Dopamine Reduced Pyroptosis and Improved Functional Recovery After Spinal Cord Injury

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Research

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Abstract

Background: Neuronal loss, demyelination, and an excessive inflammatory response accompany the pathogenesis of spinal cord injury (SCI). The inflammatory response is promoted by inflammasomes in variety diseases. Dopamine is a neurotransmitter that also functions as a regulator in NLRP3 (nucleotide-binding oligomerization domain-like receptor 3) inflammasome-dependent neuroinflammation. However, the effects and molecular mechanisms underlying the role of dopamine in SCI are little known.

Methods: Functional recovery was assessed using Basso Mouse Scale (BMS) and BMS subscore. Histopathologic damage was evaluated by H&E staining. Demyelination was evaluated using immunofluorescence staining of myelin basic protein. Neuronal loss was evaluated by immunohistochemistry staining of NeuN. Pyroptosis was assessed by flow cytometry, western blot, and cell viability and cytotoxicity assays.

Results: This study using mice showed that dopamine was significantly associated with enhanced locomotor recovery after SCI; with a reduction in NLRP3 inflammasome activation, pyroptosis, neuron and myelin loss, and histological changes. In vitro data suggested an association between dopamine and suppressed NLRP3 inflammasome activation and neuronal pyroptosis, and greater survival of neurons.

Conclusion: Thus, dopamine may be a novel and effective approach for improving recovery after SCI.

Introduction

Spinal cord injury (SCI) is a devastating disease, characterized by prominent paralysis and sensorimotor impairments [1, 2]. The pathology of SCI includes phases of both primary and secondary injury [3]. The primary injury constitutes the irreversible mechanical injury, due to direct impact on the spinal cord [4]. Secondary injury results from a complex cascade of molecular events, including neuronal loss [5, 6], excessive inflammatory response [7], demyelination [8], and oxidative stress [9]. Mounting evidence suggests that, among these, neuronal loss [10] and excessive inflammatory response [7] are especially grievous, by causing persistent damage and progressive degeneration of the spinal cord. Attenuation of secondary neuronal death [11], neuroinflammation [12], and demyelination [8] may contribute to the recovery of locomotor function.

Pyroptosis is a type of programmed cell death, activated by inflammatory caspases (caspase-1 and-4, -5, -11) in response to pathogens, mediated by the highly conserved protein, gasdermin D (GSDMD) [13]. During injury of the central nervous system, caspase-1 cleaves GSDMD to generate the N-terminal fragment of GSDMD [14, 15]. This leads to rupture of the cellular membrane and the release of cellular contents, including the proinflammatory cytokines interleukin (IL)-1β and IL-18 [16]. Pyroptosis contributes to neuronal loss in traumatic brain injury [17], SCI [18], and demyelination in multiple sclerosis [19].
Dopamine is a neurotransmitter in the brain, and serves as a chemical messenger in some peripheral parts of the body [20, 21]; it serves as an important bridge between the nervous and immune systems [22]. There is evidence that dopamine is a negative regulator for the activation of the NLRP3 (nucleotide-binding oligomerization domain-like receptor 3) inflammasome [23]. Dopamine can control NLRP3-dependent inflammation, including systemic inflammation induced by lipopolysaccharide, neuroinflammation due to neurotoxin, and peritoneal inflammation caused by monosodium urate crystal [20]. In addition, activation of the NLRP3 inflammasome leads to pro-caspase-1 changes to the subunits of caspase-1, which is involved in pyroptosis [14, 15]. However, it is not known whether dopamine administration may be protective after SCI, and its molecular mechanisms have not been elucidated.

This study investigated whether dopamine may benefit therapy for SCI. Specifically, the effects of dopamine administration after SCI were determined, especially changes in neuron and myelin loss, histopathology, and locomotor recovery. The involvement of pyroptosis in the therapeutic effects of dopamine was also explored.

**Materials And Methods**

**SCI and dopamine administration**

All surgical procedures and animal care were approved by the Hangzhou First People's Hospital's Ethics Committee and were conducted in accordance with the Animal Care guidelines of China. Adult female C57BL/6 mice (purchased from Shanghai SLAC Laboratory Animal, 8-10 wk, 20–25 g) were apportioned to receive vehicle and dopamine treatment.

