Tropical Journal of Pharmaceutical Research April 2022; 21 (4): 749-754
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i4.10

Original Research Article

Propofol combined with hyperbaric oxygen improves the prognosis of spinal cord injury in rats via MAPK/ERK signaling pathway

Ting Shen, Yan Wang, Feng Xu, Jianyu Man, Juan Zeng*
Department of Anesthesiology, The Second Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang, China

For correspondence:* ***Email:* *1076006678@qq.com;* ***Tel:*** *+86-018907347565*

Sent for review: 29 October 2021 *Revised accepted: 28 March 2022*

Abstract

Purpose: *To determine the effect of propofol combined with hyperbaric oxygen on spinal cord injury (SCI) in rats.*
Methods: *A total of 36 Sprague-Dawley (SD) rats were randomly divided into sham group (S group), model group (M group), and propofol combined with hyperbaric oxygen group (P group). The Basso, Beattie and Bresnahan (BBB) scoring system was adopted to evaluate the recovery of motor function in rats. Subsequently, levels of interleukin-18 (IL-18) and IL-1β in the spinal cord tissues were determined using enzyme-linked immunosorbent assay (ELISA). Nerve cell apoptosis in the spinal cord tissues were examined via terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Finally, the protein expression levels of MAPK/ERK in the spinal cord tissues were assessed by Western blotting.*
Results: *Compared with S group, BBB score in M group decreased at days 3 and 10 after treatment. While the BBB score of rats in P group was significantly increased ($p < 0.05$), compared with S group. The expressions of IL-18 and IL-1β were significantly lower in S and P groups than in M group ($p < 0.05$). S and P groups had lower apoptosis rate in the spinal cord tissues than in M group. Furthermore, Western blotting results showed that protein expressions of MAPK/ERK pathway were higher in S group and P group than in M group ($p < 0.05$).*
Conclusion: *Propofol, combined with hyperbaric oxygen improves the prognosis of SCI rats probably by regulating MAPK/ERK signaling pathway, thus paving way for the development of a potential treatment for the management of spinal cord injury in humans.*

Keywords: *Propofol, MAPK/ERK signaling pathway, Hyperbaric oxygen, Spinal cord injury*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Spinal cord injury (SCI) is a common serious neurological disease. Because nerve cells are non-renewable, trauma and spinal cord tumors in numerous patients become complicated with hemiplegia or paraplegia, severely affecting quality of life. Spinal cord injury repair has always been a global challenge, and the incidence rates of SCI differ widely worldwide. Although the

global incidence rate is estimated to be 2.3 cases/100,000 residents in 2007 [1], the number found in the literature varies widely. As far as Spain's specific situation is concerned, the global number shown in a few epidemiological studies is 0.8-2.3 cases/100,000 residents [2,3].

Propofol is a novel narcotic drug with sedative effects, which is rapidly metabolized *in vivo*. Patients awaken quickly after treatment, and it is a useful approach that can be adopted in treating craniocerebral injury and reducing cerebral oxygen consumption [4]. Hyperbaric oxygen relieves microcirculation disturbance in patients with severe craniocerebral injury, and ensures sufficient oxygen supply [5]. SCI, similar to craniocerebral injury, is also a nerve cell injury.

So based on the above, the effect of propofol combined with hyperbaric oxygen on SCI in rats and its possible mechanisms, were explored in this study, so as to provide new ideas for improving the cure rate of SCI patients.

EXPERIMENTAL

Materials

Rats

Specific Pathogen Free (SPF)-grade Sprague-Dawley (SD) male rats (8 weeks-old, 180-200 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China).

Animal grouping and SCI modeling

A total of 36 SD rats were randomly divided into 3 groups, namely, sham operation group (S group), SCI model group (M group) and propofol combined with hyperbaric oxygen group (P group), and each group was further assigned into two subgroups according to two treatment time points, with 6 rats in each subgroup. After anesthesia with pentobarbital, the rats were fixed on a sterilized operation board, and the fur on the back was shaved off. Following disinfection with Aierdian, a longitudinal incision was made to expose the T_9 spinous process and vertebral arch, and then this spinal cord segment was clamped for 30 sec to induce the severe injury. In the S group, the spinal cord did not undergo weight dropping, but the muscles, fascia and skin were sutured layer by layer, and the incisions were disinfected again.

