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Original Article

Dihydrotestosterone reduces neuroinflammation in spinal cord injury through NF-κB and MAPK pathway

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Abstract

Neuroinflammation induced by microglia following spinal cord injury (SCI) leads to secondary neurologic injury. Androgens including testosterone and dihydrotestosterone (DHT) show as endogenous neuroprotective factors against multiple neurologic diseases, while their therapeutic role in SCI-induced neuroinflammation and underlying mechanism remains elusive. In the study, we aimed to investigate the role of DHT against microglia-induced neuroinflammation in SCI and evaluate its protective treatment. BV2 cells were activated by neuroinflammation via LPS in vitro. Adult male C57BL/6 mice were used to establish the SCI model. BV2 cells and SCI mice were administrated DHT. Microglia activation, pro-inflammatory factors, p38 and p65 phosphorylation, glial scar, fibrotic scar, histology, and locomotor function recovery were measured, respectively. We demonstrated that DHT administration attenuates neuroinflammation in microglia through inhibition of p38 and p65 pathways. Moreover, DHT reduces microglia and astrocyte accumulation, cord fibrosis and histologic damage. Besides, DHT ameliorates locomotor functional recovery after SCI. DHT is verified to play a neuroprotective role in SCI, which fights against neuroinflammation by inhibition of p38 and p65 pathways. Therefore, Mel is defined as a promising factor in protecting neural tissue after SCI.

Keywords: Dihydrotestosterone, Spinal cord injury, Neuroinflammation, Microglia, p38 and NF-κB pathways

1. Introduction

Spinal cord injury (SCI) stands as a profoundly devastating neurologic condition, marked by severe dysfunction, paralysis, and even death [1-3]. The aftermath of trauma triggers the activation of microglia in situ, initiating a cascading neuroinflammatory response. This response, in turn, sets off the innate immune system, recognizing exogenous neurotoxic substances and pro-inflammatory stimuli [3,4]. The consequence is an accumulation of excessively activated microglia in the vicinity of the injury focus, releasing massive pro-inflammatory cytokines that induce further neuronal damage [4]. Furthermore, the mediators produced by these activated microglia exert additional stimulation on astrocytes, leading to their accumulation around the injury epicenter [5].

Despite the partial protective effect of the glial barrier against necrocytotoxins in the injury focus, neurogenesis and neurostructural remodeling are significantly impeded during the neurologic repair stage [6]. Clinically, methylprednisolone has emerged as a primary treatment against neuroinflammation, offering a crucial means to limit secondary damage, alongside surgical interventions [7]. However, its effectiveness in rescuing neurologic loss remains limited. Consequently, there is an urgent need to explore novel and effective therapeutic strategies to address the pervasive issue of SCI-induced neuroinflammation.

Dihydrotestosterone (DHT), a metabolite of testosterone, has shown notable neuroprotective effects in preclinical settings, including conditions such as Alzheimer's disease (AD) [8], Parkinson's disease (PD) [9], and multiple sclerosis (MS) [10]. These effects are attributed to its anti-apoptotic and antioxidative stress properties. Despite these promising findings, few studies have delved into the specific role of DHT in neuroinflammation. While the anti-inflammatory effects of androgens in peripheral inflammatory diseases have been documented in both animal and clinical studies [11], the impact of DHT on neuroinflammation in the context of SCI and the central nervous system remains elusive.

In light of these considerations, there is a pressing need to investigate the effects and underlying mechanisms of DHT on neural tissue post-SCI. Previous studies have indicated the protective role of gonadal hormones, such as estradiol and DHT, in safeguarding spinal motoneurons following SCI [12,13]. Notably, androgens have been reported to rescue avian embryonic lumbar spinal motoneurons from injury-induced cell death [14]. However, whether DHT administration can effectively reduce neu-

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roinflammation to exert therapeutic action in SCI has not been comprehensively explored.

Therefore, the primary objective of the present study is to probe the therapeutic effects of DHT on SCI. Additionally, we aim to mechanistically investigate the role of DHT and its associated neuroinflammation pathway in the regulation of SCI. To provide a comprehensive context, it is essential to consider the epidemiology of SCI, offering insights into the prevalence, incidence, and societal impact of this debilitating condition. Furthermore, we will briefly highlight some recent advances in the treatment of SCI, emphasizing the evolving landscape of therapeutic interventions in this field.