To impose SCI, the mice in dopamine and vehicle groups were anesthetized with 1% sodium pentobarbital (75 mg/kg). After performing a laminectomy of the T10-T11 vertebrae laminae, the exposed spinal cord in dopamine and vehicle groups was compressed using a vascular clamp (30 g force) for 1 minute, based on previous studies [24, 25]. Mice in the sham group only underwent laminectomy without crush injury to the spinal cord. After the above procedures, manual urinary bladder emptying was performed (twice a day until recovery).

Immediately after the injury, mice in dopamine group was injected intraperitoneally with dopamine (50 mg/kg, Sigma) and then repeated every 4 hours for 3 days (totally 18 times). Moreover, mice in vehicle group and sham group was injected intraperitoneally with saline and then repeated every 4 hours for 3 days (totally 18 times). The dopamine dosage was chosen based on a previous study [20]. Based on previous study [26], histology evaluation and functional assessment were only performed in dopamine and vehicle groups.

**Functional assessment**

The evaluation of locomotor recovery was performed with the 9-point Basso Mouse Scale (BMS) and 11-points BMS subscore at 1, 3, 7, 14, 21, and 28 days post-operation in an open-field test [26, 27].
assessment of hindlimb movements and coordination was made by 2 independent observers blinded to the treatment group, and the consensus score was taken (n = 9 mice in each group).

**Tissue collection**

The experimental mice underwent euthanasia with intraperitoneal injection of an overdose of sodium pentobarbital at 3 days or 28 days post-SCI. At 3 days after injury, spinal cord samples were removed for western blot and flow cytometry (10 and 5 mm, respectively, centered at the lesion epicenter). At 3 days and 28 days after injury, a spinal cord sample (5 mm, centered at the lesion epicenter) was fixed in formaldehyde solution and transversely sliced to 4-µm sections for hematoxylin and eosin (H&E) staining and immunostaining. Nine series of sections at 200-µm intervals rostral and caudal to the injury epicenter were picked up on glass slides.

**H&E staining**

For H&E staining, sections were stained with H&E reagent. The evaluation of histopathologic changes was performed using a 6-point scale determined by injured neurons in the gray matter and damage of tissue structure, as described previously [28]. The score ranged from 0 (no lesion observed) to 6 (large infarction, more than half of the gray matter area). The histologic score from 4 optical fields (high power field, 450 µm × 325 µm) in each section were averaged to obtain a final score in a blinded manner (n = 9 sections/mouse; n = 5 mice/group).

**Immunostaining**

For immunofluorescence staining of myelin basic protein (MBP), sections of spinal cord were washed with phosphate-buffered saline PBS containing 0.3% Triton X-100 for 2 hours, and incubated with anti-MBP (1:100, #ab40390, Abcam) at 4 °C overnight. Subsequently, sections were washed in PBS, incubated in the secondary antibody (1:400, #A21207, Life Technologies) at room temperature for 2 hours, and then incubated with DAPI (4′,6-diamidino-2-phenylindole) for 3 minutes. Sections were visualized with a confocal microscope (Olympus, Tokyo, Japan). The fluorescence area from the optical field in the section were averaged (n = 9 sections/mouse; n = 5 mice/group).

For immunochemistry staining, sections were incubated with 0.3% hydrogen peroxide for 10 minutes, washed in PBS, treated with EDTA (ethylenediaminetetraacetic acid) antigen retrieval solution, and then incubated with anti-NeuN (1:100, #ab177487, Abcam) at 4 °C overnight. Subsequently, sections were washed in PBS, the appropriate amount of enzyme labeled sheep anti-mouse IgG polymer (#PV-6002, Beijing Zhongshu Jinqiao Biotechnology) was added, incubated at room temperature for 20 minutes. The appropriate amount of freshly prepared chromogenic solution was added. Sections were visualized with a fluorescence microscope (Olympus, Tokyo, Japan). To obtain quantitative measurements of positive cells in the ventral horn, the number of positive cells from the optical field (high power field, 225 µm × 162 µm) in the section were analyzed to get the final data (n = 9 sections/mouse; n = 5 mice/group).