The rats in P group were put into a hyperbaric oxygen chamber for 40 min at a fixed time every day. After the pressure (2 L/min) was stabilized, the rats in P group were intraperitoneally injected with 74 mg/kg propofol every day. The rats in S group and M group were not put into the hyperbaric oxygen chamber and were intraperitoneally injected with an equivalent volume of normal saline in place of propofol. This study was approved by the Animal Ethics Committee of The Second Affiliated Hospital of University of South China Animal Center, and followed international guidelines for animal studies.

Basso, Beattie and Bresnahan (BBB) score of rats in each group

The BBB score was evaluated by technicians who are not members of the research group and familiar with BBB scoring, which reflect motor function and assess SCI or recovery of rats.

Hematoxylin and eosin (H&E) staining

The rats were sacrificed to remove spinal cord tissues, which were then fixed in 4 % paraformaldehyde for 72 h. After that, the fixed spinal cord tissues were dehydrated, embedded by paraffin and cut into about 5-um thick sections. The sections were deparaffinized in xylene, sequentially washed with alcohol at different concentrations, and washed with pure water to remove alcohol. Next, the deparaffinized sections were soaked in hematoxylin for staining and added with differentiation solution after hematoxylin was washed away with clear water. The obtained light blue sections were rinsed under running water until they turned sky blue and then soaked in eosin solution. Thereafter, the eosin solution was washed away with clear water. Following water absorption, the glass slides were put into alcohol for complete dehydration, and into xylene for transparentization. Subsequently, the successfully transparentized sections were removed, dripped with gum dropping liquid and sealed with cover glasses. Pathological changes in the spinal cord tissues dried by baking were observed under an optical microscope.

Determination of IL-18 and IL-1β in spinal cord tissues

About 0.3 g spinal cord tissues were taken for homogenization and centrifuged for 10 min at 4 °C and 1000 g to obtain the supernatant. According to the instructions on the ELISA kits, standard substance and experimental samples were added to the microplate. After the reaction well was sealed, incubation was carried out on a horizontal oscillator for 2 h at room temperature. ELISA was performed according to the

manufacturer's protocols.

Detection of apoptosis via terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

The rats were sacrificed at a set time point, and then the spinal cord tissues were fixed in 4 % paraformaldehyde, rinsed and infiltrated with 0.1 % Triton X-100 according to the instructions of TUNEL cell apoptosis detection kits. Then, the paraffin-embedded sections of spinal cord tissues were prepared, the sealed section samples were marked with fluorescent color development agent, and TUNEL-positive cells were examined under a fluorescence microscope. At least, 10 fields of view were selected to calculate the TUNEL-positive cell rate, representing the apoptosis rate of spinal cord tissue cells.

Western blotting

The proteins were extracted using RIPA buffer with protease and phosphatase inhibitor cocktails (Roche) from the spinal cord tissues, which concentration was then evaluated. Next, the proteins were mixed with sodium dodecyl sulphate-loading buffer and boiled at 95 °C for 3 min. Subsequently, 8 – 10 % polyacrylamide gel electrophoresis was applied to separate the equal amounts of proteins (Beijing ApplyGene Co., Ltd., Beijing, China), which were then transferred onto a polyvinylidene fluoride (PVDF) membrane. After sealing with 10 % skim milk, the membranes were incubated with primary antibody (1: 1000) at 4 °C overnight.

The next day, after washing thrice with the prepared 1 × Tris-buffered saline with Tween®20 (TBST), the proteins were placed into the secondary antibody corresponding to the primary antibody, incubated in a shaker at room temperature for 1 h, and then washed 3 times with 1 × TBST. Thereafter, the PVDF membrane was taken out, and electrochemiluminescence (ECL) solution (Thermo Fisher Scientific, Waltham, MA, USA) was added for exposure and image development. Lastly, ImageJ software (NIH, Bethesda, MD, USA) was utilized to analyze each protein band.

Statistical analysis

SPSS statistical analysis software (version 26.0) was adopted for data analysis. Measurement data are expressed as mean ± standard deviation. Comparison between multiple groups was done using one-way ANOVA test followed by post hoc test (least significant difference). $P < 0.05$ was considered statistically significant.

RESULTS

BBB score of rats

Compared with that before operation, the BBB score in S group did not significantly change ($p > 0.05$), suggesting that the operation does not affect the BBB score of rats. Compared with that in M group, the BBB score of rats in P group was significantly increased at 3 d and 10 d ($p < 0.01$) (Table 1).

