

Original Research Article

HSF-1 attenuates isoflurane-induced cognitive dysfunction by inhibiting TLR2 expression

Xu Jia¹, Xuezhu Huang^{1*}, Mingda Duan², Wei Qiu¹, Xuanbo Zhang¹, Xin Wang³

¹Department of Anesthesiology, Affiliated Hospital of Yanbian University, Yanji City, Jilin Province 133000, ²Department of Anesthesiology, ³Department of Otolaryngology, Hainan Hospital of General Hospital of PLA, Sanya, Hainan Province 572013, China

*For correspondence: **Email:** xzhuang6664@163.com; **Tel:** +86-0433-2660157

Sent for review: 2 June 2022

Revised accepted: 1 September 2022

Abstract

Purpose: To investigate the regulatory effects of heat shock factor 1 (HSF-1) in the progression of postoperative cognitive dysfunction (POCD).

Methods: Isoflurane (ISO)-induced POCD model in rats was established to determine the role of HSF-1 in POCD. Morris water maze test was used to evaluate the learning and memory abilities of the POCD rats while mRNA and protein levels of HSF-1 were determined by RNA extraction/quantitative real-time polymerase chain reaction (RT-qPCR) and western blot analysis, respectively.

Results: The mRNA and protein levels of HSF-1 were significantly reduced in ISO model, but OE-HSF-1 treatment significantly elevated HSF-1 level ($p < 0.05$). ISO treatment also significantly decreased escape latency but increased the decreased target quadrant of the rats, while HSF-1 upregulation reversed these effects ($p < 0.05$). Additionally, HSF-1 alleviated ISO-induced hippocampal injury, improved ISO-induced hippocampal inflammation, and inhibited ISO-induced hippocampal apoptosis. Furthermore, HSF-1 was modulated by POCD via TLR2/NF- κ B pathway ($p < 0.05$).

Conclusion: HSF-1 attenuates ISO-induced cognitive dysfunction by suppressing TLR2 expression. This activity provides a potential strategy to prevent POCD via HSF-1.

Keywords: Heat shock factor 1, Isoflurane, Cognitive dysfunction, Hippocampal inflammation

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, 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

General anesthetics have been extensively applied during surgical procedures for centuries [1]. However, temporary loss of consciousness and reactivity in patients due to the application of general anesthetics might result in adverse effects [2]. Recently, some studies have indicated that anesthesia exposure after major surgery may be associated with cognitive

dysfunction and may increase the risk of Alzheimer's disease or dementia [3,4]. Postoperative cognitive dysfunction (POCD) is one of the complications after anesthesia in surgery [5]. Postoperative cognitive dysfunction is associated with a prolonged duration of hospitalization, a reduced quality of life, and an increased mortality rate [6]. Preventing POCD in patients is crucial.

As an evolutionarily conserved stress response factor, heat shock factor (HSF) modulates the transcription of heat shock protein (HSP) and promotes chaperone activity to reduce proteolytic toxic stress in eukaryotic cells [7]. As an HSF family member, HSF-1 is a vital transcription factor involved in the mechanisms of various diseases. For example, HSF-1 regulates autophagy to inhibit the LPS-induced release of inflammatory cytokines. Alleviation of HSF-1 decreases the development of atherosclerosis by increasing the levels of cholesterol 7 α -hydroxylase (CYP7A1) and multidrug transporter (MDR1) [8]. Heat shock factor-1 regulates the JAK2/STAT3 pathway and is implicated in the development of cardiac hypertrophy induced by ischemia [9]. Although numerous studies have validated the role of HSF-1 in different diseases, whether HSF-1 participates in the development of POCD is unclear.

Hence, this study aimed to assess HSF-1 function in the development of POCD.

EXPERIMENTAL

Rat models

Twenty-four Sprague-Dawley aged 18 months were obtained from Vital-River Laboratory Animal Technology (Beijing, China) and kept with free access to water and food under a standard environment. The rats were divided into 4 groups with 6 rats in each group (control group, ISO group, ISO + vector group and ISO + OE-HSF-1 group). The rats were kept in room air for both control and ISO groups, and an anesthetic chamber with ISO (2%; Sigma-Aldrich; Merck KGaA) and 100 % oxygen were used to treat each rat for 4 h. The brain tissues were collected from the sacrificed rats, and the hippocampi were dissected out for further use. The animal study was approved by the Ethics Committee of Affiliated Hospital of Yanbian University (approval no. 20211201S024) and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [10].

