

# Cu, Zn-Superoxide Dismutase Increases the Therapeutic Potential of Adipose-derived Mesenchymal Stem Cells by Maintaining Antioxidant Enzyme Levels

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**Abstract** In the present study, we investigated the ability of Cu, Zn-superoxide dismutase (SOD1) to improve the therapeutic potential of adipose tissue-derived mesenchymal stem cells (Ad-MSCs) against ischemic damage in the spinal cord. Animals were divided into four groups: the control group, vehicle (PEP-1 peptide and artificial cerebrospinal fluid)-treated group, Ad-MSC alone group, and Ad-MSC-treated group with PEP-1-SOD1. The abdominal aorta of the rabbit was occluded for 30 min in the subrenal region to induce ischemic damage, and immediately after reperfusion, artificial cerebrospinal fluid or Ad-MSCs ( $2 \times 10^5$ ) were administered intrathecally. In addition,

PEP-1 or 0.5 mg/kg PEP-1-SOD1 was administered intraperitoneally to the Ad-MSC-treated rabbits. Motor behaviors and NeuN-immunoreactive neurons were significantly decreased in the vehicle-treated group after ischemia/reperfusion. Administration of Ad-MSCs significantly ameliorated the changes in motor behavior and NeuN-immunoreactive neuronal survival. In addition, the combination of PEP-1-SOD1 and Ad-MSCs further increased the ameliorative effects of Ad-MSCs in the spinal cord after ischemia. Furthermore, the administration of Ad-MSCs with PEP-1-SOD1 decreased lipid peroxidation and maintained levels of antioxidants such as SOD1 and glutathione peroxidase compared to the Ad-MSC alone group. These results suggest that combination therapy using Ad-MSCs and PEP-1-SOD1 strongly protects neurons from ischemic damage by modulating the balance of lipid peroxidation and antioxidants.

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## Introduction

Intervention in the thoracic or abdominal aorta occasionally causes the unpredictable complications of spinal cord ischemia and paraplegia with incidence rates ranging from 2.7 to 28% [1, 2]. The re-establishment of blood flow significantly increases the generation of reactive oxygen species (ROS) and induces lipid peroxidation in neurons due to the high concentration of unsaturated fatty acids in nervous tissue and its vulnerability to ROS [3–5]. The rabbit spinal cord ischemia model is the most well established model because New Zealand white rabbits have segmental blood supply to the spinal cord [6].

Adipose tissue-derived mesenchymal stem cells (Ad-MSCs) are multipotent stromal cells in adipose tissue and have been considered a potentially ideal source for stem cell acquisition and clinical trials because they are easy to obtain and have important immunomodulatory and anti-inflammatory properties [7]. In previous studies, we established the acquisition of Ad-MSCs from rabbits [8] and observed neuroprotective effects in the spinal cord by three weeks following ischemia [9]. Several lines of evidence demonstrate that ROS facilitate the stemness of human Ad-MSCs [10] and initiate proliferation after quiescence exit [11]. However, excess amounts of ROS decrease MSC function and induce cell damage [12]. Recently, several studies have been conducted to reduce ROS by injecting antioxidant proteins such as melatonin [13] and antioxidant genes such as Mn-superoxide dismutase (SOD2) [14] to promote the therapeutic or protective effects of Ad-MSCs.

Administration of bioactive compounds does not have full effect on target systems because of the limitations of transmembrane transport and barrier systems such as the blood–brain barrier in the central nervous system. Growing evidence demonstrates that proteins and synthetic drugs can obtain effective intracellular delivery when co-injected with a cell penetrating peptide [15]. In previous studies, we demonstrated that PEP-1 cargo proteins had neuroprotective effects against ischemic damage in the brain as well as the spinal cord [16–18].

However, there are few studies on the synergistic or additive effects of SOD1 and Ad-MSC treatment on neuronal damage in rabbits after spinal cord ischemia. Therefore, in the present study, we investigated the effects of Ad-MSCs with or without PEP-1-SOD1 fusion protein on neuronal damage in the rabbit spinal cord after ischemia/reperfusion.