**Primary neuronal culture and injury models**
Primary cortical neurons were cultured as previously described [29]. Briefly, cerebral cortices from embryonic day-14 mice were minced, dissociated with 0.25% trypsin (Invitrogen, Carlsbad, CA, USA), and then passed through a cell strainer. Cells were plated on poly-L-lysine-coated dishes at a density of $1 \times 10^6$/mL and subsequently maintained in neuronal basal medium (supplemented with 2% B27, 1% L-glutamine, and 1% penicillin-streptomycin) in a humidified incubator (5% CO$_2$ and 95% air) at 37 °C.

For oxygen-glucose deprivation (OGD), the culture medium was replaced with glucose-free Dulbecco's Modified Eagle's Medium (Gibco, Waltham, MA, USA), and then cultured in a hypoxic chamber (5% CO$_2$, 0.02% O$_2$, and 94.98% N$_2$) for 6 h at 37 °C. The dopamine (0.25 mM) was pretreated (3 h) before OGD.

**Cytokine assay**

IL-1β and IL-18 levels in supernatant were determined by enzyme-linked immunosorbent assay (ELISA) kits IL-1β (catalog # BPE30552H) and IL-18 (catalog #SEA064Mu) according to the manufacturer's instructions (Wuhan Youersheng, Wuhan, Hubei, China).

**Flow cytometry**

Spinal cord tissue (5 mm, centered at the lesion epicenter) was harvested, cut, and digested with collagenase. Subsequently, the collected cells were incubated with either anti-GSDMD and anti-NeuN (1:100 both, #ab219800 and #ab177487, respectively, Abcam), or anti-GSDMD (as above) and anti-MBP (1:100, #ab40390, Abcam). To obtain quantitative measurements of double-positive cells, a flow cytometer (BD Biosciences, San Jose, CA, USA) was used for examination and analysis (n = 10 mice/group).

Cells were collected and incubated with GSDMD (1:100, #ab219800, Abcam) for 24 h and then SYTOX Blue dead cell stain (a high-affinity nucleic acid that penetrates only compromised plasma membranes; Molecular Probes, Eugene, OR, USA) at room temperature for 10 minutes to detect the formation of membrane pores. Subsequently, the cells were examined and analyzed using a flow cytometer (BD Biosciences, San Jose, CA, USA).

**Cell viability and cytotoxicity assays**

Cell viability was determined using a Cell Counting Kit-8 (CCK-8) Detection Assay Kit (#70-CCK805, Lianke). Cells were seeded in plates, pretreated with dopamine (0.25 mM) for 3 hours, and then OGD was performed. Subsequently, CCK-8 solution was added to the culture, and the absorbance at 450 nm was measured and then cell survival rate was calculated.

The release of lactate dehydrogenase (LDH) was determined for cytotoxicity using a detection assay kit (Jiancheng, catalog #A020-1-2). The supernatant in serum-free media was filtered and then transferred to 96-well plates, and incubated with the reaction mixture. Measurement of absorbance at 450 nm was analysed for quantification of LDH concentration.
**Western blot**

The protein samples of spinal cord tissue and cells were collected and extracted using RIPA lysate buffer (Beyotime, Nanjing, Jiangsu, China), and measured with a BCA Protein Assay Kit (Beyotime). The samples were resolved via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene difluoride membranes. The membranes were blocked with non-fat dry milk and incubated serially with primary antibodies against the following: NLRP3 (1:1000, #15101, Cell Signaling Technology); apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) (1:1000, #67824, Cell Signaling Technology); caspase-1 (1:1000, #ab138483, Abcam); GSDMD (1:1000, #ab219800, Abcam); IL-1β (1:1000, #12507, Cell Signaling Technology); and IL-18 (1:500, #ab71495, Abcam). The secondary antibodies were goat-anti mouse GAM007, and goat-anti rabbit GAR0072 (both 1:5000, Lianke). The intensity of these bands was measured with enhanced chemiluminescence reagents (Perkin Elmer Life Sciences, Waltham, MA, USA). These were analyzed using MultiGauge image analysis software version 3.0 (Fujifilm Holdings, Tokyo, Japan).