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of S-100 β , neuron-specific enolase (NSE), interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α) and IL-6 were measured using respective ELISA Kits. The harvested hippocampal tissues were homogenized on ice in Tris-HCl buffer (20 mM) with protease inhibitors. After centrifuging for 10 min at 10,000 g, the supernatant was subjected to ultracentrifugation for 2 h at 150,000 g. The concentrations of S-100 β , NSE, IL-1 β , TNF- α

and IL-6 were evaluated using the Bradford protein assay.

Morris water maze test

The Morris water maze test was used to evaluate the number of times the rats crossed the platform, distance traveled in the quadrant, and escape latency time of rats, representing their learning and memory abilities. Warm water (23 °C; 30 cm depth) was placed in an open-field circular pool, and a 10-cm-diameter, white, hidden platform was positioned 1.5 cm below the water surface. The pool was separated into isolated chambers by a black curtain. The motions of the rats were recorded using an automated video tracking system, and Morris water maze test software (DigBehv-MM, Jiliang) was applied for data analysis. The rats were trained on four trials of the navigation test daily, the escape latency to the hidden platform was recorded in the first five days, the hidden platform was removed, and the number of times the former location of the platform crossed on the sixth day was recorded.

TUNEL staining

The rat hippocampal tissues were fixed with neutral-buffered formalin (10%) and then embedded with paraffin. After dewaxing and rehydration, the tissues were antigen retrieved using sodium citrate buffer (10.2 mmol/L). TUNEL staining was performed using an *in situ* cell death detection kit (catalog number: 11684817910; Roche) at 95 °C for 20 min. The number of brown nuclei (TUNEL-positive nuclei) was counted in 10 microscopic fields (400x) of each section.

Immunofluorescence (IF) staining

Following dehydration and embedment, the rat hippocampal tissues were sliced into sections using a microtome (Leica, Germany). After sealing with goat serum (10%; Solarbio, China) and Triton X-100 (0.3%; Solarbio, China) in PBS, the tissues were incubated with primary antibodies (anti-p65, ab32536; 1:100; Abcam, Shanghai, China) for 12 h at 4 °C. PBS was then applied to wash the tissues, followed by incubation with secondary antibodies (1:1000; Alexa Fluor® 594; goat anti-rabbit; Abcam) for 1 h and with 4',6-diamidino-2-phenylindole (Solarbio, China) for 5 min. A fluorescence microscope was used to observe the fluorescence, and Image-Pro Plus software was applied to analyze the results.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the hippocampal tissues using TRIzol reagent (GeneAll, Korea). Complementary DNA (cDNA) was synthesized using a commercial reverse transcription kit (BR631-096 Biofact; Korea). An Applied Biosystems 7500 real-time PCR system (Applied Biosystems) was employed for PCR. The expression of HSF-1, IL-1 β , TNF- α and IL-6 was analyzed using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as internal control. The primer sequences are presented in Table 1.

Western blot analysis

Radio immunoprecipitation assay lysis buffer (Beyotime, China) was employed to extract the proteins, whose levels were quantified using the BCA protein assay kit (Beyotime, China). After separation by 12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis, the proteins were transferred onto PVDF membranes. The membranes were blocked using 5% skimmed milk, followed by incubation with primary antibodies at 4 °C for 12 h. The membranes were rinsed with phosphate-buffered saline, and the secondary antibody was added for 2 h. Finally, enhanced chemiluminescence (ECL) kit (GE Healthcare, USA) was used to assess the protein signals. The primary antibodies used were anti-HSF-1 (ab61382; 1:1000; Abcam), anti-BAX (ab182733; 1:2000; Abcam), anti-Bcl-2 (ab196495; 1:2000; Abcam), anti-HMGB1 (ab18256; 1 μ g/mL; Abcam), anti-TLR2 (ab209217; 1:1000; Abcam), anti-MyD88 (ab219413; 1:1000; Abcam), anti-TAK1 (ab109526; 1:1000; Abcam), anti-p-TAK1 (ab109404; 1:1000; Abcam) and anti-GAPDH (ab181602; 1:10000; Abcam).

