

Original Research Article

ELAVL1 ameliorates sevoflurane-induced neurotoxicity by inhibiting NLRP3 inflammasome activation

Yan Xia¹, Kunguang Wang², Yan Chen¹, Xiaoxuan Du^{1*}

¹Department of Anesthesiology, The Sixth Clinical Hospital Of Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region 830002, ²Department of Anesthesiology, Changji People's Hospital, Changji Hui Autonomous Prefecture, Xinjiang Uygur Autonomous Region 831100, China

*For correspondence: **Email:** xxdu4522@163.com; **Tel:** +86-13999178983

Sent for review: 6 July 2023

Revised accepted: 28 November 2023

Abstract

Purpose: To investigate the role of embryonic lethal-abnormal vision like protein 1 (ELAVL1) in sevoflurane-induced neurotoxicity.

Methods: A cell model was established in HT22 cells by exposing them to 3 % sevoflurane for 12 h. The HT22 cells were transfected with siRNA ELAVL1 (si-ELAVL1) or siRNA negative control (si-NC) to knockdown ELAVL1 expression. Enzyme-linked immunosorbent assay (ELISA) was performed to assess the levels of proinflammatory factors in HT22 cell culture supernatants. Cell apoptosis was analyzed using flow cytometry while apoptosis-related proteins and NLRP3 inflammasome pathway proteins were determined by western blot.

Results: ELAVL1 was upregulated in sevoflurane-exposed HT22 cells. Sevoflurane exposure also resulted in inflammation, apoptosis and activation of NLRP3 inflammasome of HT22 cells. Importantly, knockdown of ELAVL1 inhibited inflammation and apoptosis in HT22 cells caused by sevoflurane through the inhibition of IL-6, IL-1 β and TNF- α production and by regulating Bax and Bcl-2 protein expression. Furthermore, knockdown of ELAVL1 suppressed NLRP3 inflammasome activity as reflected by the inhibition of the expression of caspase 1, ASC, IL-1 β and IL-18.

Conclusion: The findings of this study suggest that the knockdown of ELAVL1 alleviates the inflammation and apoptosis of HT22 cells induced by sevoflurane which impeded the activation of NLRP3 inflammasome, suggesting that ELAVL1 would make a good therapeutic target for sevoflurane-caused neurotoxicity.

Keywords: Sevoflurane, HT22 cells, ELAVL1, Inflammasome, Apoptosis, NLRP3

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INTRODUCTION

Sevoflurane is most commonly used as an inhalable anesthetic in clinical practice [1]. However, sevoflurane may cause neurotoxicity during prolonged use and in high concentrations [2]. Currently, sevoflurane's mechanism of

neurotoxicity is not completely understood, but many studies suggest that neuroinflammation contributes to the neuronal injury and death caused by sevoflurane [3].

Nod-like receptor protein 3 (NLRP3), a typical inflammasome, is a crucial participant in the

process of inflammatory responses [4]. It has been shown that NLRP3 is involved in neuroinflammation responses by inducing the production of proinflammatory factors, including interleukin (IL) 1 β (IL-1 β) and IL-18 [5]. Additionally, activation of NLRP3 causes neuronal death and inflammation, which promotes the development of neurological diseases [6]. Therefore, inhibition of NLRP3 activation as a potential therapeutic strategy for neurological disorders has been proposed [7].

Embryonic lethal-abnormal vision like protein 1 (ELAVL1), also called human antigen R (HuR), is an RNA-binding protein that participates in various biological processes, including transcription, RNA stability and RNA transport [8]. Recent research has identified ELAVL1 as a potential therapeutic target in neuroinflammation, as it has been implicated in this process [9]. Furthermore, studies have shown that ELAVL1 is linked to the activation of NLRP3 [10]. However, it remains unclear whether ELAVL1 is involved in sevoflurane-induced neurotoxicity via the modulation of NLRP3 activity.

EXPERIMENTAL

Cell culture and treatment

HT22 mouse hippocampal neuronal cells were purchased from Procell (Wuhan, China). The cells were kept in DMEM medium (GIBCO, Grand Island, NY, USA) containing 10 % FBS, 1 % penicillin-streptomycin at 37 °C and 5 % CO₂.