IL-1β and IL-18 expressions in rats

Rats in S group had normal spinal cord tissues with no abnormalities such as edema, inflammatory cell infiltration and hemorrhage. Rats in M group suffered from obvious tissue edema and evident hemorrhage, necrosis and local inflammatory cell infiltration for 10 days (day). Furthermore, in comparison with those in M group, the edema was notably relieved, and no obvious hemorrhage or necrosis was found locally in P group. In contrast to those in M group, the expressions of IL-1β and IL-18 in the spinal cord tissues of rats were markedly reduced in S group and decreased in P group ($p < 0.05$). Besides, in comparison with those at 3 d, the expressions of IL-1β and IL-18 in M group and P group were elevated at 10 d group ($p < 0.05$, Table 2).

Nerve cell apoptosis in rat spinal cord tissues

Compared with those in M group, apoptotic nerve cells in the spinal cord tissues of rats were significantly reduced in S group ($p < 0.01$) and decreased in P group ($p < 0.05$), with statistically significant differences.

Table 1: Comparison of BBB score of rats in each group at different time points (n = 6, $\bar{}$mean ± SD)

Group	Before operation	Day 3	Day 10
S group	21 ± 0.00	21 ± 0.00[a]	21 ± 0.00[a]
M group	21 ± 0.00	2.38 ± 0.16[b]	1.84 ± 0.13[b]
P group	21 ± 0.00	4.51 ± 0.48[bc]	9.61 ± 1.07[bd]

[a]$p > 0.05$, [b]$p < 0.01$ *vs.* before operation. [c]$p > 0.05$, [d]$p < 0.01$ *vs.* M group

Table 2: Comparison of IL-1β and IL-18 expressions in the spinal cord tissues among each group of rats (n = 6, mean ± SD)

Group	Day 3		Day 10	
	IL-1β (pg/mL)	*IL-18 (pg/mL)*	*IL-1β (pg/mL)*	*IL-18 (pg/mL)*
S group	13.61 ± 1.17	70.84 ± 6.98	14.18 ± 1.37	69.67 ± 6.51
M group	69.57 ± 7.10	238.42 ± 21.37	97.68 ± 9.27	468.16 ± 42.84
P group	38.61 ± 4.02	125.64 ± 11.62	51.69 ± 4.82	238.27 ± 24.66

[a]$P < 0.01$, *vs.* S group; [b]$p < 0.05$, *vs.* M group; [c]$p < 0.05$, *vs.* M group; at day 3, and [d]$p < 0.05$ *vs.* P group at day 3

In comparison with those at day 3, apoptotic nerve cells were increased at day 10 in M group ($p < 0.01$), but they did not change significantly at 10 d in P group ($p > 0.05$, Figure 1).

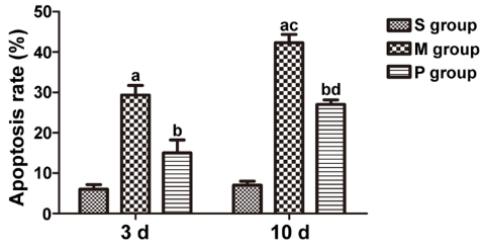

Figure 1: Comparison of the apoptosis rate in the spinal cord tissues of rats in each group. [a]$p < 0.01$, *vs.* S group; [b]$p < 0.05$, *vs.* M group; [c] $p < 0.05$, *vs.* M group at day 3, and [d]$p > 0.05$ *vs.* P group at day 3

MAPK and ERK expressions in rat spinal cord tissues

Compared with M group, S group exhibited distinctly raised expression levels of MAPK and ERK ($p < 0.01$), and P group showted raised expression levels of MAPK and ERK ($p < 0.05$, Figure 2).

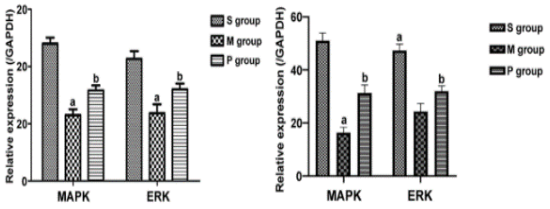

Figure 2: Comparisons of MAPK and ERK expression levels and the absorbance of protein bands in spinal cord tissues among the various groups of rats. [a]$P < 0.01$ *vs.* S group, [b]$p < 0.05$ *vs.* M group