**Statistical analyses**

All data in this study are expressed as the mean ± standard error of the mean (SEM). The BMS score was analyzed using 2-way repeated-measures analysis of variance (ANOVA) and then Bonferroni’s post hoc test. One-way ANOVA and Tukey’s post hoc test was used for comparisons with other groups. Differences were considered statistically significant at p < 0.05.

**Results**

**Dopamine enhanced behavioral recovery in mice**

First, the effects of dopamine on functional locomotor recovery were assessed using the BMS score at 1, 3, 7, 14, 21, and 28 days after SCI. Locomotor assessment at 1, 3, and 7 days after SCI showed no significant difference in BMS scores between the dopamine and vehicle groups. The BMS score of dopamine treated mice inclined to significantly elevated levels starting at 7 days post-injury and remained consistent up the end of the follow-up (28 days post-injury) compared with vehicle-treated controls (Fig. 1A). Moreover, the BMS subscore of mice in the dopamine -treated group was significantly higher compared to that of mice in the vehicle-treated group from 14 days post-injury to 28 days post-injury (Fig. 1B).

**Dopamine alleviated the severity of histopathological impairments in mice**

The effects of dopamine on histological changes were investigated via H&E staining of the spinal cords of the mice. In the vehicle mice, there was substantial impairment to the spinal cord, with significant alteration of the white matter and edema at 3 days(Fig.2A-2C) and 28 days (Fig.2D-2F). The damage was less severe in mice administrated dopamine(p=0.023 for 3 days; p=0.038 for 28 days).

**Dopamine decreased neuronal loss in mice**
To investigate the effects of dopamine on neuronal loss after SCI, an analysis was performed of NeuN in the spinal cords of the mice, using immunohistochemistry. At 3 days and 28 days post-injury, the number of NeuN-positive cells in the dopamine group was significantly higher than that of the vehicle group (p=0.008 for 3 days, Fig.3A-3C; p=0.003 for 28 days, Fig.3D-3F).

**Dopamine attenuated demyelination in mice**

To evaluate the effect of dopamine on demyelination, the MBP level was studied using immunofluorescence staining of the spinal cord at 28 days post-injury. The MBP immunoreactivity of the dopamine-treated mice was significantly higher than that of the vehicle group (p=0.032, Fig.4A-4C).

**Dopamine suppressed cell pyroptosis in mice**

To examine the effect of dopamine on the pyroptosis of neurons and oligodendrocytes, flow cytometry of NeuN and GSDMD, and MBP and GSDMD, was conducted in spinal cord samples. The number of double-positive cells of NeuN and GSDMD, and MBP and GSDMD was significantly higher after SCI((p<0.001 for NeuN and GSDMD, Fig.5A-5B; p<0.001 for MBP and GSDMD, Fig.5C-5D). It was found that dopamine treatment was associated with fewer positive cells in dopamine group(p=0.002 for NeuN and GSDMD, Fig.5A-5B; p=0.042 for MBP and GSDMD, Fig.5C-5D).

Additionally, the protein levels of GSDMD, IL-1β, and IL-18 were significantly higher in the spinal cords of vehicle mice than in the sham group(p<0.001 for GSDMD, p=0.001 for IL-1β, p=0.07 for IL-18,Fig.5E-5F). However, dopamine treatment was associated with lower protein levels of GSDMD, IL-1β and IL-18 compared with the vehicle group(P=0.016 for GSDMD, p=0.008 for IL-1β, p=0.035 for IL-18,Fig.5E-5F).

These results show that administration of dopamine can induce inhibition of pyroptosis of neurons and oligodendrocytes, and suppress neuroinflammation.

**Dopamine inhibited the NLRP3 inflammasome activation in mice**

To explore further the mechanism of dopamine on pyroptosis, the activation of NLRP3 inflammasome were detected in the spinal cords of mice. Previous studies have showed that the concentration of NLRP3 peaks at 3 days after SCI [30, 31]. In the present study, the western blot analysis conducted 3 days after SCI showed that the levels of NLRP3, ASC, and caspase-1 were significantly higher in the vehicle group compared with the sham group(p=0.011 for NLRP3, p<0.001 for ASC, p=0.013 for caspase-1,Fig.6A-6B). Nevertheless, dopamine treatment reversed the increased levels of NLRP3, ASC and caspase-1 induced by SCI( p=0.025 for NLRP3, p=0.017 for ASC, p=0.046 for caspase-1,Fig.6A-6B).