For the establishment of a neuronal injury model, the HT22 cells culture plate was placed in a chamber, which was connected to an anesthesia machine and then cells were exposed to 3 % sevoflurane (Marushi Pharmaceutical, Osaka, Japan) for 12 h. Thereafter, a further 36 h of normal culture was performed on the cells.

Cell transfection

To knockdown the expression of ELAVL1, a siRNA targeting ELAVL1 (si-ELAVL1) or a nonsense siRNA (si-NC) was transfected into HT22 cells using Lipofectamine 3000 reagent (Invitrogen, Grand Island, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

HT22 cell RNA was reversely transcribed into cDNA following total RNA extraction. Next, ELAVL1 mRNA expression was tested using qRT-PCR and utilizing the SYBR Green method (Bio-Rad, Hercules, CA, USA). The results were analysed by 2^{- $\Delta\Delta$ ct} method (Table 1).

Western blot

The HT22 cells were homogenized in RIPA lysis buffer (Beyotime, Shanghai, China). The lysate was collected and denatured at 95 °C and then subjected to SDS-PAGE. Post trans-blotting incubation with primary antibodies that were shown in Table 2 was carried out on the membrane at 4 °C overnight. Following secondary antibody incubation, color development on the blots was performed.

Table 2: Antibodies used in western blot studies

Antigen	Code	Working dilution	Supplier
ELAVL1	ab238528	1:1000	Abcam
Bax	ab182734	1:1000	Abcam
Bcl-2	ab182858	1:10,000	Abcam
NLRP3	ab270449	1:1000	Abcam
Caspase1	ab138483	1:250	Abcam
ASC	ab309497	1:1000	Abcam
IL-1 β	ab254360	1:1000	Abcam
IL-18	ab207323	1:1000	Abcam
β -actin	ab5694	1:1000	Abcam

Enzyme-linked immunosorbent assay (ELISA)

ELISA assays were conducted on the culture supernatants of HT22 cells to ascertain the production of IL-6, IL-1 β and tumor necrosis factor α (TNF- α). ELISA kits (catalog nos. R6000B, RLB00 and RTA00) were obtained from R&D Systems (Minneapolis, MN, USA).

Flow cytometric analysis

Annexin V-FICT/PI kit (Beyotime, Shanghai, China) was used to analyze apoptosis in the HT22 cells by flow cytometry. After treatment, the HT22 cells were gathered and then underwent a labeling procedure utilizing Annexin V-FICT and PI for a duration of 15 min and then apoptosis was determined.

Table 1: Primers used for qRT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
ELAVL1	CATCCCTACCACCCAACACC	CCAGACAGTGGAGACCCACAC
β -actin	TGAGCTGCGTTTTACACCCT	GCCTTCACCGTTCCAGTTTT

Statistical analysis

Each experiment was repeated three times. Data analysis was done using GraphPad Prism 8.0 software and the results presented as mean \pm SD. A t-test was employed to compare two groups, while a one-way ANOVA followed by Tukey's test was utilized to compare three or more groups. A statistical level of $p < 0.05$ was regarded as significant.

RESULTS

ELAVL1 was highly expressed in HT22 cells exposed to sevoflurane

The detection of sevoflurane on ELAVL1 expression in HT22 cells showed that sevoflurane significantly increased the mRNA and protein expression levels of ELAVL1 in HT22 cells (Figure 1 A and B ; $p < 0.05$).

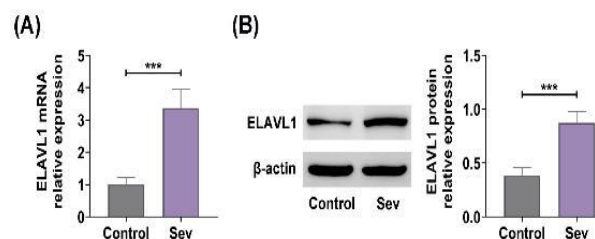


Figure 1: ELAVL1 was highly expressed in HT22 cells exposed to sevoflurane. (A) mRNA expression of ELAVL1. (B) Protein expression of ELAVL1. *** $p < 0.001$