## Experimental Procedures

### Experimental Animals

Male New Zealand white rabbits (1.2–1.5 kg) were obtained from the Experimental Animal Center (Cheonan Yonam College, Cheonan, South Korea). They were housed under standard conditions with adequate temperature (22 °C) and humidity (60%) control, a 12-h light/12-h dark cycle, and free access to food and water. The handling and care of the animals conformed to established guidelines to comply with current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). All of the experiments were conducted with an effort to minimize the number of animals used and the suffering caused by procedures employed in the present study.

### Construction of Expression Vectors

The PEP-1-SOD1 expression vector was constructed as previously described [16–18]. PEP-1-SOD1 expression vector was constructed to express the PEP-1 peptides (KETW-WETWWTEWSQPKKKRKV) as a fusion with human Cu, Zn-SOD. Firstly, two oligonucleotides (top strand) 5'-TATG AAAGAAACCTGGTGGGAAACCTGGTGGACCGAAT GGTCTCAGCCGAAAAAACAACGTAAAGTGC-3' and (bottom strand) 5'-TCGABCACCTTACGTTTTTTTTTCG GCTGAGACCATTTCGGTCCACCAGGTTTTCCACC AGGTTTTCTTCC-3' were synthesized and annealed to generate a double-stranded oligonucleotide encoding the PEP-1 peptides. The double-stranded oligonucleotide was directly ligated into *NdeI-XhoI* digested pET-15b vector (sFig. 1).

Next, on the basis of the cDNA sequence of human SOD1, two primers were synthesized. The sense primer, SOD1, 5'-CTCGAGGCGACGAAGGCCGTGTGCGTG-3', contained an *XhoI* site, and the antisense primer, SOD1, 5'-GGATCCTTATTGGGGGGATCCCAATTAC-3', contained a *BamHI* restriction site. The resulting PCR products were digested with *XhoI* and *BamHI*, eluted (Invitex, Berlin, Germany), ligated into the TA-cloning vector (Promega, Madison, WI, USA), and transformed into competent cells. This amplified cloning vector was digested with the same restriction enzymes, ligated into the PEP-1 vector using T4 DNA ligase (Takara, Otsu, Shiga, Japan), and cloned in *Escherichia coli* BL21 (DE3) cells. The expressed and purified PEP-1-SOD1 enzyme activities were similar to those of authentic human and recombinant SOD1 (data not shown) [16, 19].

To produce the PEP-1-SOD1 fusion protein, the plasmid was transformed into *E. coli* BL21 cells. The transformed bacterial cells were grown in 100 ml of LB medium at 37 °C

to a D600 value of 0.5–1.0 and induced with 0.5 mM IPTG at 37 °C for 3–4 h. Harvested cells were lysed by sonication at 4 °C in a binding buffer (5 mM imidazole, 500 mM NaCl, 20 mM Tris–HCl, pH 7.9), and the recombinant PEP-1 fusion proteins were purified. Briefly, clarified cell extracts were loaded onto a Ni<sup>2+</sup>-nitrilotriacetic acid Sepharose affinity column (Qiagen, Valencia, CA, USA) under native conditions. After the column was washed with ten volumes of a binding buffer and six volumes of a washing buffer (60 mM imidazole, 500 mM NaCl, and 20 mM Tris–HCl, pH 7.9), the fusion proteins were eluted using an eluting buffer (1 M imidazole, 500 mM NaCl, 20 mM Tris–HCl, pH 7.9). The fusion protein-containing fractions were combined, and the salts were removed with buffers (1× autoclaved PBS for desalting and 5% glycerol in 1× PBS for injection) using PD-10 column chromatography (Amersham, Brunswick, Germany). The protein concentration was estimated by the Bradford procedure using bovine serum albumin as a standard [20] and the protein was kept in 5% glycerol in 1× PBS at –70 °C until utilization.

