Original Research Article

Knockdown of TNFAIP1 mitigates sevoflurane-induced cognitive dysfunction by activating CREB/Nrf2 pathway

Jiayu Lu[1], Zhenpeng Liu[1], Wei He[1], Sichao Shao[2]*
[1]Department of Anesthesiology, The Affiliated Hospital of Beihua University, Jilin City, Jilin Province 132000, [2]Department of Anesthesiology, Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine, Wenzhou City, Zhejiang Province 325000, China

*For correspondence: **Email:** scshao5759@163.com; **Tel:** +86-13587645080

Abstract

Purpose: To investigate the role of tumor necrosis factor-induced protein 1 (TNFAIP1) and cAMP-responsive element binding protein (CREB)/nuclear factor-erythroid factor 2-related factor 2 (Nrf2) pathway in sevoflurane (SEV)-induced cognitive dysfunction.
Methods: A SEV-induced cognitive dysfunction rat model was developed. Bcl-2, Bax, heme oxygenase-1, Nrf2, p-CREB, and CREB protein levels in rat hippocampal tissue were assessed by western blot. Learning and long-term memory were evaluated using Morris water maze test. Glutathione peroxidase, malondialdehyde, and superoxide dismutase levels in hippocampal tissue were measured by enzyme-linked immunosorbent assay (ELISA). The 2,7-dichlorodihydro-fluorescein diacetate fluorescent assay was used to measure reactive oxygen species, while TUNEL staining was used to assess neuronal cell apoptosis.
Results: Knockdown of TNFAIP1 attenuated SEV-induced learning and long-term memory dysfunction (p < 0.005), oxidative stress (p < 0.005), apoptosis (p < 0.005), and inhibition of the CREB/Nrf2 signaling pathway.
Conclusion: This study demonstrates that knockdown of TNFAIP1 alleviates SEV-induced cognitive dysfunction by reversing inhibition of the CREB/Nrf2 signaling pathway.

Keywords: TNFAIP1, Postoperative cognitive dysfunction, Sevoflurane, cAMP-responsive element binding protein (CREB), Nuclear factor-erythroid factor 2-related factor 2 (Nrf2)

INTRODUCTION

Postoperative cognitive dysfunction is anesthesia-induced cognitive impairment that continues for weeks or months after surgery [1]. Numerous studies have shown that inhalation anesthesia, such as sevoflurane (SEV), can lead to neurotoxicity and postoperative cognitive dysfunction in elderly patients [2]. Inhalation anesthesia impairs learning and memory by inducing neuronal apoptosis. cAMP-response element binding protein (CREB), a member of the bZIP transcription factor superfamily, modulates expression of genes involved in the dopaminergic response in neurons [3]. In addition, CREB regulates genes involved in immunity, cellular metabolism, apoptosis, and cell proliferation [4]. A reduction in CREB

expression in the hippocampus of subjects with spatial memory impairment was recently reported [5]. Nuclear factor-erythroid 2 related factor 2 (Nrf2) regulates production of reactive oxygen species (ROS) [6] and has been shown to have neuroprotective effects; thus, it may be a possible therapeutic target for neurodegenerative diseases [7]. It has been shown that CREB1 regulates the Nrf2 signal transduction pathway through cAMP [8]. Tumor necrosis factor-induced protein 1 (TNFAIP1) was first identified in umbilical vein endothelial cells and can be induced by tumor necrosis factor α and interleukin-6 [9].

The TNFAIP1 plays major roles in DNA repair, cellular apoptosis, and multiple human diseases, and has been shown to be expressed at high levels in Alzheimer's disease in brain tissue [10]. TNFAIP1 is encoded by a highly conserved single-copy gene and functions as a substrate-specific adaptor in the regulation of the cytoskeletal structure. In addition, TNFAIP1 participates in DNA synthesis and repair partly through interactions with proliferating cell nuclear antigen and the small subunit of DNA polymerase δ [11]. TNFAIP1 contributes to b-amyloid-induced overexpression of ROS, suppression of the mitochondrial membrane potential, and neuronal cell death in human SH-SY5Y cells [12]. However, the role of TNFAIP1 in SEV-induced postoperative cognitive dysfunction remains unclear. In this study, the association between TNFAIP1 and SEV-induced cognitive dysfunction was determined by measuring neuronal apoptosis, oxidative stress, cognitive impairment, and activation of the CREB/Nrf2 pathway in mice treated with SEV and/or TNFAIP1 silencing.