DISCUSSION

At present, there are about 180,000 cases of SCI globally, and this number is still on the rise. According to an epidemiological investigation, falling as well as traffic accidents are the most common causes of SCI [6,7]. Currently, the treatment of SCI involves traditional drug therapy, surgical therapy, cell therapy, gene therapy and tissue engineering therapy [8-11]. Spinal cord injury includes trauma and tumor cell entry, causing a series of nervous system problems, such as loss of motor and sensory functions, intestinal and bladder dysfunction, spasm, neuralgia and autonomic nerve reflex disorders. The pathophysiology of SCI is traditionally divided into two stages: primary SCI and secondary SCI [12,13]. The complex pathophysiology of SCI includes pathological defects [14-16]. However, these complex pathophysiological processes still need to be studied further.

The emphasis of current treatments of SCI patients is mainly targeted at relieving early compression, nerve compression, as well as the prevention of complications [17,18]. There are many studies on nerve cell regeneration, but the protection of spinal cord nerve cells has rarely been investigated. As propofol exerts sedative effects and can reduce oxygen consumption [19], and hyperbaric oxygen provides sufficient oxygen to the body [20], the effects of propofol combined with hyperbaric oxygen on SCI in rats and its possible action mechanisms were explored in this study.

It was found in this research that propofol combined with hyperbaric oxygen could increase the BBB score of rats and contribute to motor function recovery, and these effects were more obvious at 10 d. H&E staining also revealed that propofol combined with hyperbaric oxygen effectively relieves spinal cord edema and reduces inflammatory cell infiltration. When the time was extended, the injury in M group was aggravated, and the tissue edema in P group was gradually alleviated. TUNEL assay of apoptosis also confirmed that propofol combined with hyperbaric oxygen improved the apoptosis rate of nerve cells, but it would be reduced as the treatment time was increased. In the meantime, the mechanism by which propofol combined with hyperbaric oxygen improved SCI in rats was investigated. It was discovered that the expressions of MAPK and ERK were up-regulated in the M group but down-regulated in

the P group after intervention, indicating that propofol combined with hyperbaric oxygen may play a role by activating the MAPK/ERK signaling pathway.

However, there are still some limitations in this present study. First, there were only 6 rats in each group, so the sample size was too small and needs to be expanded in further research. Second, there was a lack of the specific inhibitors that knock out gene in rats so as to confirm that the prognosis of SCI rats can be improved by activating the MAPK/ERK pathway. If propofol combined with hyperbaric oxygen is proved to be effective in animals with small side effects, the approval of the Ethics Committee can be applied for to carry out clinical experiments. This may be an efficacious way to improve the cure rate of SCI patients in the future.

CONCLUSION

Propofol combined with hyperbaric oxygen improves the prognosis of SCI in rats, probably by regulating MAPK/ERK signaling pathway. Thus, this combination treatment has potentials for use in the management of SCI in humans.

DECLARATIONS

Acknowledgement

This work was supported by Hunan Provincial Health Commission 2019 Annual Scientific Research (Grant no. C2019098) and Hengyang City guiding Plan Project (Grant no. 202121034434).

Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ting Shen and Juan Zeng designed the study and performed the experiments, Yan Wang and Feng Xu collected the data, Ting Shen and Jianyu Man analyzed the data, Ting Shen and Juan Zeng prepared the manuscript. All authors read and approved the final manuscript.

