AC-YVAD-CMK Inhibits Caspase-1-mediated Imflammatory Response to Improve Bone Marrow Stromal Cells on Neuroprotection After Sci in Rats

Xiangtong Zhang
First Affiliated Hospital of Harbin Medical University
Faling Qu
First Affiliated Hospital of Harbin Medical University
Aili Gao
Northeast Agricultural University
Yonghua Li
Sunshine Union Hospital
Yue Shi
First Affiliated Hospital of Harbin Medical University
Chenfei Jin
Fourth Affiliated Hospital of Harbin Medical University
Guoxu Zhu
First Affiliated Hospital of Harbin Medical University
Xin Chen
First Affiliated Hospital of Harbin Medical University
Yu Long
First Affiliated Hospital of Harbin Medical University
Zhenfeng Jiang
First Affiliated Hospital of Harbin Medical University
Kongbin Yang
First Affiliated Hospital of Harbin Medical University
Hongsheng Liang (lianghongsheng11@163.com)
First Affiliated Hospital of Harbin Medical University https://orcid.org/0000-0002-8662-939X

Research

Keywords: Spinal cord injury, AC-YVAD-CMK, Bone marrow stromal cells, caspase-1

Posted Date: May 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-31277/v1

Abstract

Background: Stem cell transplantation maybe an advantaged method for curing spinal cord injury (SCI). However, the adverse environment of the injured area makes the treatment effects on transplantation unsatisfactory. The aim of this study was to investigate whether AC-YVAD-CMK could change enviroment by inhibiting caspase-1-mediated imflammatory response to improve Bone marrow stromal cells (BMSCs) transplantation on neuroprotection after SCI.

Methods: Firstly, BMSCs were prepared and cultured *in vitro* and the SCI rat model was constructed. Then, AC-YVAD-CMK and BMSCs were injected into the SCI model at the same time. Western blot and ELISA were used to detect the production of inflammatory factors and neurotrophic factors in the injured area, meanwhile immunohistochemistry was employed to clarify the effect of AC-YVAD-CMK on BMSCs transplantation. Finally, we evaluated the recovery of limb motor function by BBB score in SCI rats.

Result: In the AC-YVAD-CMK + BMSCs group, the active caspase-1, IL-18 and IL-1β expressed less than those in the BMSCs group or AC-YVAD-CMK group at 1 week after SCI ($P < 0.01$). Meanwhile, increasing brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) levels, the PKH26 labeled BMSCs with some being neurofilament protein-200 (NF-200)-positive were still observed in the contusion site. Furthermore, the restoration of motor connections across the injury sites was positively correlated with the recovery of spinal cord function in cotreatment group ($P < 0.01$).

Conclusions: These results show that caspase-1-mediated inflammatory response is inhibited by AC-YVAD-CMK to enhance BMSCs transplantation to improve neuroprotection after SCI.

Introduction

Spinal cord injury (SCI) refers to complete or incomplete spinal cord motor, sensory, sphincter and autonomic dysfunctions resulted from the violent attack on spinal cord [1]. Epidemiological data show that the incidence of SCI is approximately 54 cases per million people in the United States, or approximately 17,000 new SCI cases each year. Vehicle crashes are currently the leading cause of injury followed by falls, acts of violence, and sports/recreation activities, according to the National Spinal Cord Injury Statistical Center [2]. It is one kind of devastating disease in orthopedics and causes serious physiological and psychological damage to the patients [3-5]. Researchers found that the final neurological damage of SCI is caused by two mechanisms, namely primary injury and secondary injury. Primary injury refers to tissue damage caused instantly by the mechanical force in spinal cord tissue, and the resulted nerve damage is irreversible; a series of self-destruction processes in which pathological factors aroused by primary injury participate are called secondary injuries, of which the evolution lasts up to a few hours to a few weeks [6]. This is an active adjustment process of the cell and molecular levels, reversible and able to be controlled. Up to date, there are many therapies to treat SCI, such as, inhabiting inflammation response, transplanting stem cells [7-9]. Unfortunately, there are still no effective measures for repair of the damaged spinal cord.

As we all know, caspases are cysteine proteases that are key effector enzymes of apoptotic cell death [10]. They can be activated by either internal or external stimuli to the cell. Activation of caspase-1 initiates a complex cascade of enzymes and molecules, terminating in a final pathway for cell death [11]. And caspase-1, previously known as IL-1β-converting enzyme, is a critical mediator of apoptosis after not only Central Nervous System (CNS) insults, such as trauma and ischemia, but also in chronic neurodegeneration, including Parkinson disease and multiple sclerosis (MS) [12,13]. Moreover, AC-YVAD-CMK is an irreversible tetrapeptide inhibitor of caspase and has good cell permeability [14,15]. Although it shows selectivity for caspase-1 family enzymes, it also inhibits other caspases [16]. At the same time, related research shows that AC-YVAD-CMK can protect brain tissue in the agains intracerebral hemorrhage (ICH) induced injury by inhibiting inflammatory reaction, and can improve the neurological function after cerebral hemorrhage [13,17]. Zhang F et al.concluded that AC-YVAD-CMK attenuates acute gastric injury in mice: involvement of silencing NLRP3 inflammasome activities [18]. Moreover, NKaraoğlan A et al. found that AC-YVAD-CMK inhibites posttraumatic apoptosis in a rat SCI model [19]. Therefore, the use of AC-YVAD-CMK to inhibit caspase-1-mediated inflammatory response plays a positive role in the recovery of damaged tissues.

Meanwhile, stem cells that are capable of contributing trophic support or integrating into functional synaptic networks with host tissues are being developed for therapeutic use after SCI [20,21]. Bone marrow stromal cells (BMSCs) are considered as a kind of clinical applaction stem cells for autologous transplantation and no ethical issue [22]. And we have also confirmed, BMSCs transplantation for the treatment of SCI is feasible, because BMSCs is directionally induced to differentiate into neuron-like cell and secret neurotrophic factors [23]. But the simple effect of BMSCs transplantation therapy on SCI is limited. One of the reasons is the unsatisfying survival rate and neural differentiation of BMSCs after transplantation in vivo [24]. The extremely unfavorable environments, including activation of inflammatory processes, lack of trophic support, the presence of the glial scar, all of which are inhibitory to structural repair, may contribute to the lower survival rate of BMSCs following transplantation [25, 26]. Tan Y et al. found that blockade of interleukin 6 signaling improves the survival rate of transplanted bone marrow stromal cells and increases locomotor function in mice with spinal cord injury [27]. These results show that stem cell transplantation is an effective method to treat SCI, but the inflammatory environment after transplantation may be an important reason to limit its effect.