2. Materials and Methods

2.1. Cell culture and treatment

The BV2 microglia line and was obtained from the Hubei University of Medicine. Cells were cultured in 5×5 cm^2 flasks containing 5 mL dulbecco's modified eagle medium (DMEM, Gbico, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS, Gbico, Rockville, MD, USA) and 100 U/mL penicillin-streptomycin (Gbico, Rockville, MD, USA). For cell treatment, DHT (MedChemExpress, Shanghai, China) was dissolved in 0.1% dimethyl sulfoxide (DMSO, sigma, St. Louis, MO, USA). Then the microglia medium was added to 10 nM DHT for 30 min. Lipopolysaccharide (LPS, 1 μg/mL Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMEM and added in microglia medium for 18 h.

2.2. Animals and grouping

Adult male C57/B6J mice, aged 6-8 weeks (20-22 g), were housed in Hubei University of Medicine. Standard laboratory conditions, such as 12 h/12 h light/dark cycle, available food and water, 50% humidity as well as 22 ± 1°C temperature, were provided. Mice were randomly assigned to three different experimental groups. The Sham group (Sham), SCI group, and SCI+DHT group (n=9) were established in the study. The study was approved by the animal ethical committee of Hubei University of Medicine.

2.3. Spinal cord injury

The modeling method was described as follows: mice were anesthetized intraperitoneally using xylazine (5 mg/kg) and ketamine (95 mg/kg) by normal saline, then we conducted laminectomy and impacted the 10th spinal cord using 70 kilodyne using an NYU Impactor. DHT was subcutaneously injected (5 mg/kg/day) for two weeks post SCI.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Microglia medium and tissue homogenate were collected. We centrifuged them for 5 min and isolated the supernatant. ELISA of pro-inflammatory factors was conducted using an ELISA Kit (KeyGen, Nanjing, China) according to manufacturer's instructions. The absorbance (OD value) of each well was measured at 450 nm using a spectrophotometer.

2.5. Western Blotting

Protein was extracted from cells using a Total Protein Extraction Kit (KeyGen, Nanjing, China) directed by the manufacturer's protocols. Concentration determination was employed with a Bicinchoninic Aid (BCA) Assay Kit (Thermo Scientific, Waltham, MA, USA). Then equivalent protein was performed electrophoresis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer and immuno-blocking. Incubation of primary and secondary antibodies (iNOS (1;250, Abcam, Cambridge, MA, USA), COX-2 (1;1000, Abcam, Cambridge, MA, USA), p-p65 (1:1000, Abcam, Cambridge, MA, USA), p65 (1:1000, Abcam, Cambridge, MA, USA), p-p38 (1:1000, Abcam, Cambridge, MA, USA), p38 (1:1000, Abcam, Cambridge, MA, USA), β-Actin (1;2000, Abcam, Cambridge, MA, USA), and Goat Anti-Rabbit IgG H&L (HRP) (1:2000, Abcam, Cambridge, MA, USA)), protein was exposed using an enhanced chemiluminescence (ECL) system (Tanon, China) and quantified using ImageJ software (USA).

2.6. Immunofluorescence (IF) and immunohistochemical staining (IHC)

Cord tissues were collected after mouse sacrifice. Cells and tissues were fixated with 4% paraformaldehyde (PFA) for 24 h. Following dehydration by ethanol and permeation by xylene, tissue was embedded into paraffin and cut into 6 μm sections using a rotary microtome. Roasted for 48 h, sections were deparaffined, hydrated and antigen repaired. For IF, Sections and cells were incubated with iNOS (1:100, Abcam, Cambridge, MA, USA), IBA-1 (1:300, Abcam, Cambridge, MA, USA), TNF-α (1:200, Abcam, Cambridge, MA, USA), IL-1β (1:200, Abcam, Cambridge, MA, USA), GFAP (1:200, Abcam, Cambridge, MA, USA) and fibronectin (1:200, Abcam, Cambridge, MA, USA) overnight at 4°C. For IF, sections were incubated with Alexa Fluor® 488 or 594 (1:200, Abcam, Cambridge, MA, USA) for 1 h. The nucleus was stained with diamidine phenylindole (DAPI, Mounting Medium With DAPI-Aqueous, Fluoroshield, Abcam, Cambridge, MA, USA). For IHC, sections were incubated with p-p38 (1:100, Abcam, Cambridge, MA, USA) and p-p65 (1:200, Abcam, Cambridge, MA, USA) overnight at 4°C. IHC staining using a DAB Coloring Kit (Solarbio, Beijing, China) according to manufacturer's protocols and hematoxylin was used to counterstain the nucleus. Then the images of sections were visualized and collected using a fluorescence inversion microscope system.