Hematoxylin-eosin (H&E) staining

The rat hippocampal tissues were cut into slices, and paraformaldehyde solution (4%) was applied to fix the slices. After rinsing and dehydration, the

tissues were embedded in paraffin. Hematoxylin and eosin solution was then added, and a NIKON light microscope was used to assess the pathological changes of hippocampal tissues in the rats.

Statistical analysis

Data analysis was performed using SPSS version 22.0 software, and the data were presented as means \pm standard deviation (SD). Differences between the two groups were tested using Student's t-test, while differences among multiple groups were compared by one-way ANOVA and Tukey's test. All the experiments were conducted at least 3 times. $P < 0.05$ was deemed statistically significant.

RESULTS

HSF-1 ameliorates ISO-induced cognitive dysfunction

The mRNA and protein levels of HSF-1 were reduced in the ISO model; OE-HSF-1 treatment significantly elevated the levels (Figure 1A-1B). The learning and memory abilities of the rats were identified using the Morris water maze test. ISO treatment significantly increased the escape latency and decreased the times across the stealth platform and time spent in the target quadrant, while HSF-1 upregulation reversed these effects (Figure 1C). Thus, HSF-1 ameliorates ISO-induced cognitive dysfunction.

Heat shock factor-1 (HSF-1) alleviates ISO-induced hippocampal injury

The pathological changes of the rat hippocampal tissues were assessed by hematoxylin and eosin staining. The nuclei in the hippocampal tissues were concentrated and vacuolated and arranged disorderedly and loosely after ISO treatment, whereas these effects were alleviated by increased HSF-1 expression (Figure 2 A).

Table 1: Primers used in PCR

Primer	Direction	Sequence
HSF-1	Forward	5'-TCTCCTGTCCTGTGTGCCTAGC-3'
	Reverse	5'-CAGGTCAACTGCCTACACAGACC-3'
IL-1 β	Forward	5'-ACAACTTTGGTATCGTGAAGG-3'
	Reverse	5'-CGTTATCCCATGTGTGCGAAGAA-3'
TNF- α	Forward	5'-GAGGCCAAGCCCTGGTATG-3'
	Reverse	5'-CGGGCCGATTGATCTCAGC-3'
IL-6	Forward	5'-ACTCACCTCTTCAGAACGAATTG-3
	Reverse	5'-CCATCTTTGGAAGGTTTCAGGTTG-3'
GAPDH	Forward	5'-GCCACAACGACCCCTTCATG-3'
	Reverse	5'-TGCCAGTGAGCTTCCCGTTC-3'

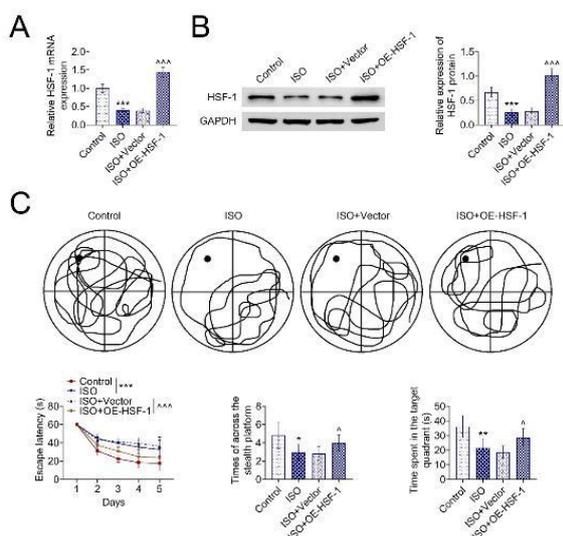


Figure 1: HSF-1 ameliorates ISO-induced cognitive dysfunction. (A&B) mRNA and protein levels of HSF-1. (C) The Morris water maze test was applied to measure the learning and memory abilities of the rats. * $P < 0.05$, *** $p < 0.001$, relative to the control group. ^ $p < 0.015$, ^^^ $p < 0.001$, relative to the ISO + vector group

