Int J Clin Exp Pathol 2015;8(8):8976-8982
www.ijcep.com /ISSN:1936-2625/IJCEP0009644

Original Article

Neuroprotective effect of methylprednisolone combined with placenta-derived mesenchymal stem cell in rabbit model of spinal cord injury

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Received April 27, 2015; Accepted June 22, 2015; Epub August 1, 2015; Published August 15, 2015

Abstract: The aim of this study was to assess the ability of the combination treatment of methylprednisolone (MP) and placenta-derived mesenchymal stem cells (PDMSCs) in a rabbit model of spinal cord injury (SCI). Rabbits were randomly divided into four groups: group 1 (control), group 2 (MP), group 3 (PDMSCs) and group 4 (MP + PDMSCs). In all groups, the spinal cord injury model was created by the weight drop method. Levels of malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined by kit. Histopathological examination was also performed. Neurological evaluation was carried out with the Tarlov scoring system. The results showed both MP and PDMSCs had neuroprotective effects, and combining the administration of MP with PDMSCs was shown a significant effect on the recovery of neurological function. Therefore, the combined use of MP and PDMSCs can be used as a potential therapeutic method for SCI.

Keywords: Neuroprotective, methylprednisolone, placenta-derived mesenchymal stem cell, spinal cord injury

Introduction

Spinal cord injury (SCI), one of the most disabling diseases, negatively affects the quality of life of patients and their families [1]. Primary trauma to spinal cord initiates a cascade of cellular and biochemical events, which lead to severely disturb motor, sensory, and autonomic functions [2, 3]. Traumatic SCI has become one of the worldwide clinical problems, its burden both for the patient and the health care system [4].

The neurological damage at the time of insult is called “primary injury”. Following the primary injury, free radical damage caused by biochemical changes associated with lipid peroxidation to cause additional deterioration of the original area is called “secondary damage” [5-8]. Oxidative stress, inflammation and lipid peroxidation appear to be the most important mechanisms that cause neuronal damage after SCI [9]. The biochemical and pathophysiologic changes after SCI have been investigated in order to develop treatments that may minimize function loss.

A variety of pharmacological agents have been used to prevent secondary damage after experimental injury. The growing evidences have demonstrated that mesenchymal stem cells provided obvious effect in the treatment of nervous system dysfunctions [10, 11]. The interest in placenta-derived mesenchymal stem cells (PDMSCs) is currently growing. Previous studies have shown that, PDMSCs loaded on the human amniotic membrane were beneficial for the treatment of radial nerve injury and promoted the healing of tendon grafts in the bone tunnel [12, 13].

Many neuroprotective drugs have been evaluated to minimize injury during the second phase of SCI, only methylprednisolone (MP) has been shown to provide benefit in large clinical trials [14-18]. In the present study, we assessed the effectiveness of MP combined with PDMSCs in a rabbit model of SCI, to assess this as a novel

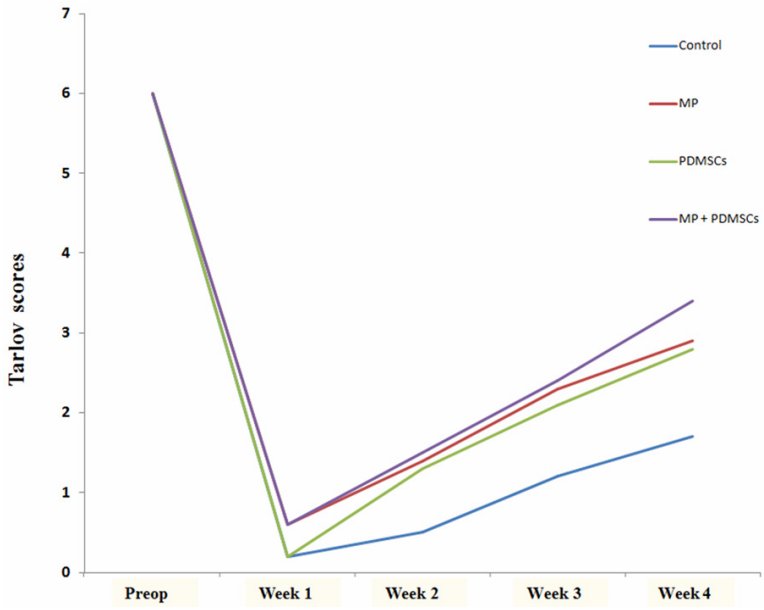

Figure 1. Tarlov score results in each study group.

