds [31]. The rats were kept on a heating pad until full recovery and placed in their cages. On the day after surgery, all the animals received intra-muscular ceftriaxone and vancomycin injections for the next two days. The intervention for each group was accomplished in the first day after the surgery. The rats in the sham group underwent lumbar punctures (LP) as previously described [32] without intrathecal injections. The control rats received a sterile water injection (0.1 cc). The MSCs group received an injection (0.1 cc) of a suspension of cells with 500,000 cells per milliliter density. MSCs, previously confirmed by flow cytometry analysis, were provided by stem cell bank of cellular and molecular research center of Guilan University of Medical Sciences. The GCSF group received an injection (0.1 cc) of GCSF (10 micrograms). The GCSF + MSCs group received an injection (0.1 cc) containing relevant cells with the same density and GCSF (10 micrograms). Daily care included emptying the bladders manually by means of the Crede maneuver (by compressing the bladders by finger until it empties). Saturated picric acid was used for autophagia of lower extremities [31]. The 5 groups were kept in separate cages in an animal room with food and water *ad libitum* with a room temperature of 24 °C and a light and darkness interval of 12 hours. Further recordings of the LSS were done at Weeks 1, 3, 5, and 7 after surgery (LSS1, LSS2, LSS3, and LSS4, respectively). Data management and analysis were done using SPSS (version 21). The present cross-sectional study was approved by ethics committee of Guilan University of Medical Sciences.

Results

Dead animals were excluded from the study and finally 9, 11, 7, 10, and 14 rats remained in the GCSF, MSCs, GCSF + MSCs, control, and sham groups, respectively. Comparison of LSS0 and LSS1 showed that there was a significant decrease in the score in all the groups except the sham group, showing that the injury was successfully made by compressing the cords, but the laminectomy alone did not result in SCI. Thereafter, the rats in the intervention and control groups showed a slow improvement in the LSS scores during 7 weeks (from LSS1 to LSS4), although this improvement was not statistically significant in neither groups (Figure 1). Furthermore, there were no significant differences in recovery amongst GCSF, MSCs, GCSF + MSCs, and control groups ($p \geq 0.05$).

Discussion

We previously showed that some degrees of spontaneous recovery occurs after SCI in rats, especially in those with partial SCI [1]. In the present study, the drop in LSS score after the surgery (LSS0 and LSS1) showed that the injury was made successfully. The laminectomy, on the other hand, was unable to make injury per se. Moreover, the animals experienced a non-significant recovery, albeit it is worth mentioning that the incensement in the LSS score of the rats were never due to using their hind limbs, and upgrades resulted from a better stability in their trunks or their body angles related to the water surface.

None of the interventions in the present study showed significant recovery after SCI induction and there were no significant differences in the recovery among different groups. Several studies have evaluated the efficacy of stem cells or growth factors in the treatment of SCI [4]; however, the techniques applied for induction of SCI differed from that used in the present study. Most studies used the weight drop model

to induce SCI, but it resulted in a partial lesion in the cord. The aneurysmal clip model was used to make complete cord lesions in the present study. Aneurysmal clips are used as an option for aneurysm treatment [33], however, it can be used for compressing the cord, a well-known model of SCI [29]. In this model, the cord is compressed directly at its anterior surface, where the motor segment exists and it is allegedly mimics human SCI to a large extent [27]. Occluding the anterior spinal artery by the clips is another mechanism in which ischemia is superimposed to contusion by clip compression.

Bone marrow MSCs provide promising approaches to treatment of CNS dysfunctions because of their ease of collection, readily genetic manipulation, fast proliferation, and their potential to be used as autografts [34]. Transplantation of MSCs into the SCI has been suggested for therapeutic benefits [35]. By way of illustration, MSCs were used in several models of the central nervous system injuries such as traumatic brain injury, traumatic SCI, and ischemic stroke. Evidence from literature suggests that MSCs may improve functional recovery after SCI [14-16]. In spite of the fact that differentiation of MSCs into cells of neural lineage both in vitro and in vivo has been suggested, it may not be a key element of functional recovery after brain injury or SCI. Neuroprotection, expression of growth factors or cytokines, provision of a regenerating environment, vascular effects, and remyelination are the other possible explanations of SCI recovery with BMSC transplantation and it is presumed that more than one of these mechanisms is involved in the functional recovery. Although MSCs have suggested for treatment of SCI, they may exert side effects and can be contraindicated in patients with a history of myelitis [28].