Therefore, we propose the hypothesis that AC-YVAD-CMK inhibited caspase-1-mediated imflammatory response to improve BMSCs transplantation on neuroprotection after SCI.

Materials And Methods

Animals

In this study, 8-10 weeks old female Sprague-Dawley (SD) rats (200-250g) were selected. The living conditions, surgical methods and postoperative care of all animals were approved by the animal experimental Committee of the First Affiliated Hospital of Harbin Medical University.

Cell isolation, culturing and labeling

Partially purified stem cell–enriched BMSCs were prepared as previously described [28]. Briefly, BMSCs were obtained from the femurs and tibias of 4-week-old female SD rats (Harbin, Heilongjiang, China) and fractionated by density centrifugation using Lymphoprep! density solution (density: 1.077; Nycomed Pharma, Oslo, Norway). After centrifugation, whole mononuclear cells were collected from the interface and extruded with 10mL of DMEM/F12. The remaining cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (Gibco BRL), 3mML-glutamine, 100U=mL penicillin, 100U=mL streptomycin sulfate, and incubated at 37°C with 95% humidity and 5% CO_2. After 48h of incubation, the nonadherent cells were removed by changing the medium. The BMSCs were passed four times before being used in the following experiments.

Before transplanting BMSCs were labeled with the red fluorescent cell tracking dye PKH26 (Sigma-Aldrich) following the manufacturer's instructions. The efficiency of cellular labeling (routinely 100%) was examined with a fluorescence microscope.

Spinal cord injury modle

The rats were anesthetized by an intraperitoneal injection with 5% chloral hydrate (30mg/kg). After adequate amounts of anesthetized, the rats were fixed on the sterile table. Using aseptic techniques, a midline incision was made in the back in the skin and musculature to expose the T9-T11 vertebrae. Then a complete laminectomy was performed on the 10th thoracic spinal cord level exposing the cord using a surgical microscope. After laminectomy, the spinal cord was compressed by placing a 50 g weight on the exposed spinal cord column for 5 min using a rectangular plate which was longitudinally oriented over the spinal cord. The plate had an area of 11.0 mm^2 (2.2×5.0 mm) and a concave shape that ensured equal distribution of the pressure on the spinal cord tissue. It was considered that the model was successful to see the spastic tail swing of rats, paralysis of upper and lower extremities after retraction and flapping of both legs and body. The wound site was rinsed with sterile phosphate-buffered saline (PBS) with 0.1% gentamicin, the muscles were sutured in layers, and the skin was closed. During the whole process, the body temperature of the rats were maintained until they woke up. After the operation, the rats were assisted to urinate 3-4 times a day until the function of urination recovered. After the operation, penicillin was injected intraperitoneally for 100 mg/kg/D × 3 D to prevent infection.

Experimental groups and intraperitoneal administration

Eighty rats were divided into four groups: (i) SCI group; (ii) AC-YVAD-CMK treatment group: intraperitoneal injection of AC-YVAD-CMK dissolved by DMSO (10mg/kg) immediately after injury, three times every other day; (iii) BMSCs treatment group: immediately after injury, $1×10^5$ BMSCs were injected into 3ul medium with microinjector to the epicenter of spinal cord contusion, and single dose DMSO with equal

volume was injected into abdominal cavity; (iiii) Combined treatment group (AC-YVAD-CMK + BMSCs): immediately after injury, inject AC-YVAD-CMK solution and BMSCs into corresponding parts.

Western blot

Immediately after the rats (n=4 for each group) were deeply anesthetized and transcardially perfused with 0.9% ice-cold saline, the spinal cord tissues around the epicenter of the lesion (10 mm in total length) were carefully isolated at the end of first week. The spinal cord tissues were digested with RIPA lysate containing PMSF and 1% protease inhibitor for 30 min and the protein concentration was determined using the BCA method and stored at −80℃. Different samples with an equal amount of protein were separated using 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. After blocked with 5% skim milk at room temperature for 1h, the members were incubated with primary antibodies against pre-caspase-1 (1:200), active-caspase-1 (1:200), IL-1β (1:100), IL-18 (1:100), and β-actin (1:1000) at 4℃ overnight. After 3 washes with TBST (Tris-buffered saline and Tween 20, PH 7.5), the membranes were appropriately incubated with secondary antibodies (HRP, 1 : 1500 dilutions) at room temperature for 2h. The images were captured by means of the Odyssey Infrared Imaging System (LI-COR, Biosciences) and the band intensities were measured with Odyssey v1.2 software (LI-COR, Biosciences).

BDNF and NT-3 assay

In the present study, we also detected the levels of brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) at the end of the first and eighth week after operation. The rats (n=4 for each group per time point) were anesthetized with 1% sodium pentobarbital (40 mg/kg) and transcardially perfused with 200 mL of ice-cold 0.1M PBS, pH 7.2. A 5-mm long segment of spinal cord containing the lesion site and substitutes was excised on dry ice. The segment was weighed and then mechanically homogenized in ice-cold 0.1M PBS. Homogenates were centrifuged for 10 min at 14,000 rpm at 4℃, and the supernatant fluid was detected with a BDNF kit (R&D Systems, Minneapolis, MN) and an NT-3 ELISA kit (Abnova, Taipei, Taiwan), according to the manufacturer's instructions. The samples were always analyzed in triplicate. Histological analysis The animals were given an overdose of Nembutal (80 mg/kg, i.p.) and were transcardially exsanguinated with 150 mL of physiological saline followed by fixation with ice-cold 4% paraformaldehyde in 0.01M PBS (pH 7.4). Spinal cords were dissected and postfixed and cryoprotected.

Immunohistochemistry

The rats (n=6 for each group) were transcardially perfused with 100ml 0.9% ice-cold saline and 100ml 4% paraformaldehyde under deep anesthesia at the end of the first and eighth week after operation. The spinal cords were harvested and fixed with 4% paraformaldehyde for 24h, then in 10% and 30% sucrose

until saturation successively and embedded them in OCT compound and sectioned them frontally at 20 mm on a cryostat. For immunofluorescence staining, the sections were blocked with 10% normal goat serum in PBST (0.1%) for 1h after pushed with PBST for 5 min × 3 times and incubated overnight at 4°C with the primary antibodies: ploYclonal anti-mouse antineurofilament protein-200 (NF-200, 1:100) to identify axons. After several pushed with PBST, the sections were incubated with FITC antibody in the darkroom. The images were acquired using inverted fluorescence microscope and confocal microscopy.