EXPERIMENTAL

Animal model

Sprague-Dawley rats (Shanghai SLAC Laboratory Animal Co. Ltd, Shanghai, China) were used in this study. All animals were housed at 22 – 24 °C under a 12 h light/dark rhythm. All animal experiments were approved by the Beihua University Laboratory Animal Ethics Committee (approval no. 2021013) and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [13]. Five rats were randomly selected for the normal group, and the other 20 rats were used to establish the cognitive dysfunction animal model. The control group remained untreated, the rats in the SEV group were placed in the inhaled anesthesia induction chamber with 2 % SEV (Maruishi

Pharmaceutical, Osaka, Japan) for 5 h. The rats in the SEV + sh-NC group were injected in the paracele with sh-NC (0.5 nmoL in 1 µL *in vivo* transfection reagent, Entranster™ - *in vivo*, Engreen, China) using a microinjector and then placed in the inhaled anesthesia induction chamber with 2 % SEV for 5 h. The rats in SEV + sh-TNFAIP1 group were injected in the paracele with sh-TNFAIP1 (0.5 nmoL in 1 µL *in vivo* transfection reagent) using a microinjector and then placed in the inhaled anesthesia induction chamber with 2 % SEV for 5 h. Hippocampal tissues samples from each rat were used for TUNEL staining, western blot, and 2,7-Dichlorodihydro-fluorescein diacetate (DCFDA) staining to observe pathological changes.

Western blot assay

Hippocampal tissues were homogenized with homogenate buffer and centrifuged at 13000*g* at 4 °C for 15 min. Protein samples (20 µg) were separated by 10 % SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were incubated with primary antibodies against Bcl-2 (1:2000, Santa Cruz Biotechnology), GAPDH (1:10000, Abcam), Bax (1:2000, Abcam), heme oxygenase-1 (HO-1; 1:2000, Santa Cruz Biotechnology), Nrf2 (1:2000, Abcam), p-CREB (1:1000, Abcam), and CREB (1:2000, Abcam) overnight at 4 °C. After washing, the membranes were incubated with horseradish peroxidase-labeled secondary antibody at 37 °C for 1 h. Detection was performed with enhanced chemiluminescence (Sigma-Aldrich). GAPDH was used as the internal control.

Enzyme-linked immunosorbent assay (ELISA)

Glutathione peroxidase (GSH-PX), malon-dialdehyde (MDA) and superoxide dismutase (SOD) levels in hippocampal tissues were measured by ELISA according to the manufacturer's protocol (R & D Systems).

DCFDA assay

Hippocampal tissues were homogenized with homogenate buffer, centrifuged, and supernatants were treated with 5 µmol/L DCFDA (Sigma, D6883). Fluorescent intensities were detected using a microplate reader, an excitation wavelength of 488 nm, and an emission wavelength of 525 nm.

TUNEL assay

Hippocampal tissues were deparaffinized, cut into coronal sections, dewaxed, and dehydrated

using an ethanol series involving 2 h of incubation in 70 % ethanol, 2 h of incubation in 96 % ethanol, and 2 h of incubation in 99 % ethanol. Finally, the sections were fixed with 4 % paraformaldehyde for 2 h, incubated in 20 % sucrose phosphate buffer at 4 °C overnight, and 20 µm transverse sections were made using a - 22 °C cutting machine. Apoptotic cells were labeled using the TUNEL staining Kit (Sigma-Aldrich) according to the manufacturer's instructions. The numbers of apoptotic neuronal cells were counted by microscopy.