### Experimental Groups and Treatment

To elucidate the effects of Ad-MSCs with or without PEP-1-SOD1 on ischemic damage, animals were divided into two groups: sham-operated (control) and ischemia groups. The latter was further divided into three subgroups: vehicle, Ad-MSc ( $2 \times 10^5$ ), and Ad-MSc with 0.5 mg/kg PEP-1-SOD1 (Ad-MSc-SOD1) treatment groups. The animals received intrathecal Ad-MSc (intrathecally) and/or intraperitoneal PEP-1-SOD1 injection once immediately after reperfusion. Ad-MSCs were prepared as described in a previous study [8], and the cells were suspended in 5  $\mu$ l artificial cerebrospinal fluid (aCSF, 119 mM NaCl, 26.2 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.3 mM MgCl<sub>2</sub>, 10 mM glucose) and then transplanted intrathecally into the cisterna magna. The same volume of vehicle (aCSF) was injected in all groups according to previously reported techniques [21, 22]. In brief, the back around the site of intrathecal injection was shaved, wiped with alcohol, and allowed to dry. A needle (gauge 30, Hamilton, Point Style 4) was positioned over the midline of the atlanto-occipital membrane to form an angle of 60 degrees with the horizontal line. No obvious behavioral sequelae (e.g. locomotor, feeding, or drinking) were found during the experiment period.

### Induction of Transient Spinal Cord Ischemia

Animals were anesthetized with a mixture of 2.5% isoflurane (Baxter, Deerfield, IL, USA) in 33% oxygen and 67% nitrous oxide. A ventral midline incision was made in the abdomen. The abdominal aorta was isolated underneath the left renal artery and occluded using a non-traumatic

aneurysm clip to spare the principal mesenteric flow to the gut. Thereafter, spinal cord ischemia was induced according to a modified method reported by Kiyoshima et al. [23]. After 30 min of occlusion, the aneurysm clip was removed, and reperfusion was observed from the abdominal aorta. Body temperature was maintained under free-regulating or normothermic ( $38.7 \pm 0.3$  °C) conditions, monitored with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA, USA), and maintained using a thermometric blanket before, during, and after surgery until the animal had completely recovered from anesthesia. Thereafter, the animal was kept on a thermal incubator (Mirae Medical Industry, Seoul, South Korea) to maintain body temperature until it was euthanized. Control animals were subjected to the same surgical procedures except the abdominal aorta was not occluded.

### Neurological Assessment

For the assessment of neurological function, modified Tarlov criteria were used [24]: zero, no voluntary hind-limb function; one, only perceptible joint movement; two, active movement but unable to stand; three, able to stand but unable to walk; or four, complete normal hind-limb motor function [25, 26]. All assessments were performed under blinded conditions to ensure objectivity, with two observers for each experiment, and assessments of control, vehicle-treated, Ad-MSc alone, and Ad-MSc with PEP-1-SOD1 groups were conducted under the same conditions. Neurological function was assessed 24 and 72 h after reperfusion because the neurological function of the animals starts to deteriorate approximately 12–24 h after reperfusion, progressing to complete delayed-onset paraplegia within the ensuing 48 h [27, 28].

### Immunohistochemistry for NeuN in the Spinal Cord

For histological analysis, animals ( $n = 5$  in each group) used in the neurological assessment were anesthetized with 2 g/kg urethane (Sigma, St. Louis, MO, USA) 72 h after ischemia/reperfusion and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.4). The animals' spinal cords were removed, and the 5–6th lumbar segments (L<sub>5</sub>–L<sub>6</sub>) of the spinal cord were postfixed in the same fixative for 12 h. The spinal cord tissues were cryoprotected by infiltration with 30% sucrose overnight, and 30- $\mu$ m-thick spinal cord sections were serially cut in the coronal plane using a cryostat (Leica, Wetzlar, Germany).