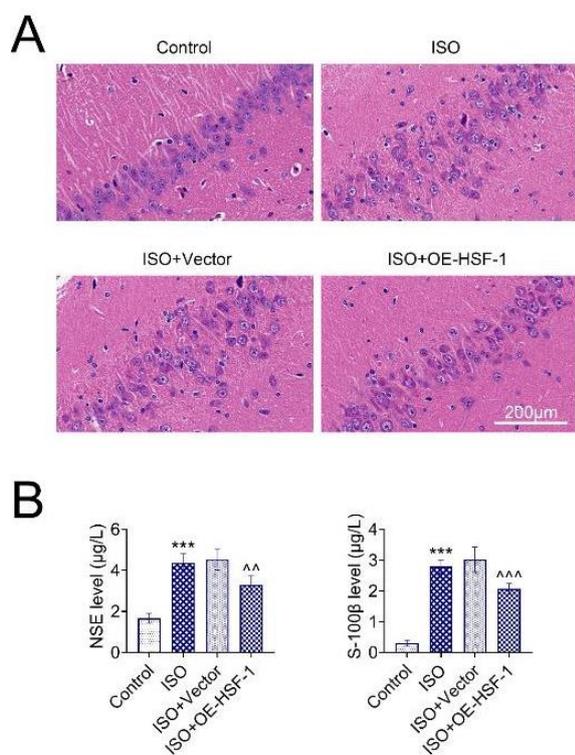


Figure 2: HSF-1 alleviates ISO-induced hippocampal injury. (A) Pathological changes in rat hippocampal tissues, as determined by hematoxylin and eosin staining. (B) Levels of S-100β and NSE. *** $p < 0.001$ relative to the control group. ^^ $P < 0.01$, ^^ $p < 0.001$, relative to the ISO + vector group

In addition, elevated HSF-1 counteracted the ISO-mediated increase in brain injury markers (S-100β and NSE) (Figure 2 B). Taken together, HSF-1 alleviates ISO-induced hippocampal injury.

Heat shock factor-1 improves ISO-induced hippocampal inflammation

Next, the role of HSF-1 in hippocampal inflammation was further investigated. qRT-PCR revealed that ISO induced the mRNA levels of hippocampal inflammation indexes (IL-1β, TNF-α and IL-6), but HSF-1 upregulation offset these effects (Figure 3 A). Similarly, the ISO-induced IL-1β, TNF-α and IL-6 levels were neutralized by HSF-1 overexpression (Figure 3B). Thus, HSF-1 improves ISO-induced hippocampal inflammation.

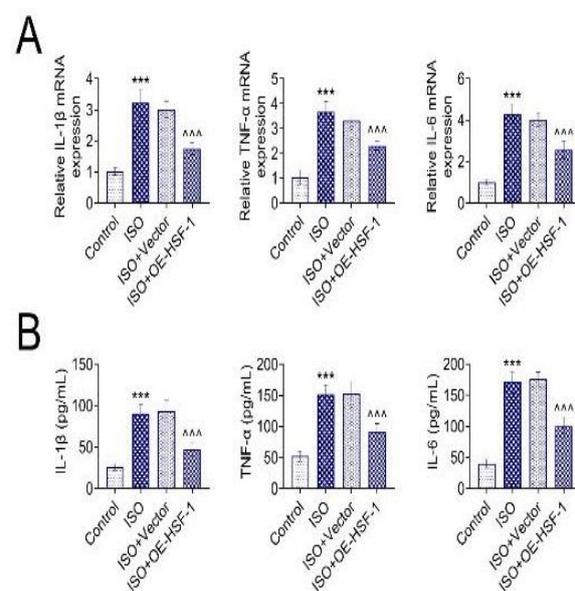


Figure 3: HSF-1 improves ISO-induced hippocampal inflammation. (A) Expression of IL-1β, TNF-α and IL-6. (B) Levels of IL-1β, TNF-α and IL-6. *** $P < 0.001$, relative to the control group. ^^^ $p < 0.001$, relative to the ISO + vector group

HSF-1 inhibits ISO-induced hippocampal apoptosis

The TUNEL assay revealed that ISO increased the hippocampal cell apoptosis, but HSF-1 overexpression reversed this effect (Figure 4 A and B). Furthermore, Bax protein levels increased, while Bcl-2 protein levels decreased, in the ISO-treated group. The upregulation of HSF-1 reversed the effect of ISO on Bax and Bcl-2 (Figure 4 C). Thus, HSF-1 inhibits ISO-induced hippocampal apoptosis.