Morris water maze test

After SEV exposure, the rats in each group were placed in a 120 cm diameter pool filled with clear water to a depth of 30 cm that contained a submerged platform ~1 cm below the water surface. For the four training days, the mice were trained four times per day using four different starting quadrants. When a rat stopped on the platform for more than 3 s within 90 s, the time was recorded as the escape latency. The fourth day involved trials to examine the consolidation of learning and memory. The submerged platform was removed and the rats were placed in the first quadrant to swim 90 s. The swimming trajectories and times it took the rats to cross the original platform were recorded.

Statistical analysis

All experiments were performed in triplicate and analyzed using GraphPad Prism Software 6.0 (GraphPad Software, La Jolla, USA). All data are presented as mean ± SEM. One-way analysis of variance (ANOVA) and t-tests were used to make comparisons. Statistically significant differences were defined as $p < 0.05$.

RESULTS

Association between TNFAIP1 and SEV-induced cognitive impairment

To assess the association between TNFAIP1 and SEV-induced cognitive dysfunction, TNFAIP1 protein expression in rat hippocampal tissue upon SEV exposure was measured by western blot. TNFAIP1 expression was markedly higher in the SEV group compared to the control group (Figure 1 A). The effect of TNFAIP1 silencing on learning and long-term memory of SEV-induced rats was evaluated by the Morris water maze test. The SEV group showed a disorderly and more prolonged swimming path compared to the control group, and this disordered, prolonged swimming path was alleviated by TNFAIP1 knockdown (Figure 1 B).

The time to find the hidden platform and the escape latency, was higher for the SEV group than the control group. However, the escape latency for the TNFAIP1 knockdown group was lower than for the SEV group (Figure 1C). On the fourth day of water maze trials, the platform was removed to evaluate the spatial-working memory of the rats. The SEV group spent less time in the target quadrant and crossed the location of the removed platform fewer times than the control group; however, TNFAIP1 inhibition increased the number of crossings (Figure 1D). These results suggested that SEV-induced learning and long-term memory dysfunction was attenuated upon TNFAIP1 silencing.
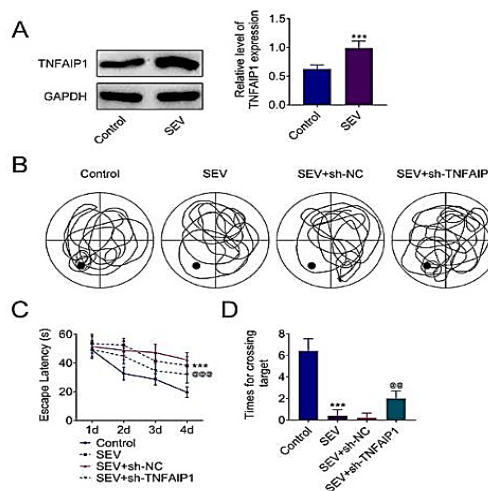

Figure 1: Knockdown of TNFAIP1 ameliorated SEV-induced cognitive dysfunction in rats. (A) TNFAIP1 expression in rat hippocampal tissue after SEV exposure. (B) The swimming paths of rats on the fourth day of training. (C) Escape latencies for each training day. (D) The number of target crossings during the trials on the fourth day. ***$p < 0.005$ vs. control, @@$p < 0.01$ *vs.* SEV

Knockdown of TNFAIP1 ameliorated SEV-induced oxidative stress

Cognitive dysfunction associates closely with the pathology status of the hippocampus. To investigate the effect of TNFAIP1 knockdown on SEV-induced hippocampus dysfunction, levels of oxidative stress-related biomarkers in the hippocampal tissues of rats were examined. GSH-PX and SOD levels were lower in the SEV group than the control group, and the MDA level was higher in the SEV group than the control group (Figure 2A). However, the abnormal levels of the antioxidant enzymes and biomolecules were ameliorated by TNFAIP1 knockdown (Figure 2 A). The DCFDA fluorescent assay was used to measure ROS in hippocampal tissues. The ROS levels in the SEV and SEV+ sh-NC

groups were higher than in the control group, and the ROS level in the SEV + sh-TNFAIP1 group was much lower than in the SEV group (Figure 2 B).
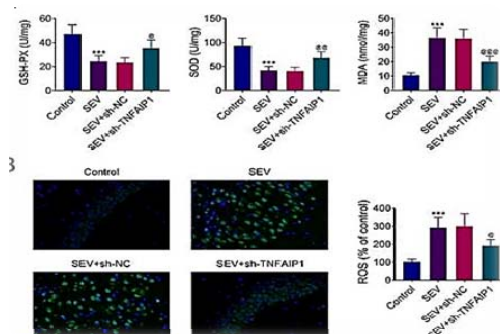