Functional assessment and electrophysiological analysis

At 1-day postoperation (1d) and weekly thereafter, the rats were functionally monitored using the well-characterized Basso–Beattie–Bresnahan (BBB) locomotor rating scale, which is graded from 0 (absence of performance) to 21 (completely normal gait performance).24 rats were placed on a 2 × 3 m^2 open field and observed for 5 mins by two observers who were blinded to the experimental grouping. They individually scored every rat at the same time, and the ultimate scores of the rats were averaged. The animals survived and exhibited no signs of autophagia throughout the course of the experiment.

Motor-evoked potentials (MEPs, n=6 for each group) were measured at the end of the first day and the eighth week after operation, as described in our previous work.26 Interelectrode impedances were maintained below 3.5–5 kX. The bandpass filter was 10–3000 Hz. Evoked responses were displayed on a Cadwell Excel monitor (S-100, Medtronic, Dantec Company, Denmark).

Quantitative and statistical analysis

For quantification, all photographs were taken using a confocal microscope (LSM 510 Meta, Zeiss, Germany). Quantitative analyses of NF-200-positive fibers, equivalent regions were analyzed for each subject (T10 level). Throughout every eighth section, the length of the excised segment of rat spinal cord was analyzed. Ten areas were chosen randomly from all the sections containing NF-200-labeled axons. Image analysis was performed with Image-Pro Plus software (Version 5.0; Media Cybernetics, The Imaging Expert, Silver Spring, MD). The percentage of each area containing NF-200-immunoreactive axons was measured, and the values in all the sections were averaged. The NF-200 immunoreactivity of each section at T10 level was expressed as a value relative to ones at T9 (taken as 100% of NF-200-labeled axons for each animal) in the experimental groups.

All the data from this study were presented as means with standard deviation (SD). All statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, IL). BBB scores were analyzed using two-way repeated-measures analysis of variance, which showed an overall significant effect of treatment. The other data were compared between groups with one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls post hoc q test. A value < 0.05 was considered to be statistically signific.

Results

Expression of caspase-1, IL-1β, and IL-18 in the difference groups

Compared with treatment groups, the levels of active caspase-1, IL-18 and IL-1β were observed most significantly increased at the end of 1 week in the control group ($P < 0.05$). Meanwhile, the active caspase-1, IL-18 and IL-1β in cotreatment group (BMSCs + AC-YVAD-CMK) expressed less than those in the BMSCs group or AC-YVAD-CMK group at 1 week after spinal cord injury ($P < 0.01$) [Fig. 1]. These data showed the caspase-1 was inhabited by AC-YVAD-CMK and the BMSCs had anti-inflammation affects.

Neurotrophin secretion in the lesion site of SCI

At the end of the first week after SCI, the levels of BDNF and NT-3 within the lesion in the AC-YVAD-CMK + BMSCs group were significantly increased, compared to the other groups ($P < 0.01$) [Fig. 2A]. Meanwhile, at the end of the eighth week, the content of BDNF or NT-3 in the SCI group was lower than those in the treatment groups ($P < 0.01$) [Fig. 2B]. In addition, we found that BMSCs transplanted therapy could increase BDNF and NT-3 levels in the spinal cord, especially in the AC-YVAD-CMK + BMSCs group ($P < 0.05$) [Fig. 2A and B].

BMSCs transplanted survival and axonal regeneration

BMSCs were labeled with the red fluorescent cell tracking by the dye PKH26 and injected in the lesion zone of spinal cord immediately after injured. After 1 week injection, both in the groups with BMSCs transplantation, the transplanted PKH26 labeled BMSCs were easily identified within the lesion zone by their red fluorescence. However, the number of PKH26 labeled cells was similar in the cotreatment group and in the BMSCs transplant alone group (data is not shown). At the 8 weeks post-injection, hardly any PKH26 labeled BMSCs could be found in or near the contusion site in the BMSCs transplant alone, but some PKH26 labeled BMSCs were still observed in the contusion site in the cotreatment group ($P < 0.01$) [Fig. 3A and B].

Meanwhile, axons regeneration were evaluated within the lesion by the end of the eighth week after injury. In the AC-YVAD-CMK + BMSCs group, a large amount of thick and long NF-200-positive fibers (48.5% ± 4.68%) were observed throughout the thoracic region of the cord. In contrast, fewer NF-200-positive fibers were found within the injury epicenter of the BMSCs group (9.05% ± 2.92%; $P < 0.01$) [Fig. 3A and C]. In addition, very few NF-200-positive fibers were observed within SCI model group (data not shown). We also found that more BMSCs may different into neuro-like cells (yellow) at the lesion site in the AC-YVAD-CMK + BMSCs group [Fig. 3A]. These data indicated that the axons were partially regenerated in the BMSCs groups, with the best results found in the AC-YVAD-CMK + BMSCs group.

The locomotor functional recovery and neural circles were re-established

As shown in Fig. 4, compared to the other groups (the second week), the BBB scores in the AC-YVAD-CMK + BMSCs group demonstrated a significant improvement in the subchronic phase (up to about the second week after the operation, $P < 0.01$ vs. other groups). The improvement was maintained throughout the whole chronic phase of recovery at the end of the experimental time. The improvement was also detected in the AC-YVAD-CMK or BMSCs group, compared to the SCI group ($P < 0.05$) at the end of eight weeks. In the AC-YVAD-CMK + BMSCs group, we observed that the rats could occasionally support plantar steps (11.330.4) at the end of the eighth week, which was better than the BMSCs group (extensive movement of all three joints, 7.670.4; $P < 0.05$) and the AC-YVAD-CMK group (5.58 0.58; $P < 0.01$).

We also measured the spinal cord conductivity at the end of the first day and the eighth week after spinal cord injury in each experimental group (Table.1). No obvious MEPs were recorded in any of the rats at the first day after operation (data not shown). However, at the end of the eighth week, both the latency and amplitude of the MEPs recovered obviously in the AC-YVAD-CMK + BMSCs group and in the AC-YVAD-CMK or BMSCs groups, compared to the SCI group ($P < 0.01$). Moreover, the MEPs in the AC-YVAD-CMK + BMSCs group also demonstrated better recovery by their electrophysiological parameters than those in the AC-YVAD-CMK or BMSCs groups ($P < 0.05$). These data indicated that the neural loop was partially regenerated in the treatment groups, with the best results found in the AC-YVAD-CMK + BMSCs group.

Table 1
Latency and Amplitude of MEP at the end of the 8th week.

Group	*N*	Latency (ms)	Amplitude (μV)
SCI	6	8.23±0.45*	77.4±5.37*
AC-YVAD-CMK	6	6.48±0.51	208.5±11.00
BMSCs	6	5.78±0.32	236.5±14.26
AC-YVAD-CMK + BMSCs	6	4.15±0.45#	529.71±2.72#

*SCI group versus all other groups, P < 0.01; #AC-YVDA-CMK + BMSCs group versus the AC-YVAD-CMK or BMSCs groups, P < 0.05. Values are mean ± SD.