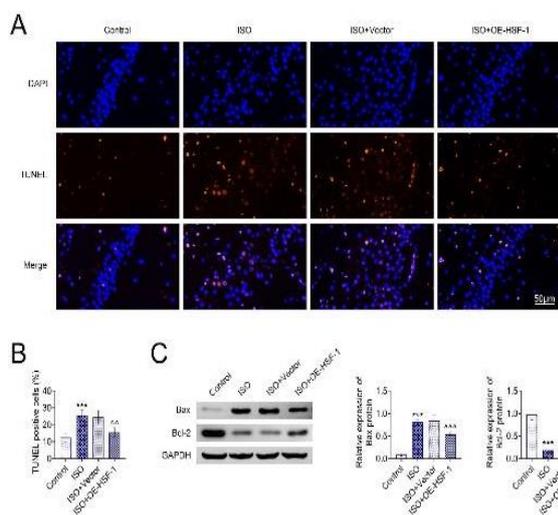


Figure 4: HSF-1 inhibits ISO-induced hippocampal apoptosis. (A-B) Hippocampal apoptosis. (C) Protein levels of Bax and Bcl-2. ^{***}*P* < 0.001 relative to the control group. [^]*p* < 0.01, ^{^^}*p* < 0.001, relative to the ISO + vector group

Heat shock factor-1 modulates TLR2/NF-κB pathway

The regulatory mechanism of HSF-1 in POCD was investigated. The protein levels of TLR2/NF-κB pathway-associated proteins, including HMGB1, TLR2, MyD88 and p-TAK1, were upregulated following ISO treatment, but these effects were offset by HSF-1 induction (Figure 5

A). Furthermore, immunofluorescence staining revealed that the nuclear translocation of p65 induced by ISO was reversed by HSF-1 induction (Figure 5 B). Hence, HSF-1 modulates the TLR2/NF-κB pathway.

DISCUSSION

Anesthesia-induced POCD is a prevalent complication in patients receiving surgery [11]. A few weeks post-surgery, POCD might occur and is associated with impaired cognitive function and elevated mortality in patients. Identifying novel biomarkers to prevent POCD is critical clinically. As a frequently used anesthetic, ISO is extensively applied in clinics and demonstrates acute and chronic effects on brain function in patients [12]. Cognitive dysfunction is induced by ISO via neuroinflammation. Therefore, the ISO-induced cognitive dysfunction model is widely used to investigate the pathogenesis of POCD. For example, ISO-mediated cognitive function is attenuated by astrocytic network reconstruction regulated by connexin 43 gap junction in mice [13].

ISO-induced cognitive dysfunction is inhibited by Angiogenic Factor with G-patch and FHA domains 1 (AGGF1) by activating the PI3K/AKT signaling pathway.