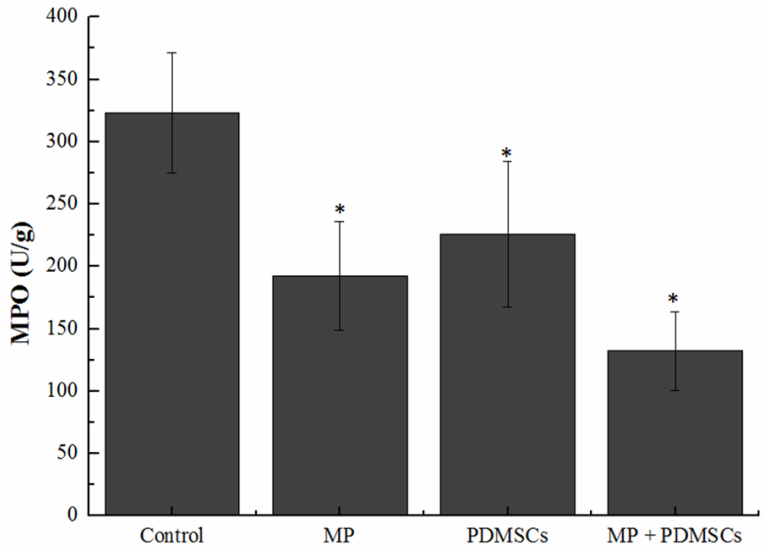

Figure 2. Tissue MPO levels in study groups. *$P < 0.01$ vs. Control group.

method for the clinical treatment of spinal cord injury.

Materials and methods

Materials

MP was obtained from Chengdu Must Biotechnology Co., Ltd. (Chengdu, China), PDMSCs were purchased from Wuhan Procell life technology Co. Ltd.

Animals

Adult female New Zeland rabbits (n = 40) weighting (2500-3500 g) were obtained from Wuxi Jiangnan technology Co. Ltd. (SPF grade, Certificate No. SCXK20150004).

Ethics statements

All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures in this study, including the use of animals, were approved by the Institutional Animal Care Committee of Binzhou Medical College.

Spinal cord injury model

All animals were anesthetized with xylazine (10 mg/kg) and ketamine hydrochloride (80 mg/kg). Laminectomies were performed at the T8-T10 level. Spinal cords were injured using the weight drop technique according to Allen's method [19]. A stainless steel rod (10 g, 3-mm diameter tip) is dropped through a height of a 10 cm to the center of the spinal cord. After the surgical and traumatic interventions, the wounds were closed in layers with silk sutures.

Experimental groups

All the rabbits were randomly divided into the following four groups (10 rabbits in each group):

Group 1: Control group, the rabbits received the equal volume normal saline (0.9% NaCl) every day. No other treatment was given to this group.

Group 2: MP group, animals received a single high dose of MP (60 mg/kg) intravenously immediately after trauma. After 4 h, the rabbits