To ensure that the histochemical and immunohistochemical data were comparable between groups, sections were carefully processed under parallel conditions. Five sections in each group were sequentially incubated with

0.3% hydrogen peroxide ( $H_2O_2$ ) in PBS for 30 min and 10% normal goat serum in 0.05 M PBS for 30 min. Sections were then incubated with mouse anti-neuronal nuclei (NeuN; 1:1000; Chemicon International, Temecula, CA, USA) overnight at room temperature. Sections were then incubated with biotinylated goat anti-mouse IgG, followed by a streptavidin-peroxidase complex (1:200; Vector, Burlingame, CA, USA). Immunostaining was visualized by reaction with DAB in 0.1 M Tris-HCl buffer (pH 7.2). Sections were dehydrated and mounted on gelatin-coated slides in Canada balsam (Kanto, Tokyo, Japan).

The measurement of NeuN-positive cells in all groups was performed using an image analysis system equipped with a computer-based CCD camera (software: Optimas 6.5, CyberMetrics, Scottsdale, AZ, USA) in a tissue area observed under 100× primary magnification. In brief, NeuN-positive neurons were counted at the center of the ventral horn of the spinal cord. Images were converted to grayscale, and NeuN-positive neurons were automatically selected according to the intensity of NeuN immunostaining. Cell counts were obtained for each group by averaging the counts from five sections, and the cell number was reported as a percentage of that obtained from the control group.

#### **Tissue Malondialdehyde Analyses in L<sub>5</sub>–L<sub>6</sub> Spinal Cord Homogenates**

Animals ( $n=5$  in each time point/each group) from control, vehicle-treated, Ad-MSC alone, and Ad-MSC with PEP-1-SOD1 groups were used to measure malondialdehyde (MDA) formation in L<sub>5</sub>–L<sub>6</sub> spinal cord homogenates using a commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA) 24 and 72 h after ischemia/reperfusion. The MDA concentration was determined from a standard curve and expressed as nmol/g of wet tissue. All assays were conducted in triplicate.

#### **Tissue SOD1 and Glutathione Peroxidase Analyses in L<sub>5</sub>–L<sub>6</sub> Spinal Cord Homogenates**

Animals ( $n=5$  in each time point/each group) from control, vehicle-treated, Ad-MSC alone, and Ad-MSC with PEP-1-SOD1 groups were used to measure SOD1 and glutathione peroxidase (GPx) in L<sub>5</sub>–L<sub>6</sub> spinal cord homogenates using commercial kits (Cayman, Ann Arbor, MI, USA) 24 and 72 h after ischemia/reperfusion. Readings were obtained using a spectrophotometer (DU 640B, Beckman, Fullerton, CA, USA). The final SOD1 and GPx activity levels were expressed as units of enzymatic activity per mg of protein sample (U/mg and nmol/min/mg, respectively). One unit of SOD1 is defined as the amount of enzyme necessary to achieve 50% dismutation of the superoxide radical. All assays were conducted in triplicate.

#### **Statistical Analysis**

The data are shown as mean ± SEM. Differences among the means were statistically analyzed by one-way analysis of variance followed by Bonferroni's post-hoc test, using GraphPad Prism 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was considered at  $P < 0.05$ .

#### **Results**

##### **Effect of Ad-MSCs with or Without PEP-1-SOD1 on Hindlimb Behavioral Function**

In the control group, animals did not show any behavioral changes in motor functions 24 and 72 h after sham operation and had an average neurological score of four. In the vehicle-treated group, animals were unable to stand and extended their hindlimbs in sitting position, with average neurological scores of 0.9 and 0.7 at 24 and 72 h after ischemia/reperfusion, respectively. In the Ad-MSC-treated group, most animals could stand but not walk at 24 h after ischemia/reperfusion. The motor function was aggravated 72 h after ischemia/reperfusion with the average neurological score decreasing from 2.5 to 1.6. In the Ad-MSC-treated group with PEP-1-SOD1, many animals could walk and motor function was maintained (3.3–3.1) by 72 h after ischemia/reperfusion (Fig. 1).