Knockdown of ELAVL1 reduced the inflammation of HT22 cells induced by sevoflurane

To determine whether ELAVL1 was involved in sevoflurane-induced cell inflammation, si-ELAVL1 was transfected into HT22 cells to interfere with ELAVL1 expression. The results of the western blot study showed that ELAVL1 protein level was lower in Sev+si-ELAVL1 group than in Sev+si-NC group (Figure 2 A; $p < 0.05$). Moreover, ELISA results showed that sevoflurane exposure alone resulted in elevation of IL-6, IL-1 β and TNF- α release in HT22 cells, while si-ELAVL1 group exhibited lower levels of those factors compared to si-NC group after exposure to sevoflurane (Figure 2 B; $p < 0.05$). The data suggest that ELAVL1 silencing mitigated sevoflurane-induced inflammation in HT22 cells.

Knockdown of ELAVL1 inhibited sevoflurane-induced apoptosis in HT22 cells

As shown in Figure 3 A, the degree of apoptosis

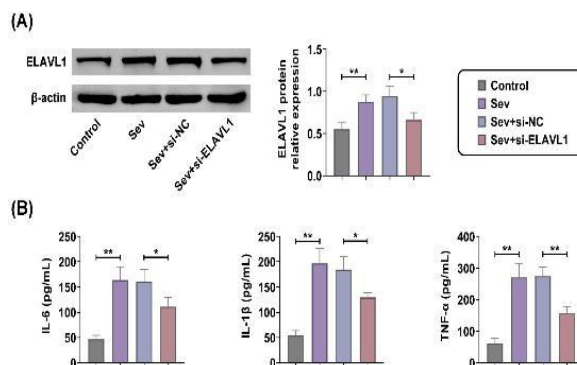


Figure 2: Knockdown of ELAVL1 reduced the inflammation of HT22 cells induced by sevoflurane. (A) Expression of ELAVL1 protein. (B) Proinflammatory factors (IL-6, IL-1 β and TNF- α). * $P < 0.05$, ** $p < 0.01$

in sevoflurane treated HT22 cells was greater than in the control group. Importantly, HT22 cells transfected with si-ELAVL1 showed lower level of apoptosis than in si-NC-transfected group after sevoflurane exposure. However, the western blot results showed that sevoflurane treatment enhanced Bax expression but reduced Bcl2 expression, and this was reversed by si-ELAVL1 (Figure 3 B). The data suggest that ELAVL1 knockdown inhibited the apoptosis of HT22 cells induced by sevoflurane.

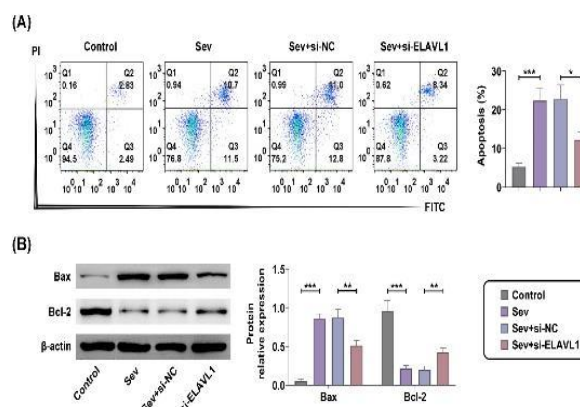


Figure 3: Knockdown of ELAVL1 inhibited sevoflurane-induced apoptosis in HT22 cells. (A) Representative (left) and statistical data (right) of cell apoptosis. (B) Apoptosis-related proteins (Bax and Bcl-2). * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Knockdown of ELAVL1 suppressed the activation of NLRP3 inflammasome

In the present study, western blot result further revealed that HT22 cells showed increase in NLRP3, caspase 1, ASC, IL-1 β and IL-18 expressions after sevoflurane exposure ($p < 0.05$). However, HT22 cells knocked down with ELAVL1 showed decreased expression of NLRP3, caspase 1, ASC, IL-1 β and IL-18 in

comparison with si-NC-transfected cells after exposure to sevoflurane (Figure 4 A and B; $p < 0.05$). The data indicate that ELAVL1 silencing inhibited the activation of NLRP3 inflammasome caused by sevoflurane in HT22 cells.