Discussion

Locomotor function recovery after SCI is still a challenging issue in clinical practice. Many ways were used to repair SCI, such as stem cell transplantation, new materials and noval drugs. The combination therapies may be an effective way to promote restorate motor function after SCI. In the present study, We decided to use caspase-1 inhibitor (AC-YVAD-CMK) to repair SCI on the basis of BMSCs transplantation. This study was designed on the basis that AC-YVAD-CMK inhibited caspase-1 active inflammation to

improve the local microenvironment for promoting the survival of BMSCs transplanted to crue SCI. In this study, our results demonstrated that the combination therapy (AC-YVAD-CMK + BMSCs) was more effective on reducing the expression of the inflammatory factors (IL-1β and IL-18) than single use AC-YVAD-CMK or BMSCs. In addition, the BDNF, NT-3 levels and the number of NF-200-positive fibers within the lesion site were significantly increased at the end of the eighth week. Then the survival rate of grafted BMSCs was the most promoted in the AC-YVAD-CMK + BMSCs group. Finally, we found that locomotor function was obviously improved in the cotreatment group. Thus, the results of our study suggested that AC-YVAD-CMK inhibited caspase-1-mediated inflammatory response to enhance BMSCs to improve neuroprotection after SCI.

As we all know, it has been reported that the mechanism of SCI is very complex, including inflammation reaction, the less nurotrophic factors and neurons lost within the lesion site, which may inhibit axonal regrowth [29]. Inflammation is one of the main mechanisms after the acute SCI [30,31]. Meanwhile, previous studies have revealed that caspase-1 appeared to play a role in tissue injury by participating in the inflammatory pathways [32]. Activation of caspase-1 in a cell can lead to cleavage and activation of additional molecules of the same or other proteases, leading to an amplified protease cascade [9]. Li M et al. [33] have firstly shown that caspase-1 is activated in neurons after SCI. They demonstrated a 17-fold increase in caspase-1 activity in injured animals when compared with sham operated mice at 24 hours after trauma. Similarly, Fink KB and colleagues [34] revealed that inhibition of caspase-1 cascade causes a reduction of posttraumatic brain injury. What's more, IL-1β is a mediator of inflammation with direct neurotoxic effects [10]. It is secreted by microglias, astrocytes, macrophages, and neutrophils in the CNS, and is proteolytically cleaved by caspase-1 from pro-IL-1β [35]. Researchers have revealed that AC-YVAD-CMK inhibits the mature of IL-1β [14,15]. In an experimental study, Ray AM et al. [36] observed that AC-YVAD-CMK provided prolonged neuroprotection against neuronal death induced by oxygen and glucose deprivation in hippocampal slices, and they considered that its effect was not directly a result of inhibition of IL-1β. Combining with these research results, in the present study, AC-YVAD-CMK was used to inhibit caspase-1 activity implicated to change the terrible environment. Our findings also demonstrate that the effect of AC-YVAD-CMK shown by inhabiting the caspase-1 expresion. The levels of caspase-1 at the same period was significantly decreased. Similarly, IL-1β and IL-18 were also decreased after the AC-YVAD-CMK treatment. Therefore, AC-YVAD-CMK can effectively inhibit caspase-1-mediated inflammatory response after tissue injury and promote functional recovery.

Meanwhile, most of the studies showed that BMSCs transplanted alone could improve the locomotor function following spinal cord injury [18,19,21]. Previous studies have demonstrated that BMSCs could secret neurotrophins to improve the axonal regeneration, such as BDNF, NT-3 [20]. Neurotrophins provide a powerful survival signal for neurons and support axonal regrowth. Among these neurotrophins, BDNF and NT-3 are significant members of the neurotrophic factors family and play important roles in neuronal injury repair [37]. Furthermore, it has been demonstrated that many stem cells can increase the amount of BDNF and NT-3 after spinal cord transaction, such as BMSCs, neural stem cells and Schwann cells [20]. However, the locomotor function was limited improved. The researchers found the survival of transplanted BMSCs was too low to playing its important affects on proving the axonal regeneration,

such as secreting neurotrophic factors or different into neural like cells. Then, Satin AM et al. used platelet-rich plasma and hyaluronic acid to improve the survival rate of BMSCs and got satisfactory results [38]. In our study, we chose BMSCs with AC-YVAD-CMK to enhance the survival rate of BMSCs transplanted. We found that the levels of BDNF and NT-3 were obviously increased in the spinal cord tissue surrounding the lesion site. Finally, we detected that NF-200 positive fibers were significantly increased within the lesion site in the treatment groups, especially in the AC-YVAD-CMK + BMSCs group. It has been demonstrated that the number and the thickness of NF-200 positive fibers may indicate more axonal regeneration. Therefore, our results indicated that the axons were successfully regenerated and motor function was recovered. From evaluate local motorfuction, our results show that higher BBB scores were obtained at the end of eight weeks after surgery in the treatment group (specailly in the AC-YVAD-CMK + BMSCs group), compared to the control group. In addition, the electrophysiology results demonstrated that the latency and amplitude of the MEPs were obviously recovered in the experiment groups. The results suggested that AC-YVAD-CMK with BMSCs could effectivly repair SCI.

Conclusions

In summar, we found that the positive effects of AC-YVAD-CMK combination with BMSCs on the improved locomotor functional through anti-caspase-1-mediated inflammatory response, survival rate of BMSCs, and activation of BDNF and NT-3 and improvement of axonal regeneration in SCI rat. These results suggest that the application of AC-YVAD-CMK on the basis of BMSCs transplantation may provide a promising treatment strategy for the neuroprotection after SCI.

Abbreviations

SCI: Spinal cord injury; BMSCs: Bone marrow stromal cells; BBB: Basso–Beattie–Bresnahan; BDNF: Brain derived neurotrophic factor; NT-3: Neurotrophin-3; NF-200: Neurofilament protein-200; CNS: Central Nervous System ; MS: Multiple sclerosis; ICH: Intracerebral hemorrhage; SD rats: Sprague-Dawley rats; PBS: Phosphate-buffered saline; MEPs: Motor-evoked potentials; SD: Standard deviation; ANOVA: Analysis of variance.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University and all procedures using animals were guided by the regulations of Harbin Medical University on the administration of experimental animals.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Natural Science Foundation of China (No. 81041082, 81571108, 81501050), the Project of Harbin science and technology innovation talents (No. 2017RAXXJ042), and the Project of scientific research and practical innovation for Postgraduates of Harbin Medical University (No. YJSSJCX2019-25HYD).