##### **Effect of Ad-MSCs with or Without PEP-1-SOD1 on Cell Survival**

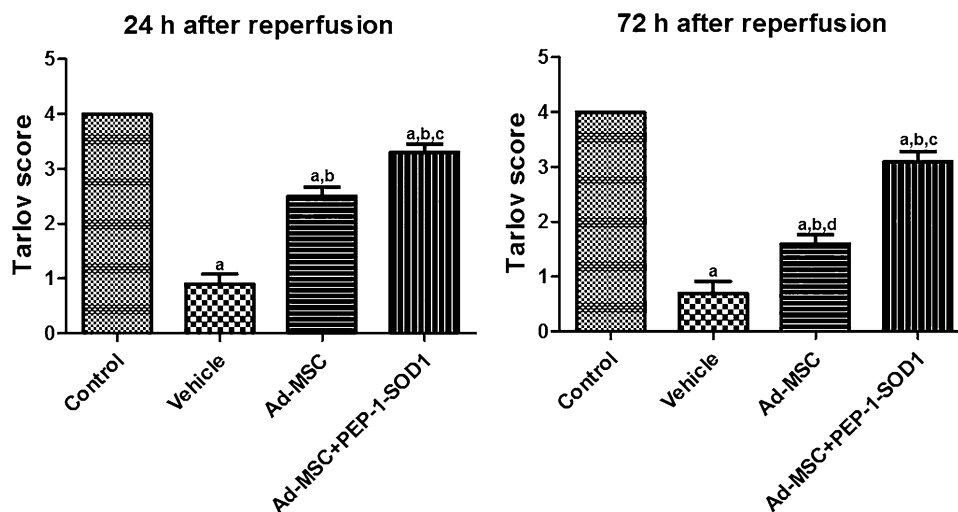
In the control group, NeuN-immunoreactive neurons were abundantly observed in the ventral horn of the spinal cord (Fig. 2a). In the vehicle-treated group, a few NeuN-immunoreactive neurons were detected in the ventral horn of the spinal cord (Fig. 2b). In this group, the number of NeuN-immunoreactive neurons was 27.6% of the control group cell count (Fig. 2e). In the Ad-MSC-treated group with or without PEP-1-SOD1, numerous NeuN-immunoreactive neurons were observed in the ventral horn of spinal cord (Fig. 2c, d). In these groups, the number of NeuN-immunoreactive neurons was more abundant in the Ad-MSC-treated group with PEP-1-SOD1 (87.6% of control) than in the Ad-MSC alone group (63.6% of control) (Fig. 2e).

##### **Effect of Ad-MSCs with or Without PEP-1-SOD1 on Lipid Peroxidation in L<sub>5</sub>–L<sub>6</sub> Spinal Cord Homogenates**

In the control group, MDA levels were 2.17 and 2.20 nmol/g protein in L<sub>5</sub>–L<sub>6</sub> spinal cord homogenates 24



**Fig. 1** Scores for neurological function in each animal as measured by modified Tarlov criteria in the control, vehicle-treated, Ad-MSC alone, and Ad-MSC-treated group with PEP-1-SOD1 (Ad-MSC + PEP-1-SOD1) 24 and 72 h after sham surgery or ischemia/reperfusion ( $n = 10$  per group; <sup>a</sup> $P < 0.05$  versus the control group; <sup>b</sup> $P < 0.05$  versus the vehicle-treated group; <sup>c</sup> $P < 0.05$  versus the Ad-MSC alone group; <sup>d</sup> $P < 0.05$  versus 24 h after ischemic group). All data are shown as means  $\pm$  SEM



and 72 h after sham operation, and there were no significant differences between time points. In the vehicle-treated group, MDA levels were significantly increased to 271.4 and 360.4 % of control group 24 and 72 h after ischemia/reperfusion, respectively. In the Ad-MSC-treated group, MDA levels were also significantly increased 24 and 72 h after ischemia/reperfusion compared to those in the control group. However, MDA levels were significantly lower than those in the vehicle-treated group 24 and 72 h after ischemia/reperfusion. In the Ad-MSC-treated group with PEP-1-SOD1, MDA levels were increased, but significantly lower than those in the vehicle-treated group 24 and 72 h after ischemia/reperfusion. In addition, MDA levels were significantly lower in this group compared to the Ad-MSC alone group 72 h after ischemia/reperfusion (Fig. 3).