2.7. Behavioral assessment

Mice in each group were allowed to free movement in an open field for 4 min at 1days, 3 days, 7 days, 14 days, and 28 days post SCI. Two researches scored sports situations according to the Basso Mouse Scale (BMS) in a blind way. Then data were collected and analyzed in statistics.

2.8. Statistical analysis

Data were displayed as the means ± SD. The difference in statistics between the two groups was assessed using Student's *t*-test. Difference among more than two groups was evaluated via one-way or two-way ANOVA. Data were collected and analyzed using Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS Inc., Chicago, IL, USA). $P<0.05$ means statistical significance.

3. Results

3.1. DHT reduces LPS-induced inflammatory activation in BV2 microglia

To provoke an inflammatory response in microglia, we performed LPS administration for stimulation. Then the released pro-inflammatory cytokines in medium, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 were detected using ELISA, showing that the expressions of TNF-α, IL-1β and IL-6 were significantly increased following LPS treatment, whereas DHT reduced the levels of the above pro-inflammatory cytokines in LPS treated microglia medium (Figure 1A-1C). To verify inflammatory activation in BV2 microglia, the representative mediator inducible nitric oxide synthase (iNOS) and the microglial marker ionized calcium-binding adaptor molecule-1 (IBA-1) were visualized using IF, the Figure 1D showed that LPS administration significantly increased iNOS (Red) expressions in microglia (Green), however, DHT administration reduced iNOS expression in microglia. It was indicated that DHT decreased inflammatory microglia activation after LPS stimuli. Furthermore, the pro-inflammatory cytokines in cells including TNF-α and IL-1β were examined using IF, exhibiting that the levels of TNF-α (Red) and IL-1β (Green) elevated markedly post LPS utilization while synthetic inflammatory cytokines reduced in microglia with DHT treatment (Figure 1E). Hence, it is proved that DHT could reduce LPS-induced inflammatory microglia activation.

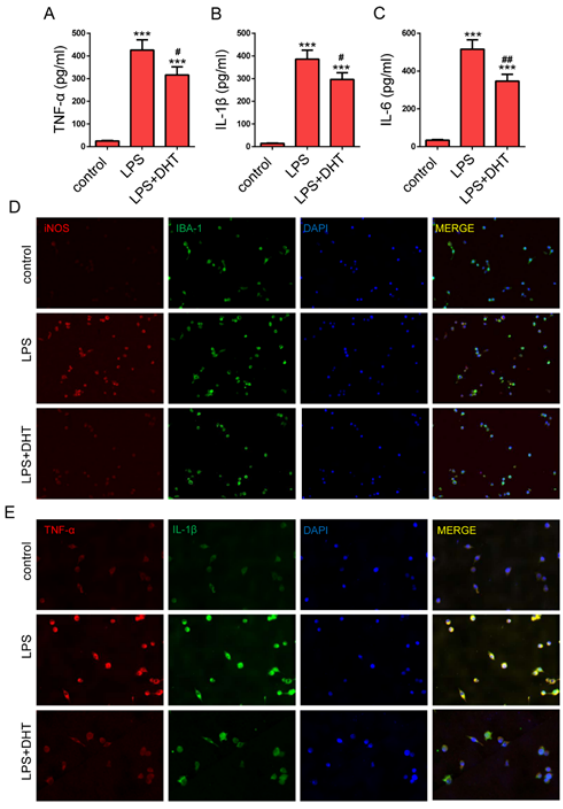

Fig. 1. DHT reduces LPS-induced inflammatory activation in BV2 microglia. (A-C) Representative ELISA of TNF-α, IL-1β and IL-6 in control, LPS and LPS+DHT treated microglia medium. (D) Representative IF of iNOS (red) and COX-2 (green) in control and LPS-treated microglia, (magnification: 100×). (E) Representative IF of TNF-α (red) and IL-1β (green) in control, LPS and LPS+DHT treated microglia, (magnification: 200×). "***" P<0.001, "**" P<0.01 and "*" P<0.05 *vs.* control group and "###" P<0.001, "##" P<0.01 and "#" P<0.05 means *vs.* LPS group with statistical significance.