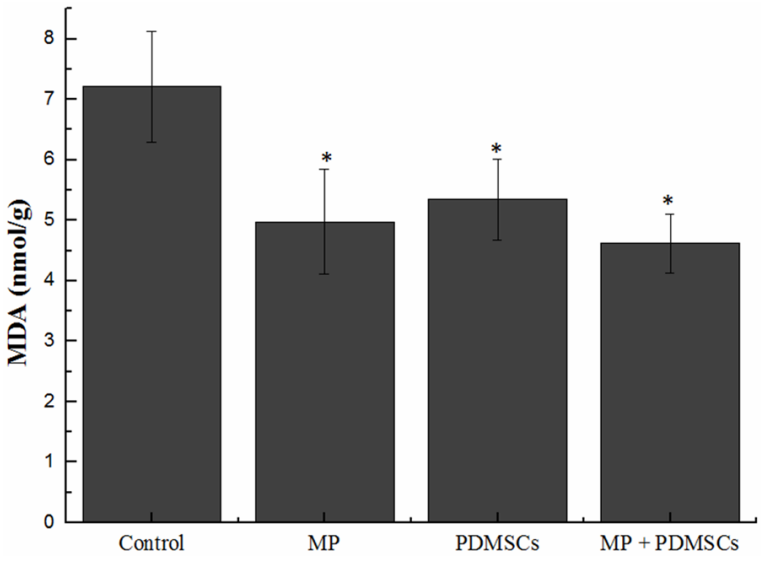

Figure 3. Tissue MDA levels in study groups. *$P < 0.05$ vs. Control group.

received intravenous 30 mg/kg dose of MP again. Then, the rabbits received intravenous 30 mg/kg dose of MP twice a day, for consecutive 3 days [20, 21].

Group 3: PDMSCs group, 6 days after creating the spinal cord transection injury, 0.2 ml 1 × 10^6 cells/ml of cell suspension was injected into the SCI rabbits using a micro syringe.

Group 4: MP + PDMSCs group, treated similarly to group 2, but after 6 days, 0.2 ml 1 × 10^6 cells/ml of cell suspension was injected into the injured spinal cord using a micro syringe.

Neurological evaluation

Neurological functions were assessed before surgery and at 1, 2, 3, 4 weeks after surgery. Neurological situation was assessed using Tarlov scoring system [22]. A score from 0 to 6 was assigned to each animal as follows: score 0, spastic paraplegia and no movement of the lower limbs; score 1, spastic paraplegia and slight movement of the lower limbs; score 2, good movement of the lower limbs, but inability to stand; score 3, able to stand but unable to walk; score 4, able to walk, but not lasting; score 5, able to walk, but unable to run; score 6, complete recovery of hind-limb function.

Biochemical analysis

After neurological evaluation, rabbits were killed with overdose pentobarbital. Trauma site being at the epicenter, 2 cm length spinal cord segments were removed en block. Tissue samples were immediately stored at -80°C. On the day of analysis, the tissues were homogenized in physiologic saline solution and centrifuged 1780 × g for 20 min at 4°C. The supernatant was collected and quantitatively assayed for the levels of MPO, MDA, SOD, GSH-Px and CAT. The detection of these substances used ELISA kits according to the manufacturer's instructions (Nanjing Jiancheng Co.).

Histopathological evaluation

The cord specimens obtained at 4 week post-injury were prepared for histological study. Each cord segment was immersed in 4% formaldehyde for 72 h. The specimens were then embedded in paraffin wax, cut into 5 μm thick sections and stained with hematoxylin and eosin (H&E). The specimens were examined under a light microscope, blinded to the study for congestion, hemorrhage, edema, necrosis, and neuronal viability.

Statistical analysis

Statistical analysis was performed by using SPSS 17.0. Statistical comparisons between the groups were tested with the Kruskal-Wallis test, and the Mann-Whitney U test was used for dual comparisons. In each test, data were reported as the mean ± SD, and $P < 0.05$ was considered to indicate statistically significant.