#### Effect of Ad-MSCs with or Without PEP-1-SOD1 on SOD1 Activity

In the control group, the activity of SOD1 was similarly detected in L<sub>5</sub>–L<sub>6</sub> spinal cord homogenates 24 and 72 h after sham operation. In the vehicle-treated group, the activity of SOD1 was increased 24 h after ischemia/reperfusion, but significantly decreased 72 h after ischemia/reperfusion to lower levels compared to the control group. In the Ad-MSC alone group, SOD1 activity was significantly increased 24 h after ischemia/reperfusion with higher levels compared to those of the vehicle-treated group. In addition, SOD1 activity was significantly decreased and lower than that in the control group 72 h after ischemia although it was higher than that in the vehicle-treated group. In the Ad-MSC-treated group with PEP-1-SOD1, SOD1 activity was significantly increased 24 h after ischemia/reperfusion and showed significantly higher levels 72 h after ischemia/reperfusion compared to those in the vehicle-treated and control groups (Fig. 3).

#### Effect of Ad-MSCs with or Without PEP-1-SOD1 on GPx Activity

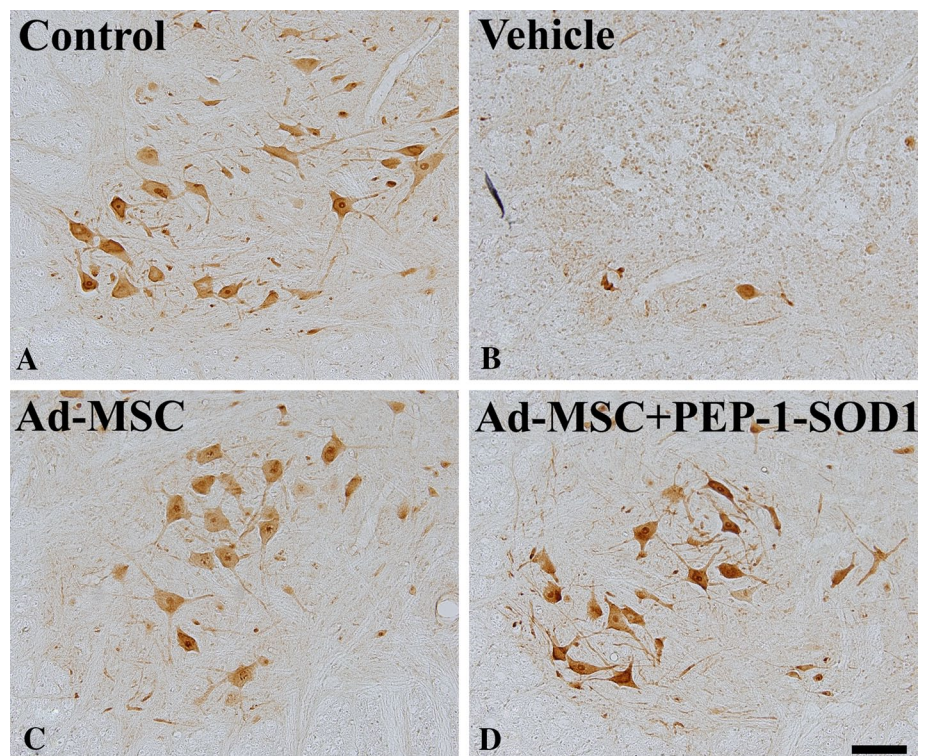
In the control group, similar GPx activity was observed in L<sub>5</sub>–L<sub>6</sub> spinal cord homogenates 24 and 72 h after sham operation. In the vehicle-treated group, GPx activity was significantly decreased 24 and 72 h after ischemia/reperfusion, compared to those in the control group, respectively. In the Ad-MSC alone group, GPx activity was significantly decreased 24 h after ischemia/reperfusion compared to that in the control group, but it showed slightly higher activity than that in the vehicle-treated group. In this group, GPx activity was significantly higher 72 h after ischemia/reperfusion compared to that in the vehicle-treated group. In the Ad-MSC-treated group with PEP-1-SOD1, GPx activity significantly increased 24 and 72 h after ischemia/reperfusion compared to those in the vehicle-treated group, and GPx levels were similar to those of the control group 24 and 72 h after ischemia/reperfusion (Fig. 3).