Figure 2: Silencing of TNFAIP1 attenuated SEV-induced oxidative stress in hippocampal neuronal cells of rats. (A) GSH-PX, SOD, and MDA levels in rat hippocampal tissues. (B) ROS levels in rat hippocampal tissues. *** $p < 0.005$ vs. control, $@p < 0.05$, $@@p < 0.01$, $@@@p < 0.005$ vs. SEV

Knockdown of TNFAIP1 decreased SEV-induced apoptosis of hippocampal neurons

TUNEL staining was used to examine cellular apoptosis of hippocampal neurons in rats. More apoptotic cells were observed in the SEV and SEV + sh-NC groups than in the control group, and the number of apoptotic cells decreased in the SEV + sh-TNFAIP1 group when compared to the SEV and SEV + sh-NC groups (Figure 3 A and B). To confirm this observation, expression of the apoptosis-related proteins Bax and Bcl-2 were evaluated. Western blot analysis revealed higher Bax expression and lower Bcl-2 expression in the SEV and SEV + sh-NC groups when compared to the control, and these SEV-induced expressions were reversed in the SEV + sh-TNFAIP1 group (Figure 3 C). Taken together, these data revealed that knockdown of TNFAIP1 decreased SEV-induced cell apoptosis of hippocampal neurons.

TNFAIP1 regulated CREB/Nrf2 signaling pathway in SEV-treated rats

To investigate the effect of TNFAIP1 silencing on the CREB/Nrf2 signaling pathway in SEV-treated rats, expression of Nrf2, HO-1, and p-CREB1 in rat hippocampal tissues was analyzed by western blot. Nrf2, HO-1, and p-CREB1 expression in hippocampal tissue was lower in the SEV and SEV + sh-NC groups than in the control group (Figure 4). Nrf2, HO-1, and p-CREB1 expression in hippocampal tissue was higher in the SEV + sh-TNFAIP1 group than in

the SEV and SEV + sh-NC groups. These results indicated that downregulation of TNFAIP1 relieved SEV-induced hippocampal neuronal dysfunction via the CREB/Nrf2 signaling pathway.
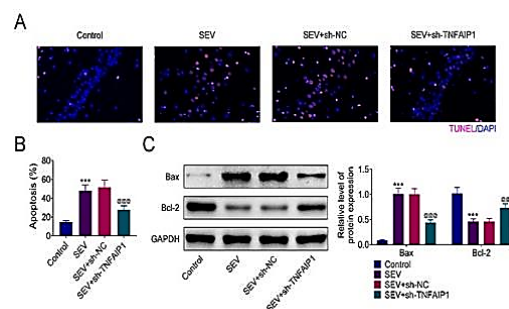

Figure 3: Knockdown of TNFAIP1decreased SEV-induced apoptosis of hippocampal neurons. (A) TUNEL staining of rat hippocampal tissues. (B) Quantitative analysis of apoptotic cells. (C) Bax and Bcl-2 protein levels were measured by western blot. *** $p < 0.005$ vs. control. $@@p < 0.01$, $@@@p < 0.005$ vs. SEV
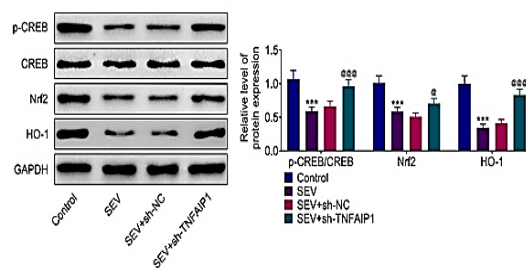