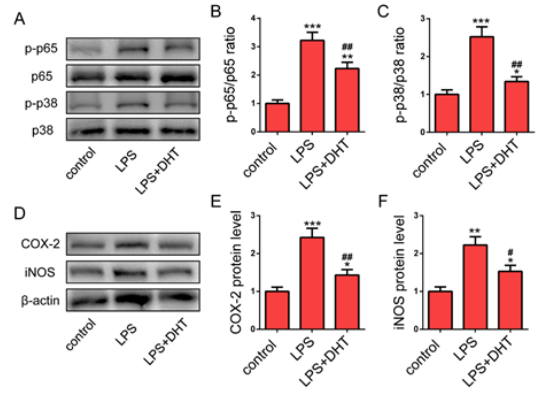

Fig. 2. DHT treatment inhibits TLR4-mediated p65 and MAPK-p38 pathways in inflammatory microglia. (A) Representative western blotting including p-p65, p65, p-p38 and p38 in control, LPS and LPS+DHT treated microglia. (B-C) Quantification of the ratios of p-p65/p65 and p-p38/p38. (D) Representative western blotting including COX-2 and iNOS in control, LPS and LPS+DHT treated microglia. (E-F) Quantification of the protein levels of COX-2 and iNOS. "***" P<0.001, "**" P<0.01 and "*" P<0.05 *vs.* control group and "###" P<0.001, "##" P<0.01 and "#" P<0.05 means *vs.* LPS group with statistical significance.

3.2. DHT treatment inhibits TLR4-mediated p65 and MAPK-p38 pathways in inflammatory microglia

Further, we investigated whether DHT inhibited toll-like receptor 4 relative signaling pathways to exert an anti-inflammation effect. Western blot exhibited increased expressions of phosphor-NF-κB p65 (p-p65) and phosphor-MAPK p38 (p-p38) in LPS-induced inflammatory microglia, whereas DHT treatment decreased p-p65 and p-p38 protein levels (Figure 2A-2C). We also detected the expressions of iNOS and cyclooxygenase-2 (COX-2), the protein levels of iNOS and COX-2 exhibited consistent expressions with p-p65 and p-p38. (Figure 2D-2F). The above results indicate that DHT attenuates inflammatory response in microglia via down-regulation of NF-κB p65 and MAPK p38 pathways.

3.3. Administration of DHT attenuates systemic neuroinflammation after SCI

We next evaluated the possible therapeutic effect of DHT in SCI mice. Firstly, we measured the microglia activation via IF, founding massive inflammatory microglia neighboring the injured centre following SCI, but DHT treatment reduced activated microglia surrounding injured focus (Figure 3A). IHC staining showed increased p-p65 and p-p38 in injured site after SCI, however, DHT administration reduced p-p65 and p-p38 positive area in injured site at 3 days post-trauma (Figure 3B). ELISA showed that the expressions of TNF-α, IL-1β and IL-6 dramatically increased in injured tissue while treatment with DHT decreased the levels of the pro-inflammatory cytokines (Figure 3C-3E). Hence, we demonstrate that DHT reduces neuroinflammation in injured tissue via inhibition of NF-κB and MAPK p38 pathways.