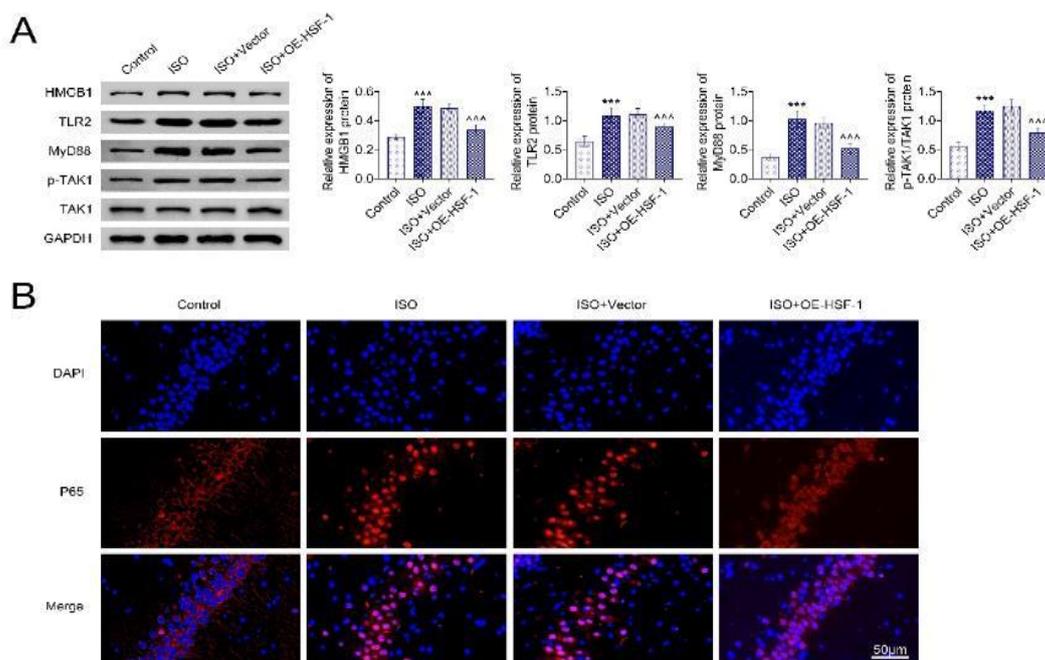


Figure 5: HSF-1 modulates TLR1/NF-κB pathway. (A) Expression levels of HMGB1, TLR2, MyD88, p-TAK1 and TAK1. (B) Nuclear translocation status of p65. ^{***}*P* < 0.001 relative to the control group. ^{^^}*p* < 0.001 relative to the ISO + vector group

Dual-specificity phosphatase 14 (DUSP14) exerts a neuroprotective role to suppress the ISO-mediated inflammatory response, cognitive impairment and pyroptosis by modulating the NLRP3 inflammasome in aged rats [14]. PYRIN-containing Apaf1-like protein 1 (PYPAF1) promotes the cognitive dysfunction induced by ISO in aged rats after surgery [15].

In the present study, to further probe the role of HSF-1 in POCD, we constructed an ISO-induced POCD model in rats. HSF-1 expression was decreased in the ISO-induced POCD model, and HSF-1 ameliorated ISO-induced cognitive dysfunction in rats. In addition, ISO-induced hippocampal injury and inflammation as well as hippocampal apoptosis were alleviated by HSF-1 overexpression. Thus, HSF-1 is involved in attenuating ISO-induced cognitive dysfunction in the POCD model.

In a previous study by Shang *et al* [15], HSF-1 negatively regulated HMGB1 expression HMGB1 to improve asthma via the TLR4/MyD88/NF- κ B signaling pathway. Thus, HSF-1 might modulate ISO-induced cognitive dysfunction by mediating TLR/NF- κ B pathway. Furthermore, several studies have confirmed the essential role of the TLR/NF- κ B signaling pathway in various diseases. For example, the TLR/NF- κ B signaling pathway is suppressed in MPTP-induced Parkinson's disease by cordycepin [16]. In diabetic nephropathy rats, renal function is ameliorated by inhibiting the TLR/NF- κ B pathway and inflammation following the application of umbelliferone [17]. MicroRNA-181a binds with CRY1 to mediate glomerular sclerosis and renal tubular epithelial injury by inactivating the TLR/NF- κ B pathway in rats with chronic kidney diseases. The TLR/NF- κ B signaling pathway is involved in miR-146a-modulated repair and inflammation in the spinal cord injury rat model [18]. The function of TLR/NF- κ B pathway in ISO-induced cognitive dysfunction has rarely been investigated. Here, HSF-1 overexpression suppressed the levels of TLR2/NF- κ B pathway-related proteins in ISO-induced cognitive dysfunction rats. Additionally, HSF-1 elevation attenuated the nuclear translocation of p65 induced by ISO. Taken together, HSF-1 modulates the TLR2/NF- κ B pathway in POCD.

CONCLUSION

The findings of this study show that HSF-1 attenuates ISO-induced cognitive dysfunction by suppressing TLR2 expression, highlighting the potential of HSF-1 in the development of prevention and treatment strategies for POCD.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed 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. Xu Jia and Xuezhu Huang designed and performed the study. Mingda Duan supervised the data collection and analyzed and interpreted the data. Wei Qiu, Xuanbo Zhang and Xin Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All the authors read and approved the manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Si J, Jin Y, Cui M, Yao Q, Li R, Li X. Neuroprotective effect of miR-212-5p on isoflurane-induced cognitive