In the normal and ischemic brains, G-CSF was demonstrated to enhance migration and differentiation into neurons of bone marrow-derived cells [36]. On the other hand, G-CSF-mediated mobilization of bone marrow-derived cells may help spinal cord

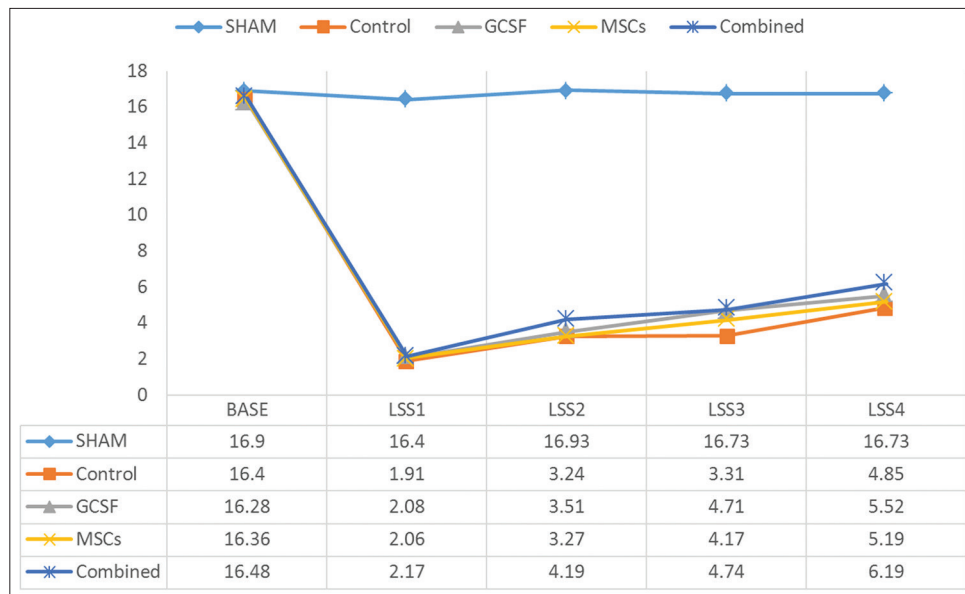


Figure 1. Locomotor scores in GCSF, MSCs, Combined (GCSF+MSCs), Control, and Sham groups (Base=before surgery, LSS1: LSS score measured a day after surgery, LSS2: LSS score measured 3 weeks after surgery, LSS3 LSS score measured 5 weeks after surgery, LSS4: LSS score measured 7 weeks after surgery).

tissue restoration [37]. G-CSF was shown to activate the JAK-STAT signaling pathway, decrease levels of the pro-inflammatory cytokine, tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and IL-8, and increase the levels of IL-1 receptor antagonist [38]. G-CSF has anti-inflammatory and immunomodulatory effects and inhibits the overproduction of pro-inflammatory cytokines from lipopolysaccharid (LPS)-stimulated monocytes [39]. It may thus inhibit pro-inflammatory cytokine toxicity, neutrophil activation and infiltration. It was also shown that, in the astrocytes, G-CSF inhibited the spinal cord ischemia-induced activation of p38. Furthermore, it resulted in the up-regulation of Akt and ERK activities in the neuronal cells. Thus, the potential neuroprotective property of G-CSF in neuronal injury is likely be mediated by its anti-inflammatory and anti-apoptotic influences upon the astrocytes and neuronal cells, respectively. SCI recovery depends on the pre-interventional neurological status of the patients. Despite therapy, complete SCI may not show significant recovery. However, partial injuries, may have a better recovery [40].

Conclusion

The findings from the present study suggest that MSGs and G-CSF fail to cure complete SCI in rats. Despite recent advances of stem-cell therapy in complete SCI, there are still concerns about its efficacy, and most of the available treatments can be applied for the recovery of partial SCIs.

Conflict of interest

The authors declare that there is no conflict of interest with regard to the present study.

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