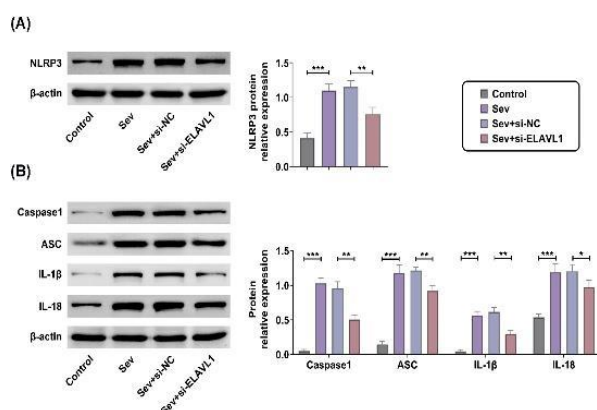


Figure 4: Knockdown of ELAVL1 suppressed the activation of NLRP3 inflammasome. (A) Expression of NLRP3 protein. (B) Caspase 1, ASC, IL-1 β and IL-18 protein expressions. * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$

DISCUSSION

In this study, it has been shown that knockdown of ELAVL1 alleviated sevoflurane-induced inflammation and apoptosis in HT22 cells. More importantly, ELAVL1 knockdown inhibited NLRP3 activation and NLRP3 inflammasome formation.

Previous research have revealed that ELAVL1 plays a role in brain injury diseases. For example, ELAVL1 was upregulated in rat brain tissue following I/R injury and ELAVL1 silencing improved infarct volume and nerve dysfunction caused by I/R [11]. The knockdown of ELAVL1 alleviated SK-N-SH cell damage induced by MPP⁺ through the inhibition of cell apoptosis and enhancement of cell viability [12]. The inhibition of ELAVL1 may promote locomotor functional recovery and relieve neuroinflammation in spinal cord injury (SCI) rats by restraining the M1 polarization of microglia [13]. This study, for the first time, found that ELAVL1 was highly expressed in sevoflurane-treated HT22 cells. Further experimental data indicate that knockdown of ELAVL1 suppressed the release of proinflammatory factors and cell apoptosis of HT22 cells induced by sevoflurane, suggesting that ELAVL1 knockdown could mitigate sevoflurane-caused neuronal injury.

Many inflammatory disorders trigger the activity of NLRP3 inflammasome and neuroinflammation is no exception [14]. NLRP3-mediated neuroinflammation, being the central component

of the inflammatory response, contributes significantly to neuronal damage [15]. An NLRP3 inflammasome consists of proteins NLRP3, adapter protein ASC and caspase-1 [16]. When the cells are stimulated, NLRP3 is activated and interacts with ASC and caspase-1, which drives the release of IL-1 β and IL-8, resulting in inflammatory responses [17]. A previous study showed that the inhibition of the activation of NLRP3 inflammasome may ameliorate surgery/sevoflurane-caused cognitive impairment postoperatively [18]. In addition, research has indicated that NLRP3/caspase-1 pathway inhibition ameliorated sevoflurane-induced neuroinflammation and cognitive impairment in rats [19]. The data in this study showed that knockdown of ELAVL1 partially reversed NLRP3, ASC, caspase-1, IL-1 β and IL-8 expression in HT22 cells induced by sevoflurane. This suggests that ELAVL1 knockdown effectively suppressed sevoflurane-induced NLRP3 inflammasome activation, which might be the underlying mechanism for the amelioration of neuronal damage caused by sevoflurane.

CONCLUSION

The findings of this study indicate that knockdown of ELAVL1 protects against inflammation and apoptosis of sevoflurane-induced HT22 cells, possibly by inhibiting NLRP3 inflammasome activity. These data suggest that ELAVL1 may be a suitable therapeutic target for sevoflurane-caused neurotoxicity.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

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. Yan Xia, Kunguang Wang, Yan Chen, Xiaoxuan Du designed the study and carried them out, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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