3.4. Effect of DHT mitigates glial accumulation and fibrosis focus in injured site

Furthermore, we investigated the effect of DHT treatment on protection of neural repair and locomotor func-

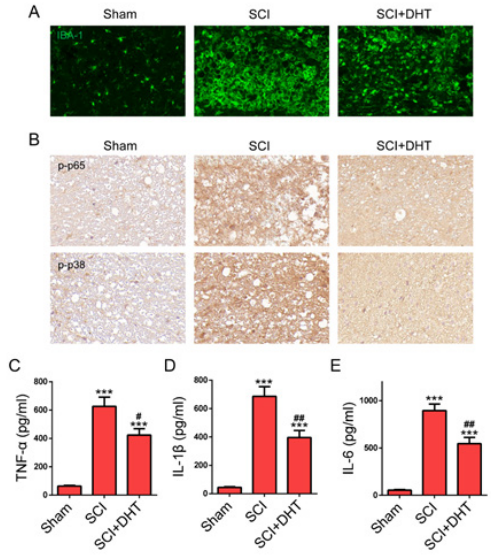

Fig. 3. Administration of DHT attenuates systemic neuroinflammation after SCI. (A) Representative IF of IBA-1 (green) in Sham, SCI and SCI+DHT at 3 days post-SCI, (magnification: 200×). (B) Representative IHC of p-p65 and p-p38 in Sham, SCI and SCI+DHT at 3 days post-SCI, (magnification: 400×). (C-E) Representative ELISA of TNF-α, IL-1β and IL-6 in Sham, SCI and SCI+DHT group at 3 days post SCI. (E) Quantification of Sirt-1 protein level. "***" P<0.001, "**" P<0.01 and "*" P<0.05 *vs.* control group and "###" P<0.001, "##" P<0.01 and "#" P<0.05 means *vs.* LPS group with statistical significance.

tional recovery. IF staining showed that SCI resulted in accumulation of excessive microglia (IBA-1 positive) and astrocytes (GFAP positive) around the epicenter at 28 days post-trauma, whereas administration of DHT reduced the area of glial scar in neurologic tissue (Figure 4A). Then we further measured the fibrosis degree in injured site using IF, showing that the positive region of fibronectin in injured focus significantly decreased in injured site after DHT treatment at 28 days post SCI (Figure 4B). Moreover, we evaluated BMS scores in each group at 1 day, 3 days, 7 days, 14 days and 28 days, respectively. The BMS scores in the Sham group were at 9 points during 4 weeks, scores in other groups were at 0 points on 1 day after trauma, the scores in the SCI+DHT group were significantly higher than those in the SCI group and SCI+Mel+EX527 group beginning at 7 days post-SCI and continued to 28 days (Figure 4C). Hence, the results proved that administration of DHT protects neural tissue from gliosis and fibrosis, improving locomotor functional recovery after SCI.

4. Discussion

Neuroinflammation is one of the important physiological and pathological events after SCI [15,16]. In this study, we demonstrated that DHT played an anti-inflammatory role in LPS-mediated inflammatory microglia by inhibiting NF-κB and P38 signaling pathways. In addition, in vivo experiments showed that administration of DHT significantly attenuated the level of neuroinflammation post-SCI, thus reducing gliosis and tissue fibrosis associated with inflammatory response, and eventually promoting the recovery of locomotor function in mice. Aberrant microglia activation and extensive neuroinflammation in neuropathy play a lethal role in the pathogenesis of aggravating neural injury [17]. Previous studies [18,19] have shown that androgens inhibited the progression of inflammatory responses by inhibition of macrophage activation. Therefore, DHT potentially plays an anti-inflammatory role in systemic diseases associated with neuroinflammation. Inflammatory microglia respond rapidly to LPS stimulation and thereby release inflammatory mediators such as NO, PGE2, TNF-α and IL-1β [8,20]. Androgen has been found to reduce the expression of inflammatory factors including TNF-α, IL-1β, and IL-6 in peripheral neuroinflammatory diseases as an alternative therapy. Here, we found that DHT inhibited the activation of pro-inflammatory microglia and reduced proinflammatory cytokines including iNOS, COX-2, TNF-α, IL-1β and IL-6, and the reduction of these inflammatory mediators is also regulated via DHT in injured spinal cord. These findings are consistent with previous results that androgen therapy reduces TNF-α and IL-1β [8,21]. As adverse effects after neuroinflammation, glial chemotaxis and long-term stimulation of chronic inflammation mediate the formation of glial scar and the occurrence of fibrosis in lesion after SCI. Previous studies have exhibited that glial hyperplasia and alternative fiber repair could hinder the recovery of neurogenic function, while the effective reduction of glial scar area and the pathogenesis of fibrosis promoted the recovery of neural tissue [22-24]. We discovered that DHT also reduced aggregation of glial cells and fibronectin levels at the last phase of SCI, which may be related to the negative regulation of neuroinflammation in the early phase. In terms of the mechanism by which DHT downregulates inflammation, previous studies have demonstrated that TLR4 expression is significantly reduced in macrophages and endothelial cells treated with androgens [25]. Moreover, androgen supplementation inhibited increased expression of TLR4 in castrated animals [26]. Therefore, we hypothesized that DHT might inhibit the pathogenesis of neuroinflammation in microglia cells through TLR4-related signaling pathways. TLR4 mediates inflammation through downstream signa-