**Dopamine inhibited pyroptosis and promoted survival in the primary cultured neurons**

The western blot analysis revealed that OGD was associated with significantly higher levels of GSDMD, relative to the control cells (p=0.006, Fig.7A-7B). Nevertheless, the GSDMD level in the dopamine group was lower than that in the vehicle group(p=0.023, Fig.7A-7B). In addition, the levels of the pro-inflammatory cytokines IL-1β and IL-18 in the culture supernatant were investigated. The levels of IL-1β
IL-1β and IL-18 were higher in OGD cells compared with the control cells (p=0.003 for IL-1β, Fig.7C; p=0.019 for IL-18, Fig.7D), whereas the levels of IL-1β and IL-18 in the dopamine group prominently lower compared with those in the vehicle group (p=0.018 for IL-1β, Fig.7C; p=0.032 for IL-18, Fig.7D).

To validate the effects of dopamine on pyroptosis and survival after SCI, primary neurons were exposed to OGD to mimic the SCI model in vitro. We detected that the significant higher number of double-positive cells of GSDMD and SYTOX Blue staining in the vehicle group compared with the control group ((p=0.004, Fig.7E,7F). However, dopamine treatment was associated with fewer positive cells (p=0.035, Fig.7E,7F).

Subsequently, the effects of dopamine on OGD-induced cell survival rate and neurotoxicity were investigated by CCK-8 and LDH assay, respectively. Compared with the control group, the cell survival rate was significantly lower (p<0.001, Fig.7G), and LDH activity markedly higher (p<0.001, Fig.7H), in the vehicle group. However, the cell survival rate was higher (p=0.002, Fig.7G), and LDH activity (p=0.003, Fig.7H) lower in the dopamine group compared to the vehicle group.

**Dopamine inhibited NLRP3 inflammasome activation in the primary cultured neurons**

To confirm further the mechanisms of dopamine on pyroptosis, the protein levels of NLRP3, ASC, caspase-1 was examined in the primary neurons. The western blot analysis showed that OGD was associated with an evident increase in NLRP3, ASC, and caspase-1, compared with the control group (p=0.015 for NLRP3, p=0.010 for ASC, p=0.048 for caspase-1, Fig.8A-8B). Nevertheless, our results showed that the level of NLRP3, ASC, and caspase-1 was lower in the dopamine group compared to the vehicle group (p=0.049 for NLRP3, p=0.029 for ASC, p=0.041 for caspase-1, Fig.8A-8B).

**Discussion**

This study showed that SCI induced pyroptosis, in vivo and in vitro. It was also found that dopamine prevented NLRP3 inflammasome activation, inhibited pyroptosis of neurons and oligodendrocytes, and alleviated the expressions of proinflammatory cytokines in vivo and in vitro after SCI. Importantly, the data revealed that dopamine reduced the loss of neuron and myelin, attenuated tissue impairments, and promoted locomotor recovery after SCI. Thus, these results suggest that dopamine has protective effects against SCI.

IL-1β and IL-18 are important factors in released intracellular contents associated with pyroptosis [32]. IL-1β has the detrimental effects not only on tissue integrity after SCI (in terms of glial activation and lesion size), but also on the plasticity of axons [33]. Moreover, IL-1β can inhibit the functional recovery after neural stem cell transplant administered to treat SCI in rats [34], and may downgrade the prognosis of SCI [35]. Importantly, downregulation of IL-1β after traumatic SCI may be potentially protective, for reducing secondary impairments and improving the outcomes [36]. Furthermore, IL-18 levels correlated with the severity of SCI [37]. In the present study, systemic treatment with dopamine after SCI was associated with lower levels of IL-1β and IL-18. This suggests that dopamine may function to control inflammation.
In the present study, after SCI and an increase in proinflammatory cytokines, a substantial loss of neurons and myelin was noted. Neuronal loss and demyelination have been identified as key features in secondary injury, and as promising therapeutic targets for improving the outcome after SCI [39, 40]. The importance of neuronal death is evident in the progression of SCI, and paralysis after SCI is due to interruption of axons and the failure of neurons to regenerate [41]. Importantly, preserving survival of motor neurons after traumatic SCI can improve functional recovery [42]. In addition, inhibition of demyelination improved recovery in a mouse model of SCI [43]. The present results showed that dopamine after SCI was significantly associated with a reduction in neuronal loss and demyelination, and an amelioration of the histopathological outcomes. These findings suggest that dopamine alleviates SCI-mediated tissue impairment.