Results

Neurological evaluation

Figure 1 demonstrates Tarlov score changes throughout the experiment. There was no statistically significant difference between groups before SCI surgery. However, postoperative neurological evaluations indicated that all groups showed severe neurological deficits. The Tarlov score of the PDMSCs group was significantly higher than that of the control group ($P < 0.01$). The Tarlov score of the MP group

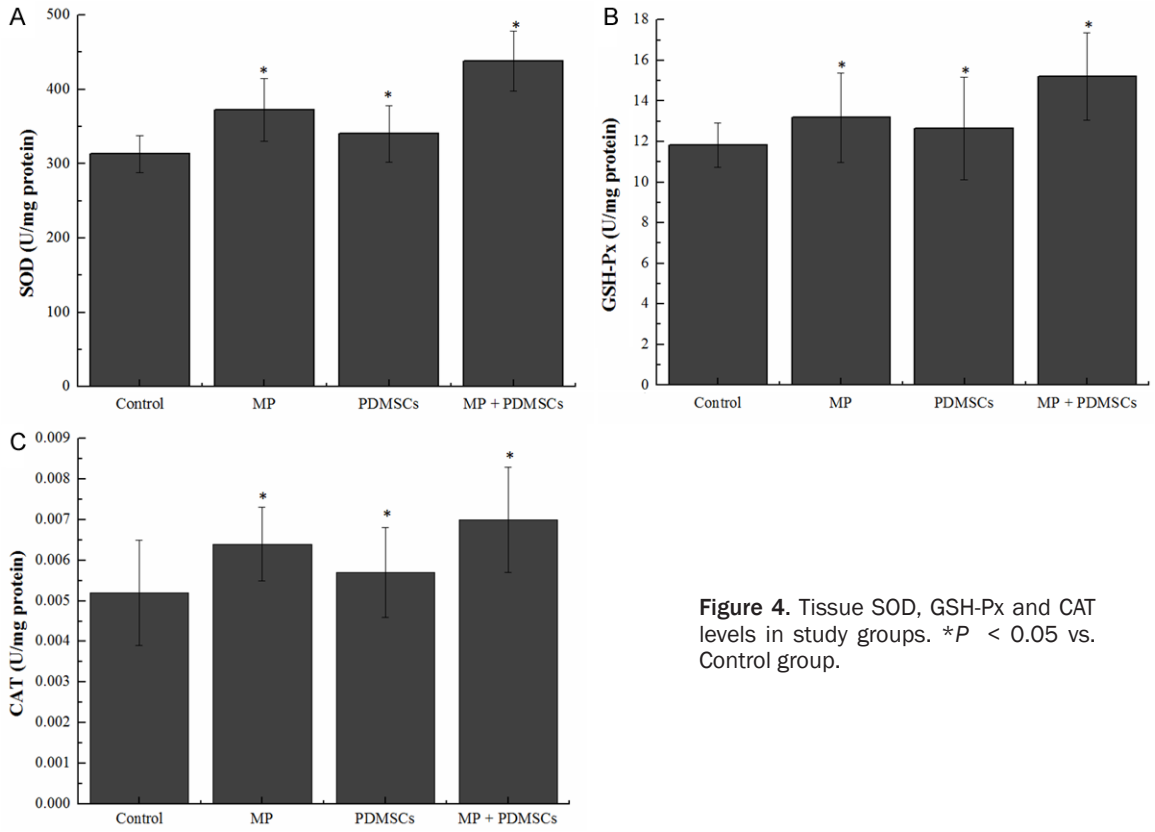

Figure 4. Tissue SOD, GSH-Px and CAT levels in study groups. *P < 0.05 vs. Control group.