Open Access

REFERENCES

1. *Lee BB, Cripps RA, Fitzharris M, Wing PC. The global map for traumatic spinal cord injury epidemiology: update 2011, global incidence rate. Spinal Cord 2014; 52(2): 110-116.*
2. *Mao X, Jiang Z, Shi C, Lu J, Rao G. Eriodictyol attenuates spinal cord injury by activating Nrf2/HO-1 pathway and inhibiting NF-kappa B pathway. Trop J Pharm Res 2020; 19(8): 1611-1617.*
3. *Perez K, Novoa AM, Santamarina-Rubio E, Narvaez Y, Arrufat V, Borrell C, Cabeza E, Cirera E, Ferrando J, Garcia-Altes A, et al. Incidence trends of traumatic spinal cord injury and traumatic brain injury in Spain, 2000-2009. Accid Anal Prev 2012; 46: 37-44.*
4. *Wang Y, Yao F, Lin Y, Xiao S. A comparative study of the analgesic effects of sevoflurane and propofol in children following otolaryngology surgical procedures: A pilot study. Trop J Pharm Res 2021; 20(1): 211-217.*
5. *Bragin DE, Statom GL, Nemoto EM. Induced Dynamic Intracranial Pressure and Cerebrovascular Reactivity Assessment of Cerebrovascular Autoregulation After Traumatic Brain Injury with High Intracranial Pressure in Rats. Acta Neurochir Suppl 2018; 126: 309-312.*
6. *Ning GZ, Yu TQ, Feng SQ, Zhou XH, Ban DX, Liu Y, Jiao XX. Epidemiology of traumatic spinal cord injury in Tianjin, China. Spinal Cord 2011; 49(3): 386-390.*
7. *Wu Q, Li YL, Ning GZ, Feng SQ, Chu TC, Li Y, Hao Y, Wu QL. Epidemiology of traumatic cervical spinal cord injury in Tianjin, China. Spinal Cord 2012; 50(10): 740-744.*
8. *Sabapathy V, Tharion G, Kumar S. Cell Therapy Augments Functional Recovery Subsequent to Spinal Cord Injury under Experimental Conditions. Stem Cells Int 2015; 2015: 132172.*
9. *Yousefifard M, Rahimi-Movaghar V, Nasirinezhad F, Baikpour M, Safari S, Saadat S, Moghadas JA, Asady H, Razavi TS, Hosseini M. Neural stem/progenitor cell transplantation for spinal cord injury treatment; A systematic review and meta-analysis. Neurosci 2016; 322: 377-397.*
10. *Lin XY, Lai BQ, Zeng X, Che MT, Ling EA, Wu W, Zeng YS. Cell Transplantation and Neuroengineering Approach for Spinal Cord Injury Treatment: A Summary of Current Laboratory Findings and Review of Literature. Cell Transplant 2016; 25(8): 1425-1438.*
11. *Dumont CM, Margul DJ, Shea LD. Tissue Engineering Approaches to Modulate the Inflammatory Milieu*

following Spinal Cord Injury. Cells Tissues Organs 2016; 202(1-2): 52-66.

12. *Ahuja CS, Nori S, Tetreault L, Wilson J, Kwon B, Harrop J, Choi D, Fehlings MG. Traumatic Spinal Cord Injury-Repair and Regeneration. Neurosurg 2017; 80(3S): S9-S22.*
13. *Freund P, Seif M, Weiskopf N, Friston K, Fehlings MG, Thompson AJ, Curt A. MRI in traumatic spinal cord injury: from clinical assessment to neuroimaging biomarkers. Lancet Neurol 2019; 18(12): 1123-1135.*
14. *Sabelstrom H, Stenudd M, Reu P, Dias DO, Elfineh M, Zdunek S, Damberg P, Goritz C, Frisen J. Resident neural stem cells restrict tissue damage and neuronal loss after spinal cord injury in mice. Sci 2013; 342(6158): 637-640.*
15. *Lukovic D, Stojkovic M, Moreno-Manzano V, Jendelova P, Sykova E, Bhattacharya SS, Erceg S. Concise review: reactive astrocytes and stem cells in spinal cord injury: good guys or bad guys? Stem Cells 2015; 33(4): 1036-1041.*
16. *Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV. Astrocyte scar formation aids central nervous system axon regeneration. Nature 2016; 532(7598): 195-200.*
17. *Ahuja CS, Wilson JR, Nori S, Kotter M, Druschel C, Curt A, Fehlings MG. Traumatic spinal cord injury. Nat Rev Dis Primers 2017; 3: 17018.*
18. *Haisma JA, van der Woude LH, Stam HJ, Bergen MP, Sluis TA, Post MW, Bussmann JB. Complications following spinal cord injury: occurrence and risk factors in a longitudinal study during and after inpatient rehabilitation. J Rehabil Med 2007; 39(5): 393-398.*
19. *Mickey BJ, White AT, Arp AM, Leonardi K, Torres MM, Larson AL, Odell DH, Whittingham SA, Beck MM, Jessop JE, et al. Propofol for Treatment-Resistant Depression: A Pilot Study. Int J Neuropsychopharmacol 2018; 21(12): 1079-1089.*
20. *Bragin DE, Statom GL, Nemoto EM. Induced Dynamic Intracranial Pressure and Cerebrovascular Reactivity Assessment of Cerebrovascular Autoregulation After Traumatic Brain Injury with High Intracranial Pressure in Rats. Acta Neurochir Suppl 2018; 126: 309-312.*