Authors' contributions

XZ and FQ performed all the experiments and contributed to the experiment design and manuscript writing. AG, YL,YS and CJ contributed to the data analysis and interpretation and drafted the manuscript. GZ, XC,YL, ZJ and KY contributed to the experiment conception and revised the manuscript. HL contributed to the conception and design, data analysis and interpretation and final revised of the manuscript. All authors read and approved the final manuscripapproval of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Author details

[1]Department of Neurosurgery, The First Affiliated Hospital of Harbin Medical University, Harbin, P. R. China. [2]School of Life Science, Northeast Agricultural University, Harbin, P. R. China. [3]Sunshine Union Hospital, Weifang, P. R. China. [4]Department of Vertigo examination room, The First Affiliated Hospital of Harbin Medical University, Harbin, P. R. China. [5]Department of Clinical Laboratory, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, P. R. China.

References

1. Steinemann S, Galanis DJ, Cheng J, Velasco BK, Biffl WL. Unique Epidemiology of Spinal Cord Injury in Hawai'i: Wave-related Incidents. Hawaii J Health Soc Welf. 2019;78:365–370.
2. Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance. J Spinal Cord Med. 2016;39:493–494.
3. Chhabra HS, Arora M. Demographic profile of traumatic spinal cord injuries admitted at Indian Spinal Injuries Centre with special emphasis on mode of injury: a retrospective study. Spinal Cord. 2012;50:745–754.
4. Adhikari SP, Gurung G, Khadka B, Rana C. Factors influencing depression in individuals with traumatic spinal cord injury and caregivers' perceived burden in a low-income country: a cross-sectional study [published online ahead of print, 2020 Mar 23] Spinal Cord. 2020;10.1038/s41393-020-0451-5.
5. Mahooti F, Raheb G, Alipour F, Hatamizadeh N. Psychosocial challenges of social reintegration for people with spinal cord injury: a qualitative study [published online ahead of print, 2020 Mar 20]. Spinal Cord. 2020;10.1038/s41393-020-0449-z.
6. Liang F, Li C, Gao C, Li Z, Yang J, Liu X, et al. Effects of hyperbaric oxygen therapy on NACHT domain-leucine-rich-repeat- and pyrin domain-containing protein 3 inflammasome expression in rats following spinal cord injury. Mol Med Rep. 2015;11:4650-6.
7. Jiao J, Zhao G, Wang Y, Ren P, Wu M. MCC950, a Selective Inhibitor of NLRP3 Inflammasome, Reduces the Inflammatory Response and Improves Neurological Outcomes in Mice Model of Spinal Cord Injury. Front Mol Biosci. 2020;7:37.
8. Shahrezaie M, Mansour RN, Nazari B, Hassannia H, Hosseini F, Mahboudi H, et al. Improved stem cell therapy of spinal cord injury using GDNF-overexpressed bone marrow stem cells in a rat model. Biologicals. 2017;50:73-80.
9. Suzuki H, Ahuja CS, Salewski RP, Li L, Satkunendrarajah K, Nagoshi N, et al. Neural stem cell mediated recovery is enhanced by Chondroitinase ABC pretreatment in chronic cervical spinal cord injury. PLoS One. 2017;12:e0182339.
10. Nakajima YI, Kuranaga E. Caspase-dependent non-apoptotic processes in development. Cell Death Differ. 2017;24:1422-1430.
11. Ren R, Wu S, Cai J, Yang Y, Ren X, Feng Y, et al. The H7N9 influenza A virus infection results in lethal inflammation in the mammalian host via the NLRP3-caspase-1 inflammasome. Sci Rep. 2017;7:7625.
12. Mendiola AS, Cardona AE. The IL-1β phenomena in neuroinflammatory diseases. J Neural Transm (Vienna). 2018;125:781-795.
13. Furlan R, Martino G, Galbiati F, Poliani PL, Smiroldo S, Bergami A, et al. Caspase-1 regulates the inflammatory process leading to autoimmune demyelination. J Immunol. 1999;163:2403-9.
14. Zhang F, Wang L, Wang JJ, Luo PF, Wang XT, Xia ZF. The caspase-1 inhibitor AC-YVAD-CMK attenuates acute gastric injury in mice: involvement of silencing NLRP3 inflammasome activities. Sci Rep. 2016;6:24166.

15. Wu B, Ma Q, Khatibi N, Chen W, Sozen T, Cheng O, et al. Ac-YVAD-CMK Decreases Blood-Brain Barrier Degradation by Inhibiting Caspase-1 Activation of Interleukin-1β in Intracerebral Hemorrhage Mouse Model. Transl Stroke Res. 2010;1:57-64.
16. Suzuki H, Sozen T, Hasegawa Y, Chen W, Zhang JH. Caspase-1 inhibitor prevents neurogenic pulmonary edema after subarachnoid hemorrhage in mice. Stroke. 2009;40:3872-5.
17. Liang H, Sun Y, Gao A, Zhang N, Jia Y, Yang S,et al. Ac-YVAD-cmk improves neurological function by inhibiting caspase-1-mediated inflammatory response in the intracerebral hemorrhage of rats. Int Immunopharmacol. 2019;75:105771.
18. Zhang F, Wang L, Wang JJ, Luo PF, Wang XT, Xia ZF. The caspase-1 inhibitor AC-YVAD-CMK attenuates acute gastric injury in mice: involvement of silencing NLRP3 inflammasome activities. Sci Rep. 2016;6:24166.
19. Karaoğlan A, Kaya E, Akdemir O, Sağmanligil A, Bilguvar K, Cirakoğlu B, et al. Neuroprotective effects of Ac.YVAD.cmk on experimental spinal cord injury in rats. Surg Neurol. 2008;69:561-7.
20. Silvestro S, Bramanti P, Trubiani O, Mazzon E. Stem Cells Therapy for Spinal Cord Injury: An Overview of Clinical Trials. Int J Mol Sci. 2020;21:659.
21. Gazdic M, Volarevic V, Arsenijevic A, Erceg S, Moreno-Manzano V, Arsenijevic N, et al. Stem Cells and Labeling for Spinal Cord Injury. Int J Mol Sci. 2016;18:6.
22. Li H, Ghazanfari R, Zacharaki D, Lim HC, Scheding S. Isolation and characterization of primary bone marrow mesenchymal stromal cells. Ann N Y Acad Sci. 2016;1370:109-18.
23. Liang H, Liang P, Xu Y, Wu J, Liang T, Xu X. DHAM-BMSC matrix promotes axonal regeneration and functional recovery after spinal cord injury in adult rats. J Neurotrauma. 2009;26:1745-57.
24. Liang H, Li C, Gao A, Liang P, Shao Y, Lin T, et al. Spinal duraplasty with two novel substitutes restored locomotor function after acute laceration spinal cord injury in rats. J Biomed Mater Res B Appl Biomater. 2012;100:2131-40.
25. Schäfer S, Berger JV, Deumens R, Goursaud S, Hanisch UK, Hermans E. Influence of intrathecal delivery of bone marrow-derived mesenchymal stem cells on spinal inflammation and pain hypersensitivity in a rat model of peripheral nerve injury. J Neuroinflammation. 2014;11:157.
26. Ide C, Nakano N, Kanekiyo K. Cell transplantation for the treatment of spinal cord injury - bone marrow stromal cells and choroid plexus epithelial cells. Neural Regen Res. 2016;11:1385-1388.
27. Tan Y, Uchida K, Nakajima H, Guerrero AR, Watanabe S, Hirai T, et al. Blockade of interleukin 6 signaling improves the survival rate of transplanted bone marrow stromal cells and increases locomotor function in mice with spinal cord injury. J Neuropathol Exp Neurol. 2013;72:980-93.
28. Tajima F, Deguchi T, Laver JH, Zeng H, Ogawa M. Reciprocal expression of CD38 and CD34 by adult murine hematopoietic stem cells. Blood .2001;97:2618-24.
29. Tashiro S, Nakamura M, Okano H. The prospects of regenerative medicine combined with rehabilitative approaches for chronic spinal cord injury animal models. Neural Regen Res 2017;12:43-46.