#### Discussion

Hypoxia/ischemia in the spinal cord is induced by occlusion of thoracoabdominal aorta, and the failure in supply of oxygen and glucose increases excitatory amino acids, inflammation, and lipid peroxidation, ultimately inducing neuronal damage [29–33]. In a previous study, we observed that Ad-MSCs protect neurons from ischemic damage after ischemia/reperfusion, although behavioral outcomes worsened by 3 days after ischemia/reperfusion [8]. However, the neuroprotective actions were maintained three weeks after ischemia [9].

In the present study, we administered Ad-MSCs from rabbit adipose tissue immediately after spinal cord ischemia to improve the neuroprotective potential. The administration of Ad-MSCs with PEP-1-SOD1 significantly increased

**Fig. 2** Immunohistochemistry for NeuN in the ventral horn of the spinal cord in L<sub>5</sub>–L<sub>6</sub> segments of control (**a**), vehicle-treated (**b**), Ad-MSC alone (**c**), and Ad-MSC-treated group with PEP-1-SOD1 (**d**) 72 h after the sham operation or ischemia/reperfusion. Scale bar 100  $\mu$ m. **g** The relative number (%) of NeuN<sup>+</sup> neurons per section for each group ( $n=5$  per group; <sup>a</sup> $P<0.05$  versus the control group; <sup>b</sup> $P<0.05$  versus the vehicle-treated group; <sup>c</sup> $P<0.05$  versus the Ad-MSC alone group). All data are shown as means  $\pm$  SEM