- dysfunction by inhibiting neuroinflammation. *Toxicol Mech Methods* 2021; 31(7): 501-506.
2. Qian. D, Chen. C, Dong. B, Qiu. Y. Up-regulation of miR-20a ameliorates sevoflurane anesthesia-induced cognitive impairment in rats by targeting EphA4. *Trop J Pharm Res* 2021; 20(4): 727-733.
 3. Chen L, Xie W, Xie W, Zhuang W, Jiang C, Liu N. Apigenin attenuates isoflurane-induced cognitive dysfunction via epigenetic regulation and neuroinflammation in aged rats. *Arch Gerontol Geriatr* 2017; 73: 29-36.
 4. Lim. H-k, Lee. S-y, Wu. C-w, Lai. JC-y, Ho. Y-h, Ku. H-c. Comparative study between oral acetaminophen and lidocaine spray on endotracheal tube-related sore throat in adult intensive care. *Signa Vitae* 2021; 17(5): 71-76.
 5. Liu L, Shang L, Jin D, Wu X, Long B. General anesthesia bullies the gut: a toxic relationship with dysbiosis and cognitive dysfunction. *Psychopharmacology* 2022; 239(3): 709-728.
 6. Chen MH, Liao Y, Rong PF, Hu R, Lin GX, Ouyang W. Hippocampal volume reduction in elderly patients at risk for postoperative cognitive dysfunction. *J Anesth* 2013; 27(4): 487-492.
 7. Kühnel A, Schilling D, Combs SE, Haller B, Schwab M, Multhoff G. Radiosensitization of HSF-1 Knockdown Lung Cancer Cells by Low Concentrations of Hsp90 Inhibitor NVP-AUY922. *Cells* 2019; 8(10).
 8. Krishnamurthy K, Glaser S, Alpini GD, Cardounel AJ, Liu Z, Ilangovan G. Heat shock factor-1 knockout enhances cholesterol 7 α -hydroxylase (CYP7A1) and multidrug transporter (MDR1) gene expressions to attenuate atherosclerosis. *Cardiovasc Res* 2016; 111(1): 74-83.
 9. Yuan L, Qiu L, Ye Y, Wu J, Wang S, Wang X, Zhou N, Zou Y. Heat-shock transcription factor 1 is critically involved in the ischaemia-induced cardiac hypertrophy via JAK2/STAT3 pathway. *J Cell Mol Med* 2018; 22(9): 4292-4303.
 10. Council NR. *Guide for the care and use of laboratory animals*. 2010.
 11. Qin J, Ma Q, Ma D. Low-dose Sevoflurane Attenuates Cardiopulmonary Bypass (CPB)- induced Postoperative Cognitive Dysfunction (POCD) by Regulating Hippocampus Apoptosis via PI3K/AKT Pathway. *Curr Neurovasc Res* 2020; 17(3): 232-240.
 12. Song J, Chu S, Cui Y, Qian Y, Li X, Xu F, Shao X, Ma Z, Xia T, Gu X. Circadian rhythm resynchronization improved isoflurane-induced cognitive dysfunction in aged mice. *Exp Neurol* 2018; 306: 45-54.
 13. Dong R, Han Y, Jiang L, Liu S, Zhang F, Peng L, Wang Z, Ma Z, Xia T, Gu X. Connexin 43 gap junction-mediated astrocytic network reconstruction attenuates isoflurane-induced cognitive dysfunction in mice. *J Neuroinflammation* 2022; 19(1): 64.
 14. Que YY, Zhu T, Zhang FX, Peng J. Neuroprotective effect of DUSP14 overexpression against isoflurane-induced inflammatory response, pyroptosis and cognitive impairment in aged rats through inhibiting the NLRP3 inflammasome. *Eur Rev Med Pharmacol Sci* 2020; 24(12): 7101-7113.
 15. Zhang X, Fan X, Li F, Qiu J, Zhang Y. Effects of PYRIN-containing Apaf1-like protein 1 on isoflurane-induced postoperative cognitive dysfunction in aged rats. *Mol Med Rep* 2020; 22(2): 1391-1399.
 16. Cheng C, Zhu X. Cordycepin mitigates MPTP-induced Parkinson's disease through inhibiting TLR/NF- κ B signaling pathway. *Life Sci* 2019; 223: 120-127.
 17. Wang HQ, Wang SS, Chiufai K, Wang Q, Cheng XL. Umbelliferone ameliorates renal function in diabetic nephropathy rats through regulating inflammation and TLR/NF- κ B pathway. *Chin J Nat Med* 2019; 17(5): 346-354.
 18. Lv ZC, Cao XY, Guo YX, Zhang XD, Ding J, Geng J, Feng K, Niu H. Effects of MiR-146a on repair and inflammation in rats with spinal cord injury through the TLR/NF- κ B signaling pathway. *Eur Rev Med Pharmacol Sci* 2019; 23(11): 4558-4563.