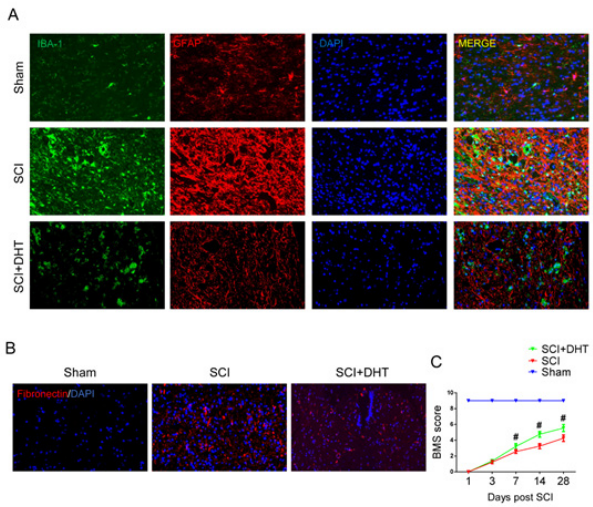

Fig. 4. Effect of DHT mitigates glial accumulation and fibrosis focus in injured site. (A) Representative IF of IBA-1 (green) and GFAP (red) in Sham, SCI, SCI+DHT group at 28 days post-SCI, (magnification: 200×). (B) Representative IF of fibronectin in Sham, SCI, SCI+DHT group at 28 days post SCI, (magnification: 200×). (C) Representative BMS scores in Sham, SCI, SCI+DHT group at 1, 3, 7, 14, and 28 days post-SCI. "###" P<0.001, "##" P<0.01 and "#" P<0.05 means vs. LPS group with statistical significance.

ling pathways NF-κB and MAPKs [27]. Consistent with previous findings, DHT inhibited phosphorylation of NF-κB pathway and subsequent inflammatory responses. However, we further found that DHT inhibited phosphorylation of the TLR4-independent NF-κB p65 and MAPK p38 pathways in LPS-treated microglia and mouse spinal cord after SCI. Meanwhile, multiple studies have shown that DHT inhibits the expression of several MAPKs pathways, such as JNK, p38 and ERK signaling, in vitro and in vivo. In the current study, we verified the poor locomotor function in mice following trauma, however, treatment with DHT improved the recovery of locomotor function in SCI mice. Earlier studies likewise showed the neuroprotective role of DHT in LPS-induced acute encephalitis, in which cognitive impairment was ameliorated via DHT [28]. However, the mechanism concerning DHT reducing glial scar and fibrosis is still unknown and needs to be investigated in further studies. In summary, DHT treatment reduces post-SCI neuroinflammation through TLR4-mediated NF-κB and MAPK-P38 signaling pathways. The results implicate that the neuroprotection of DHT in SCI model improves behavioral function and DHT administration may be a selection of adjuvant therapy or drug combination to acute neuroinflammation in early phase of SCI.

5. Conclusion

The present research certifies that DHT reduces inflammation response in LPS-induced microglia and SCI mice, which inhibits NF-κB and MAPK-P38 signaling pathways. The neurohistology and behavioral recovery are thereby improved following DHT administration in SCI.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was approved by the animal ethical committee of Hubei University of Medicine Animal Center.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Jiarui Wei and Tao Li designed the study and performed the experiments, Shengyuan Lin and Bin Zhang collected the data, Shengyuan Lin, Bin Zhang and Xing Li analyzed the data, Jiarui Wei and Tao Li prepared the manuscript. All authors read and approved the final manuscript.