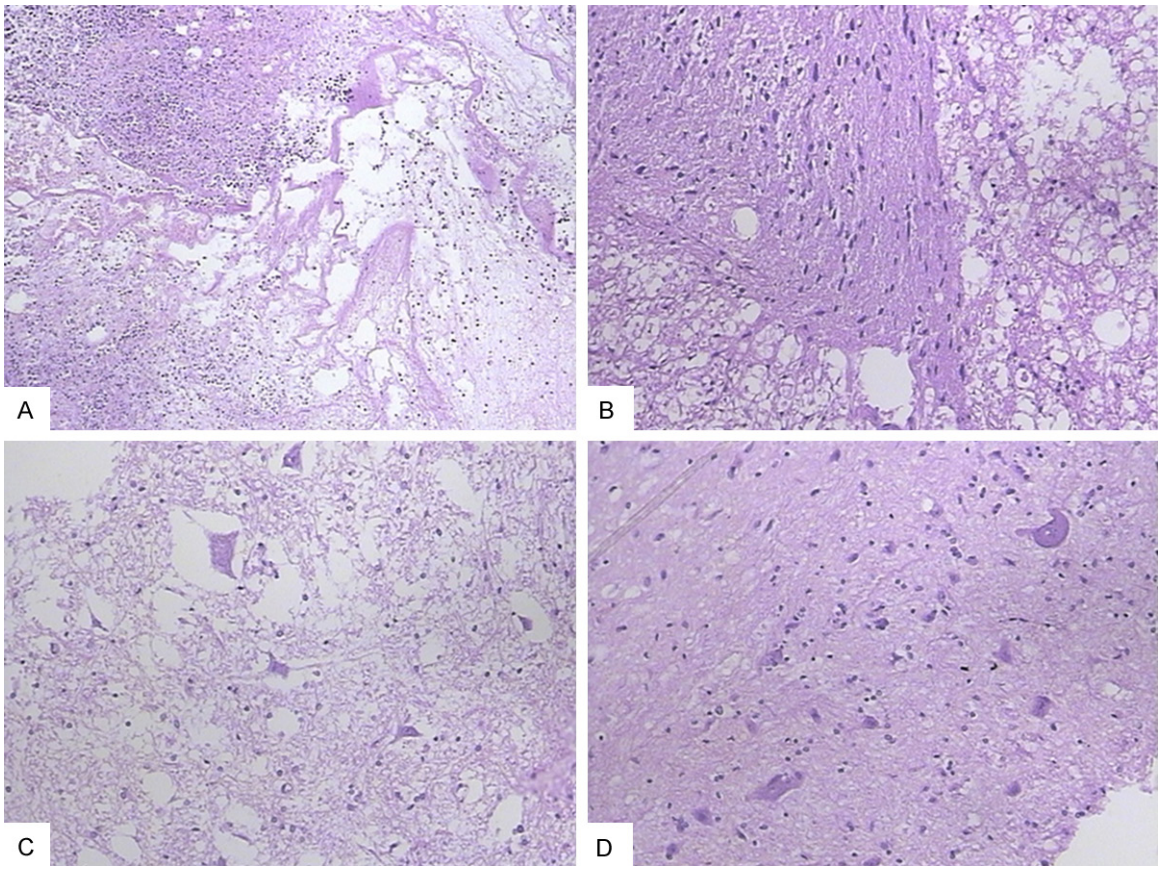

Figure 5. Histopathological photomicrographs of spinal cord tissue. A. Control group (He x100). B. PDMSCs group (He x200). C. MP group (He x200). D. MP + PDMSCs group (He x200).

was also significantly higher than that of the control group ($P < 0.01$). Meanwhile, the combined use of MP and PDMSCs has the best result in neurological evaluation.

Tissue MPO analysis

Statistically differences were observed between the control and the treatment groups with regard to the tissue MPO level (**Figure 2**). Treatment with PDMSCs led to a statistically decrease in the MPO level in tissue compared with the control group ($P < 0.01$). Similarly, the MP group also showed statistically decline in the MPO level in tissue compared with the control group ($P < 0.01$). However, the MP + PDMSCs group has the most obvious effect in the decrease of the MPO level.

Tissue MDA analysis

When the tissue MDA levels were compared between the groups, statistically significant differences were observed ($P < 0.05$). The MDA level was significantly higher in the control group compared to the other groups. As shown in **Figure 3**, the treatment of PDMSCs or MP kept MDA at a lower level than control group. However, MP + PDMSCs controlled MDA better than just PDMSs or MP alone.

Tissue SOD, GSH-Px and CAT analysis

As shown in **Figure 4**, the SOD, GSH-Px and CAT levels were lowest in the control group and the highest in the PDMSCs + MP group. Treatment with MP or PDMSCs attenuated the decline in the level of SOD, GSH-Px and CAT. However, the effect of combined therapy is better than MP or PDMSCs single treatment.