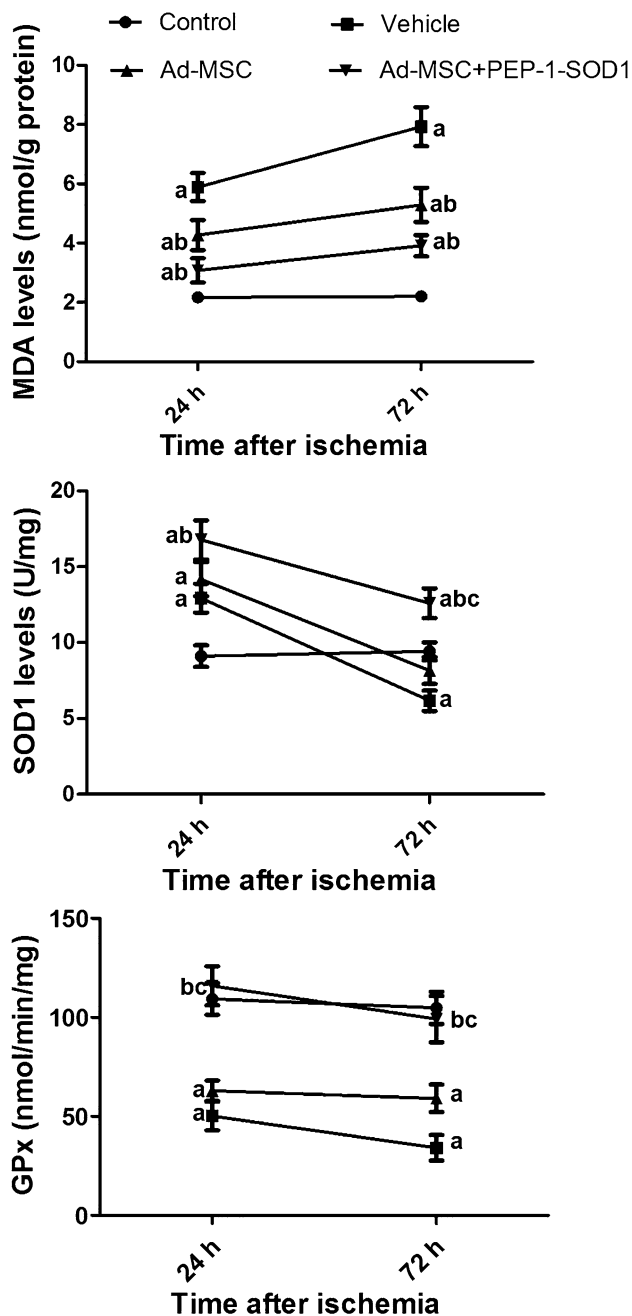


Tarlov's neurological score as well as neuronal survival compared to those of the Ad-MSC alone group. In addition, the neuroprotective effects were stronger than those observed in the PEP-1-SOD1 alone treatment as previously reported by our group [18]. This result suggests a possible role for ROS in reducing the neuroprotective potential of Ad-MSC treatment.

In the present study, we observed lipid peroxidation in spinal cord homogenates because the unsaturated fatty acids concentrated in this tissue are highly susceptible to oxidative damage, leading to DNA damage and lipid peroxidation [29, 33–35]. MDA levels were significantly increased 24 h after ischemia/reperfusion, and this result is consistent with a previous study showing that tissue MDA levels in spinal cord were significantly increased 24 h after ischemia

[33]. The administration of Ad-MSCs alone significantly decreased MDA levels in the spinal cord, and this result suggests that Ad-MSCs alone could reduce the ROS in the spinal cord. This result is supported by a previous study, which found that the supernatant of cat Ad-MSCs significantly lowered ROS production when incubated with neutrophils [36]. In the present study, the administration of Ad-MSCs with PEP-1-SOD1 further decreased MDA levels in the spinal cord.

In the present study, we also observed the activity of SOD1 and GPx in the spinal cord 24 and 72 h after ischemia. These two enzymes exhibited different changes in activity after ischemia/reperfusion. SOD1 activity significantly increased in spinal cord homogenates 24 h after ischemia/reperfusion and decreased 72 h after ischemia, while GPx



**Fig. 3** Levels of malondialdehyde (MDA, **a**) and activity of Cu, Zn-superoxide dismutase (SOD1, **b**) and glutathione peroxidase (GPx, **c**) in the L<sub>5</sub>–L<sub>6</sub> spinal cord samples from control, vehicle-treated, Ad-MSC alone, and Ad-MSC with PEP-1-SOD1 groups 24 and 72 h after sham operation or ischemia/reperfusion ( $n=5$  per each time point in each group; <sup>a</sup> $P<0.05$  versus the control group; <sup>b</sup> $P<0.05$  versus the vehicle-treated group; <sup>c</sup> $P<0.05$  versus the Ad-MSC alone group). All data are shown as means  $\pm$  SEM

activity decreased gradually following spinal cord ischemia. The increase in SOD1 activity may be closely related to a compensatory mechanism to reduce lipid peroxidation in the spinal cord. This result was supported by a previous study in which SOD1 activity peaked on day one post-surgery and

thereafter decreased gradually after ischemia/reperfusion [37]. In the ischemic brain, SOD1 activity was also shown to increase 12–24 h after ischemia/reperfusion in the hippocampal CA1 region in the gerbil [17]. The administration of Ad-MSCs increased SOD1 and GPx activity in the spinal cord after ischemia/reperfusion. One report found that mild oxidative stress induced by short-term hypoxia to Ad-MSCs significantly increases SOD activity, but not catalase or GPx activity [38]. In the present study, the administration of Ad-MSCs with PEP-1-SOD1 more pronouncedly increased SOD1 and GPx activity to levels similar to those of the control group and maintained them up to 72 h after ischemia/reperfusion.

In conclusion, PEP-1-SOD1 potentiates the neuroprotective effects of Ad-MSCs against ischemic damage by increasing and maintaining SOD1 and GPx activity in the spinal cord.

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