To explore further the mechanism of neuronal loss, demyelination, and changes in proinflammatory cytokines, pyroptosis was analyzed in vivo and in vitro. Pyroptosis is a pattern of cell death, which is consistent with the release of proinflammatory cytokines such as IL-1β and IL-18 [44]. Pyroptosis is closely associated with neuronal loss [45]. Exposure to zinc may cause inflammasome-mediated pyroptosis in olfactory neurons [46]; and 27-hydroxycholesterol contributes to the pathogenesis of neuronal death by inducing pyroptosis [45]. Valproic acid attenuated neuronal impairment that was due to ischemic/reperfusion injury by inhibiting pyroptosis [47]. The present results indicated that SCI induced pyroptosis of neurons and oligodendrocytes. However, dopamine reduced pyroptosis in vivo and in vitro. Moreover, administration of dopamine diminished the loss of neurons and myelin and improved neuronal survival. Altogether, this study showed that dopamine can attenuate SCI-mediated tissue impairment.

To investigate the mechanisms underlying the effect of dopamine on pyroptosis, the protein levels of NLRP3, ASC, caspase-1 in the spinal cord and primary cultured neurons were determined via western blot. Earlier studies have shown that the NLRP3 inflammasome activation can induce pyroptosis [48]. NLRP3 inflammasome activation mediates pyroptosis induced by radiation in bone marrow-derived macrophages [49], and pyroptosis caused by human rhinovirus and inflammation [50]. Moreover, suppression of NLRP3 inflammasome activation was previously reported to control pyroptosis and the inflammatory response [51]. Inhibition of NLRP3 inflammasome activation with MCC950 alleviated isoflurane-induced pyroptosis and cognitive impairment in aged mice [52]. Melatonin protects endothelial cell against pyroptosis via the MEG3/miR-223/NLRP3 axis in atherosclerosis [53]. In the present study, we observed that dopamine significantly reduced the levels of NLRP3, ASC, and caspase-1. These results further suggest that dopamine-mediated suppression of pyroptosis presumably depends on the alleviation of NLRP3 inflammasome activation.

**Conclusions**

In summary, this study provides evidence that dopamine after SCI is effective at reducing loss of neurons and myelin, and suppression of inflammation. The effects of dopamine included inhibition of pyroptosis of neurons and oligodendrocytes, reduction of proinflammatory cytokines, increase of neuronal survival, and attenuation of NLRP3 inflammasome activation. Importantly, all of the biological effects of
dopamine treatment significantly improved locomotor function and protected against secondary tissue damage. These results support the concept that administration of dopamine may be an effective therapeutic strategy for improving recovery after SCI.

**Abbreviations**

SCI: Spinal cord injury; GSDMD: gasdermin D; IL: interleukin; NLRP3: nucleotide-binding oligomerization domain-like receptor 3; BMS: Basso Mouse Scale; H&E: hematoxylin and eosin; MBP: myelin basic protein; EDTA: ethylenediaminetetraacetic acid; OGD: oxygen-glucose deprivation; ELISA: enzyme-linked immunosorbent assay; CCK-8: Cell Counting Kit-8; LDH: lactate dehydrogenase; ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain.

**Declarations**

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**Author's contributions**

LLZ and WJ wrote the paper. LLZ, MQL, WJ and FH performed the experiments. GMD analyzed the data. WJ designed the research and revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed in this study are included in this published article and its additional files.

**Ethics approval and consent to participate**

All protocols were approved by the Animal Care and Use Committee of Hangzhou First People's Hospital and Zhejiang University, and conformed to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 1

Dopamine (DA) improved locomotor recovery after SCI. Mice treated with DA show significant improvement in locomotor functions assessed by the Basso Mouse Scale (BMS) score (A) and Basso Mouse Scale subscore (B). Each value represents the mean ± SEM (n=9 mice/group). All values are presented as the mean±SEM. * P<0.05, **P<0.01, ***P<0.001 versus vehicle group.