Figure 4: Effect of the TNFAIP1 knockdown on the CREB/Nrf2 signaling pathway in SEV-treated rats**.** Expression of CREB/Nrf2 signaling pathway-related proteins in hippocampal tissue analyzed by Western blot (left). Quantitative analysis of the levels of CREB/Nrf2 pathway-related proteins in hippocampal tissue (right). ***$p < 0.005$ vs. control, $@@@p < 0.005$ vs. SEV

DISCUSSION

Sevoflurane is a frequently used inhaled anesthetic for clinical operations. However, it has been shown that SEV exposure can induce nerve damage. It has also been shown that SEV use in elderly people increases the incidence of postoperative cognitive dysfunction [14,15]. It has recently been shown that neuronal cell apoptosis, neurodegeneration, mitochondrial dysfunction and integrity impairment are the major mechanisms of SEV-induced neurotoxicity [16]. Previous studies suggested that SEV anesthesia induces autophagy injury in hippocampal neurons, thereby contributing to cognitive disorder [17]. The inflammatory

response of the immune system has been shown to promote neurotoxicity and cognitive dysfunction in aged rats [18]. Nevertheless, the underlying pathogenesis of SEV-induced postoperative cognitive dysfunction remains unclear. Previous research reported that TNFAIP1 is involved in cognitive function and may cause neurotoxicity [19]. The expression of TNFAIP1 protein was markedly increased in a transgenic *C. elegans* Alzheimer's disease model. Therefore, this study investigated the potential of TNFAIP1 in the treatment of SEV-induced postoperative cognitive dysfunction. In this study, it has been shown that SEV-induced TNFAIP1 expression *in vivo*. However, in rats in which TNFAIP1 was silenced, SEV-induced learning and long-term memory dysfunction was attenuated.

Improvement of postoperative cognitive dysfunction in aged rats has been associated with reductions in mitochondrial dysfunction, oxidative stress, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and levels of neurotrophic factors [20,21]. Therefore, mitochondrial dysfunction-mediated ROS overexpression is increasingly considered a major contributor to postoperative cognitive dysfunction. Activation of NADPH oxidase and overexpression of ROS trigger microglia and amplify production of multiple pro-inflammatory cytokines. The major source of ROS in phagocytes is NADPH oxidase, which is overproduced in Alzheimer's disease and critical for microglia-mediated amyloid neurotoxicity [21]. In this study, it has been established that knockdown of TNFAIP1 ameliorated SEV-induced oxidative stress.

Inhibition of TNFAIP1 reversed the decline in cognitive function and increase in hippocampal cell apoptosis that were observed in rats with SEV-induced cognitive dysfunction. In the Morris water maze test, the SEV group displayed a chaotic and longer swimming path, which improved with TNFAIP1 knockdown. The TNFAIP1 knockdown group had a lower escape latency and crossed the position of the removed platform more times than the SEV group. A recent study showed that patients with postoperative cognitive dysfunction had lower TNFAIP1 expression [22]. A previous study also showed degeneration of hippocampal neurons by neuronal cell apoptosis during postoperative cognitive dysfunction. Moreover, overexpression of TNFAIP1 impairs neuronal cell migration within the embryonic cortex and changes the morphology of immature neurons. Silencing of TNFAIP1 reduced apoptosis of hippocampal neurons in SEV-treated rats.

The CREB/Nrf2 signaling pathway has been reported to be essential for neuroprotection and memory acquisition. Rescue of suppressed CREB phosphorylation prevents IL-1β-induced cellular injury. It has been shown that inhibition of the CREB/Nrf2 signaling pathway by knockdown of CREB1 or use of an inhibitor of the CREB signaling pathway reduced learning and memory, increased cellular apoptosis, intensified cognitive dysfunction, and inhibited hippocampal cell proliferation [5].

CONCLUSION

This study shows that inhibition of TNFAIP1 upregulates CREB/Nrf2 pathway while knockdown of TNFAIP1 significantly increases Nrf2, HO-1, and p-CREB1 expression in hippocampal tissue and therefore alleviates SEV-induced cognitive dysfunction via the CREB/Nrf2 signaling pathway.

DECLARATIONS

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest 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. Jiayu Lu designed the study and supervised data collection. Zhenpeng Liu and Wei He analyzed and interpreted the data. Sichao Shao prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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