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Non.

References

1. Silva NA, Sousa N, Reis RL, Salgado AJ (2014) From basics to clinical: a comprehensive review on spinal cord injury. Prog Neurobiol 114:25-57. doi: 10.1016/j.pneurobio.2013.11.002
2. Rong W, Li H, Yang H, Yuan B, Zhu J, Deng J et al (2023) Ezetimibe attenuates functional impairment via inhibition of oxidative stress and inflammation in traumatic spinal cord injury. Cell Mol Biol 69:175-180. doi: 10.14715/cmb/2023.69.6.26
3. Jain NB, Ayers GD, Peterson EN, Harris MB, Morse L, O'Connor KC et al (2015) Traumatic spinal cord injury in the United States, 1993-2012. Jama 313:2236-2243. doi: 10.1001/jama.2015.6250
4. Liu Z, Song B (2022) Expression Pattern of Ngb in Astrocytes after Spinal Cord Injury and the Clinical Significance. Cell Mol Biol 67:160-166. doi: 10.14715/cmb/2021.67.6.22
5. Liu G, Fan G, Guo G, Kang W, Wang D, Xu B et al (2017) FK506 Attenuates the Inflammation in Rat Spinal Cord Injury by Inhibiting the Activation of NF-kappaB in Microglia Cells. Cell Mol Neurobiol 37:843-855. doi: 10.1007/s10571-016-0422-8
6. Orr MB, Gensel JC (2018) Spinal Cord Injury Scarring and Inflammation: Therapies Targeting Glial and Inflammatory Responses. Neurotherapeutics 15:541-553. doi: 10.1007/s13311-018-0631-6
7. Evaniew N, Noonan VK, Fallah N, Kwon BK, Rivers CS, Ahn H et al (2015) Methylprednisolone for the Treatment of Patients with Acute Spinal Cord Injuries: A Propensity Score-Matched Cohort Study from a Canadian Multi-Center Spinal Cord Injury Registry. J Neurotrauma 32:1674-1683. doi: 10.1089/neu.2015.3963
8. Yao PL, Zhuo S, Mei H, Chen XF, Li N, Zhu TF et al (2017) Androgen alleviates neurotoxicity of beta-amyloid peptide (Abeta) by promoting microglial clearance of Abeta and inhibiting microglial inflammatory response to Abeta. Cns Neurosci Ther 23:855-865. doi: 10.1111/cns.12757
9. Snyder B, Duong P, Trieu J, Cunningham RL (2018) Androgens modulate chronic intermittent hypoxia effects on brain and behavior. Horm Behav 106:62-73. doi: 10.1016/j.yhbeh.2018.09.005
10. Zhu ML, Bakhru P, Conley B, Nelson JS, Free M, Martin A et al (2016) Sex bias in CNS autoimmune disease mediated by androgen control of autoimmune regulator. Nat Commun 7:11350. doi: 10.1038/ncomms11350
11. Brown AS, Levine JD, Green PG (2008) Sexual dimorphism in the effect of sound stress on neutrophil function. J Neuroimmunol 205:25-31. doi: 10.1016/j.jneuroim.2008.08.005
12. Sengelaub DR, Xu XM (2018) Protective effects of gonadal hormones on spinal motoneurons following spinal cord injury. Neural Regen Res 13:971-976. doi: 10.4103/1673-5374.233434
13. Sengelaub DR, Han Q, Liu NK, Maczuga MA, Szalavari V, Valencia SA et al (2018) Protective Effects of Estradiol and Dihydrotestosterone following Spinal Cord Injury. J Neurotrauma 35:825-841. doi: 10.1089/neu.2017.5329
14. Gould TW, Burek MJ, Ishihara R, Lo AC, Prevette D, Oppenheim RW (1999) Androgens rescue avian embryonic lumbar spinal motoneurons from injury-induced but not naturally occurring cell death. J Neurobiol 41:585-595. doi: 10.1002/(sici)1097-4695(199912)41:4<585::aid-neu13>3.0.co;2-#
15. Wang C, Zhang L, Ndong JC, Hettinghouse A, Sun G, Chen C et al (2019) Progranulin deficiency exacerbates spinal cord injury by promoting neuroinflammation and cell apoptosis in mice. J Neuroinflammation 16:238. doi: 10.1186/s12974-019-1630-1
16. Brommer B, Engel O, Kopp MA, Watzlawick R, Muller S, Pruss H et al (2016) Spinal cord injury-induced immune deficiency syndrome enhances infection susceptibility dependent on lesion level. Brain 139:692-707. doi: 10.1093/brain/awv375
17. Cogut V, Bruintjes JJ, Eggen B, van der Zee EA, Henning RH (2018) Brain inflammatory cytokines and microglia morphology changes throughout hibernation phases in Syrian hamster. Brain Behav Immun 68:17-22. doi: 10.1016/j.bbi.2017.10.009