30. da Silva Alves E, de Aquino Lemos V, Ruiz da Silva F, Lira FS, Dos Santos RV, Rosa JP, et al. Low-grade inflammation and spinal cord injury: exercise as therapy? Mediators Inflamm. 2013;2013:971841.
31. Kwiecien JM, Dabrowski W, Dąbrowska-Bouta B, Sulkowski G, Oakden W, Kwiecien-Delaney CJ, et al. Prolonged inflammation leads to ongoing damage after spinal cord injury. PLoS One. 2020;15:e0226584.
32. Song L, Pei L, Yao S, Wu Y, Shang Y. NLRP3 Inflammasome in Neurological Diseases, from Functions to Therapies. Front Cell Neurosci. 2017;11:63.
33. Li M, Ona VO, Chen M, Kaul M, Tenneti L, Zhang X, et al. Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse model of traumatic spinal cord injury. Neuroscience. 2000;99:333-42.
34. Fink KB, Andrews LJ, Butler WE, Ona VO, Li M, Bogdanov M, et al. Reduction of post-traumatic brain injury and free radical production by inhibition of the caspase-1 cascade. Neuroscience. 1999;94:1213-8.
35. Monteleone M, Stow JL, Schroder K. Mechanisms of unconventional secretion of IL-1 family cytokines. Cytokine. 2015;74:213-8.
36. Ray AM, Owen DE, Evans ML, Davis JB, Benham CD. Caspase inhibitors are functionally neuroprotective against oxygen glucose deprivation induced CA1 death in rat organotypic hippocampal slices. Brain Res. 2000;867:62-9.
37. Keefe KM, Sheikh IS, Smith GM. Targeting Neurotrophins to Specific Populations of Neurons: NGF, BDNF, and NT-3 and Their Relevance for Treatment of Spinal Cord Injury. Int J Mol Sci. 2017;18:548.
38. Satin AM, Norelli JB, Sgaglione NA, Grande DA. Effect of Combined Leukocyte-Poor Platelet-Rich Plasma and Hyaluronic Acid on Bone Marrow-Derived Mesenchymal Stem Cell and Chondrocyte Metabolism. Cartilage. 2019:1947603519858739.