Histopathological evaluation

Figure 5 showed representative imagings of H&E stained cord specimens of all groups. Hemorrhage, edema, and neuronal viability parameters were significantly decreased in PDMSCs, MP, and MP + PDMSCs groups compared with those in control group. Between the treatment groups, the best significant decrease in congestion, hemorrhage, edema, and neuronal viability parameters was achieved in MP + PDMSCs group.

Discussion

SCI pathophysiology is very complex, involving in histopathological, electrophysiological and chemical changes in affected cells and tissues [23, 24]. It's characterized by large lipid content and high oxygenation, which are easily damaged by free-radical induced lipid peroxidation. Several endogenous antioxidant enzymes, such as SOD, GSH-Px and CAT, can resist oxidative damage. Protecting the spinal cord from oxidative damage using antioxidant therapies is an important investigation in SCI. Therefore, many pharmacological agents have been used in experimental spinal injury. Among them, only MP has been shown to provide benefit in large clinical trial [25]. Our study also confirmed that MP significantly ameliorated the impairment of SCI.

With the progression of stem cell research, stem cell transplantation has been suggested as a potential method for restoring SCI. PDMSCs have a strong ability to proliferate and a low immunogenicity, which contribute to their strengths as a novel seed cell for the treatment of SCI. Therefore, in this study, MP combination with PDMSCs in a rabbit model of SCI to assess this as a novel method for the clinical treatment of SCI.

MPO activity is a reliable marker of neutrophil infiltration to the injured tissue, and is associated with the number of neutrophils infiltrating the spinal cord and their activity [26]. We demonstrated that SCI injury caused a significant increase in MPO activity in spinal cord tissue. While the MP + PDMSCs treatment SCI rabbit significantly decreased MPO activity.

Oxidative damage plays an important role in the neuronal damage after SCI. There are some enzymes in cellular protection against damage from oxygen-derived free radicals, such as SOD, GSH-Px and CAT. SOD is an enzyme extensively used as a biochemical indicator of pathological states associated with oxidative stress [27]. GSH-Px acts as an enzymatic antioxidant

both intracellularly and extracellularly in conjunction with various enzymatic processes that reduce hydrogen peroxide (H_2O_2) and hydroperoxides [28]. CAT, an enzyme that plays an important role as part of the system for reactive oxygen species (ROS) detoxification converts H_2O_2 into harmless byproducts [29]. MDA is the end-product of the oxygen-derived free radicals and lipid oxidation, which reflects the damage caused by reactive oxygen species [30]. We found that in the experiment, with MDA significantly increased after SCI, SOD, GSH-Px and CAT significantly reduced, suggesting that lipid peroxidation in damage localization increased, it is also the result of increased free radicals. However, MP or PDMSCs treatment could ameliorate these abnormalities, and the effect of combined therapy was better than MP or PDMSs sole treatment.

Moreover, the weight drop method caused paraplegia in all animals. Both MP and PDMSCs treatments protected spinal cord from injury and improved neurological function as determined by Tarlov scores, and the combined use of MP and PDMSCs has the best result in neurological evaluation.

Histopathological evaluation includes congestion, hemorrhage, edema, and neuronal viability parameters. Hemorrhage, edema, and neuronal viability parameters were significantly decreased in treatment groups compared with those in the control group. Between the treatment groups, the best significant decrease in congestion, hemorrhage, edema, and neuronal viability parameters was achieved in MP plus PDMSCs group.

In conclusion, we reported a treatment for SCI with MP combination with PDMSCs, which can significantly improve nervous system dysfunctions. The effects of MP + PDMSCs were first investigated in the current study. The results imply that the combined use of MP and PDMSCs can be a novel method for the clinical treatment of spinal cord injury.

Acknowledgements

This work was supported by Shandong Province Outstanding Young Scientist Research Award Fund (No. BS2010SF029).

Disclosure of conflict of interest

None.

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