18. Lin TH, Izumi K, Lee SO, Lin WJ, Yeh S, Chang C (2013) Anti-androgen receptor ASC-J9 versus anti-androgens MDV3100 (Enzalutamide) or Casodex (Bicalutamide) leads to opposite effects on prostate cancer metastasis via differential modulation of macrophage infiltration and STAT3-CCL2 signaling. Cell Death Dis 4:e764. doi: 10.1038/cddis.2013.270
19. Samir M, Glister C, Mattar D, Laird M, Knight PG (2017) Follicular expression of pro-inflammatory cytokines tumour necrosis factor-alpha (TNFalpha), interleukin 6 (IL6) and their receptors in cattle: TNFalpha, IL6 and macrophages suppress thecal androgen production in vitro. Reproduction 154:35-49. doi: 10.1530/REP-17-0053
20. Zhou YL, Yan YM, Li SY, He DH, Xiong S, Wei SF et al (2020) 6-O-angeloylplenolin exerts neuroprotection against lipopolysaccharide-induced neuroinflammation in vitro and in vivo. Acta Pharmacol Sin 41:10-21. doi: 10.1038/s41401-019-0261-5
21. Vignozzi L, Morelli A, Sarchielli E, Comeglio P, Filippi S, Cellai I et al (2012) Testosterone protects from metabolic syndrome-associated prostate inflammation: an experimental study in rabbit. J Endocrinol 212:71-84. doi: 10.1530/JOE-11-0289
22. Li J, Jia Z, Xu W, Guo W, Zhang M, Bi J et al (2019) TGN-020 alleviates edema and inhibits astrocyte activation and glial scar formation after spinal cord compression injury in rats. Life Sci 222:148-157. doi: 10.1016/j.lfs.2019.03.007
23. Wu D, Klaw MC, Connors T, Kholodilov N, Burke RE, Tom VJ (2015) Expressing Constitutively Active Rheb in Adult Neurons after a Complete Spinal Cord Injury Enhances Axonal Regeneration beyond a Chondroitinase-Treated Glial Scar. J Neurosci 35:11068-11080. doi: 10.1523/JNEUROSCI.0719-15.2015
24. Zhou T, Zheng Y, Sun L, Badea SR, Jin Y, Liu Y et al (2019) Microvascular endothelial cells engulf myelin debris and promote macrophage recruitment and fibrosis after neural injury. Nat Neurosci 22:421-435. doi: 10.1038/s41593-018-0324-9
25. Nead KT, Sinha S, Nguyen PL (2017) Androgen deprivation therapy for prostate cancer and dementia risk: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis 20:259-264. doi: 10.1038/pcan.2017.10
26. Leimgruber C, Quintar AA, Garcia LN, Petiti JP, De Paul AL, Maldonado CA (2013) Testosterone abrogates TLR4 activation in prostate smooth muscle cells contributing to the preservation of a differentiated phenotype. J Cell Physiol 228:1551-1560. doi: 10.1002/jcp.24314
27. Lu YC, Yeh WC, Ohashi PS (2008) LPS/TLR4 signal transduction pathway. Cytokine 42:145-151. doi: 10.1016/j.cyto.2008.01.006
28. Yang L, Zhou R, Tong Y, Chen P, Shen Y, Miao S et al (2020) Neuroprotection by dihydrotestosterone in LPS-induced neuroinflammation. Neurobiol Dis 140:104814. doi: 10.1016/j.nbd.2020.104814