Figures

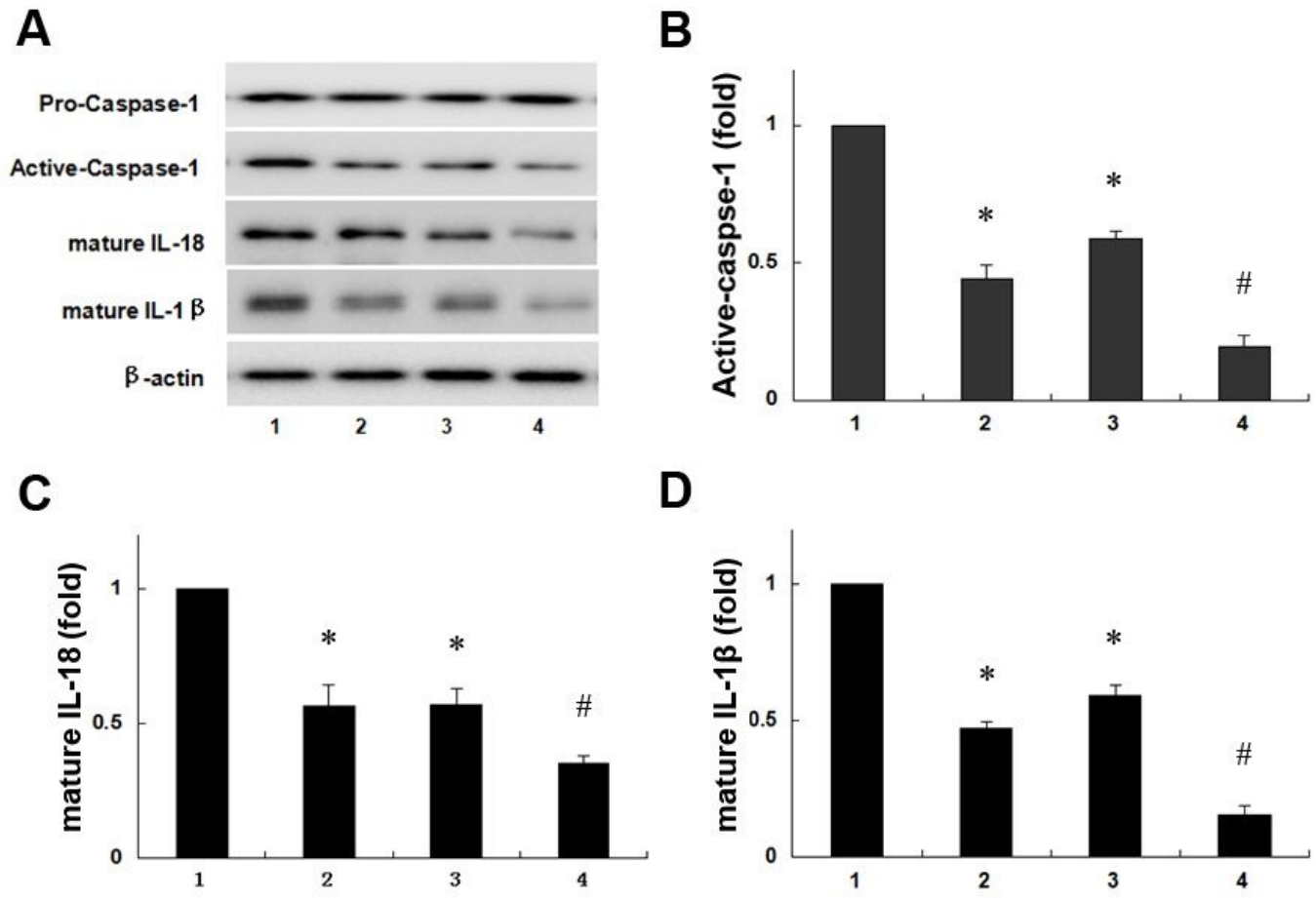

Figure 1

A: The images of pro-caspase-1, active-caspase-1, and mature IL-1β/IL-18 were assayed by western blot. (B-C) Quantification of protein active-caspase-1, mature IL-1β and mature protein IL-18 in all groups. *P < 0.05, AC-YVAD-CMK or BMSCs group vs SCI Control group, #P < 0.01, AC-YVAD-CMK + BMSCs vs other groups. 1: Control group; 2: AC-YVAD-CMK group; 3: BMSCs group; 4: AC-YVAD-CMK + BMSCs group. Values are mean ± SD.

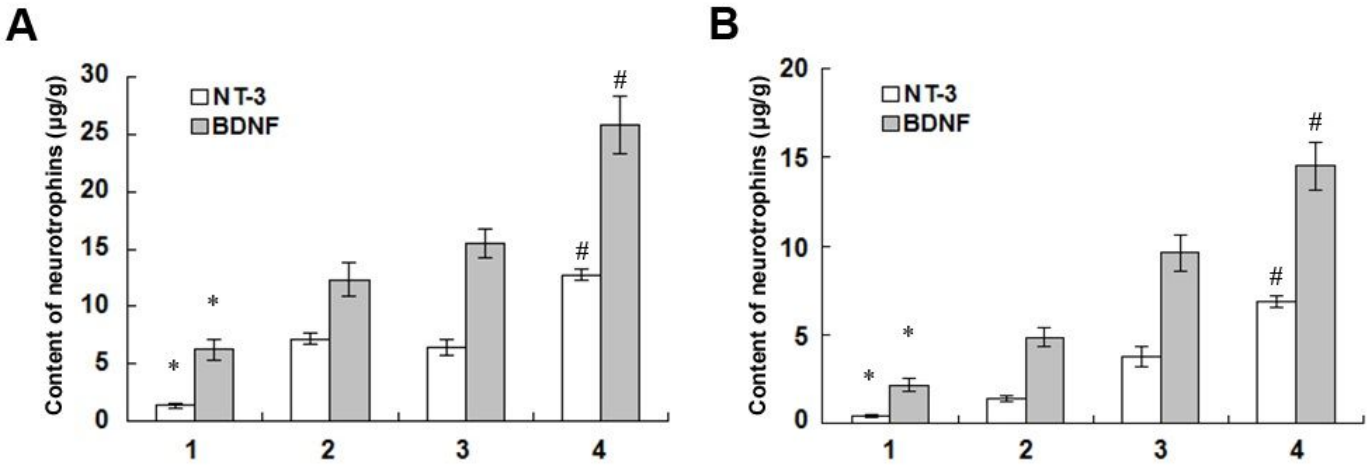

Figure 2

A: At the end of the first week, note that BDNF and NT-3 in the AC-YVAD-CMK + BMSCs group were significantly higher than the other groups (#P < 0.05). B: At the end of the eighth week, the levels of BDNF and NT-3 were obviously increased in the AC-YVAD-CMK, BMSCs and AC-YVAD-CMK + BMSCs groups, compared to the SCI group (*P < 0.01), especially in the AC-YVAD-CMK + BMSCs group (AC-YVAD-CMK + BMSCs group vs. AC-YVAD-CMK or BMSCs group, #P < 0.05). 1: SCI group; 2: AC-YVAD-CMK group; 3: BMSCs group; 4: AC-YVAD-CMK + BMSCs group. Values are mean ± SD.