Figure 2

Dopamine (DA) attenuates histopathological damage after SCI. Representative micrographs showing H&E staining alterations in DA-treated and vehicle-treated mice in tissue sections at 400 um caudal to the
injury epicenter 3 days (2A-2C) and 28 days (2D-2F) post-injury (n=5 mice/group). Scale bars: 50 um. All values are presented as the mean±SEM. * P<0.05 versus vehicle group.

**Figure 3**

Dopamine (DA) reduces neuron loss after SCI. Representative micrographs showing sparing of ventral horn neurons in DA-treated and vehicle-treated mice in tissue sections at 400 um caudal to the injury epicenter 3 days (3A-3C) and 28 days (3D-3F) post-injury (n=5 mice/group). Scale bars: 50 um. All values are presented as the mean±SEM. **P<0.01 versus vehicle group.

**Figure 4**

Dopamine (DA) attenuates demyelination after SCI. Representative micrographs showing myelin area at the injury epicenter in section stained with MBP from DA-treated and vehicle-treated mice 28 days (4A-


Figure 5

Dopamine (DA) controls pyroptosis in mice after SCI. A: Representative fluorescence activated cell sorter (FACS) analysis dot plots showing the dynamics of double-positive cells of NeuN and GSDMD. B: Graph showing the quantitative analysis of double-positive cells of NeuN and GSDMD. Note that DA treatment reduced the number of double-positive cells of NeuN and GSDMD. C: Representative fluorescence activated cell sorter (FACS) analysis dot plots showing the dynamics of double-positive cells of MBP and GSDMD. D: Graph showing the quantitative analysis of double-positive cells of MBP and GSDMD. Note that DA treatment reduced the number of double-positive cells of MBP and GSDMD. E, F: Western blot analysis showing different inflammatory intracellular pathway and cytokines in contused spinal cord at 3d after injury. Note that DA treatment reduced the levels of GSDMD, IL-1β and IL-18 at the lesion site after SCI. (n=5 mice/group). All values are presented as the mean±SEM.* P<0.05, ** P<0.01, ***P<0.001 versus corresponding sham or vehicle group.
Dopamine (DA) suppresses NLRP3 inflammasome activation in mice after SCI. Western blot analysis showing NLRP3, ASC, caspase-1 in contused spinal cord at 3d after injury. Note that DA treatment reduced the levels of NLRP3, ASC, caspase-1 at the lesion site after SCI. (n=5 mice/group). All values are presented as the mean±SEM.* P<0.05, ** P<0.01 versus corresponding sham or vehicle group.
Figure 7

Dopamine (DA) controls pyroptosis in the primary cultured neurons. A,B: Western blot analysis showing GSMDD in the primary cultured neurons. Note that DA treatment reduced the level of GSMDD after OGD. C,D: Cytokine protein level profile 3 days after DA treatment is characterized by a reduced expression of proinflammatory cytokine IL-1β and IL-18 as indicated by ELISA analysis. E: Representative fluorescence activated cell sorter (FACS) analysis dot plots showing the dynamics of double-positive cells of SYTOX Blue staining and GSDMD. F: Graph showing the quantitative analysis of double-positive cells of SYTOX Blue staining and GSDMD. Note that DA treatment reduced the number of double-positive cells of SYTOX Blue staining and GSDMD. G: Graph showing the quantitative analysis of cell survival rate determined by CCK8 assay. H: Graph showing the quantitative analysis of LDH activity in culture supernatants. Scale bars: 10 mm. All values are presented as the mean±SEM. * P<0.05, ** P<0.01, ***P<0.001 versus corresponding sham or vehicle group.

Figure 8

Dopamine (DA) suppresses NLRP3 inflammasome activation in the primary cultured neurons. Western blot analysis showing NLRP3, ASC, caspase-1 in the primary cultured neurons. Note that DA treatment reduced the levels of NLRP3, ASC, caspase-1 in the primary cultured neurons after OGD. All values are presented as the mean±SEM. * P<0.05, ** P<0.01 versus corresponding sham or vehicle group.