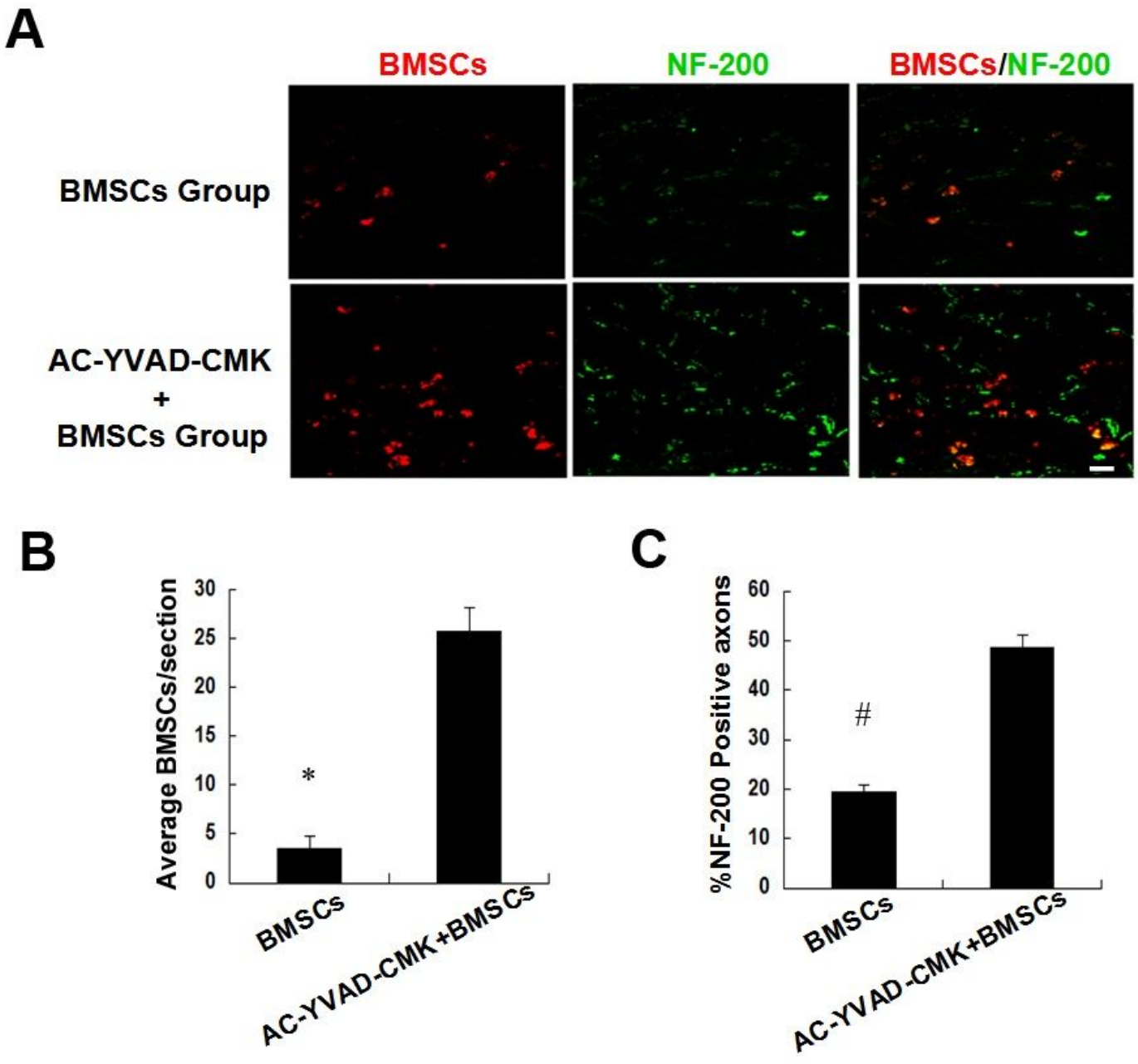

Figure 3

A: Few NF-200-positive fibers (green) were found throughout the lesion in the BMSCsl group, and fewer BMSCs (red) in the site. In the AC-YVAD-CMK + BMSCs group, there were more BMSCs in the lesion and

more thick and long NF-200-positive fibers passed through the region of the traumatic cord. B: Quantitative measurements of the survival BMSCs axons at the end of the eighth week (*P < 0.01). C: Quantitative measurements of the NF-200-positive axons at the end of the eighth week after SCI. Note that AC-YVAD-CMK + BMSCs group versus the BMSCs group (#P < 0.01); Values are expressed as mean ± SD. Scale bar: 20 μm in A.

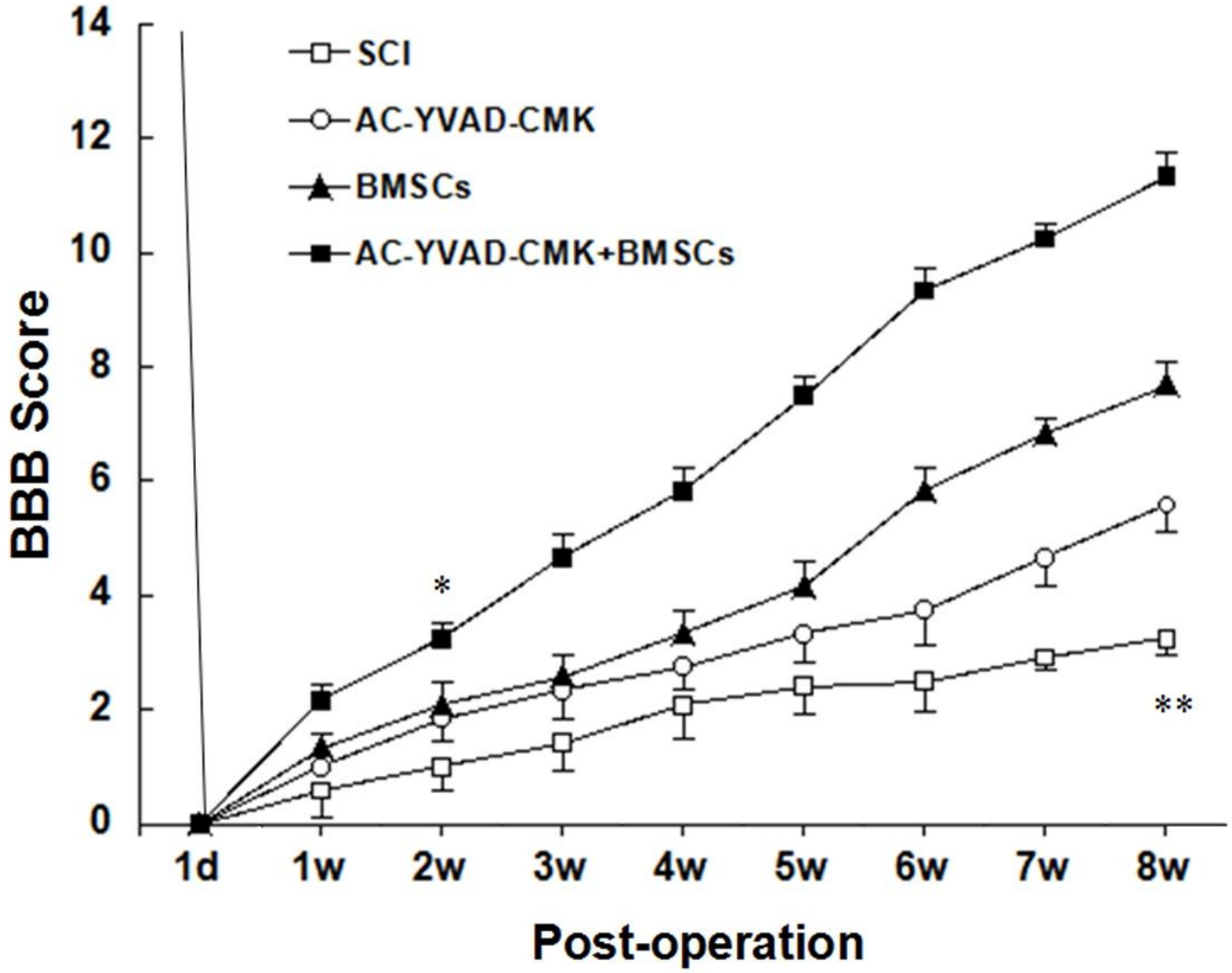

Figure 4

For the BBB scores obtained, the AC-YVAD-CMK + BMSC group showed significant improvement in open-field locomotion compared with the AC-YVAD-CMK, BMSCs and SCI groups at all time points from the second week PO (*P < 0.01; SNK q test). At the end of eight weeks, AC-YVAD-CMK or BMSCs groups compared to the SCI group (**P < 0.05).