

## Original Research Article

# C1q/tumor necrosis factor (TNF)-associated protein 6 (CTRP6) ameliorates the cognitive dysfunction induced by sevoflurane by activating AMPK/SIRT1 pathway in rats

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### Abstract

**Purpose:** To evaluate the possible effects of C1q/tumor necrosis factor (TNF)-associated protein 6 (CTRP6) on postoperative cognitive dysfunction (POCD), including the potentially-related signaling pathway.

**Methods:** Behavioral analysis and cognitive impairment were assessed in each group. Immunoblots were used to determine the level of CTRP6 following sevoflurane-induced nerve injury. Hippocampal neurons were identified using Nissl staining, while inflammatory response following neuronal injury was monitored by enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR). The involvement of adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1) pathway was determined using immunoblot.

**Results:** CTRP6 alleviated sevoflurane-induced cognitive dysfunction in rats ( $p < 0.001$ ). Moreover, CTRP6 reduced sevoflurane-induced nerve injury and inflammation in rats ( $p < 0.05$ ).

**Conclusion:** CTRP6 ameliorates sevoflurane-induced cognitive dysfunction by activating AMPK/SIRT1 pathway, thus offering a novel target for POCD treatment.

**Keywords:** Postoperative cognitive dysfunction (POCD), C1q/Tumor necrosis factor (TNF) associated protein 6 (CTRP6), Nerve injury, Inflammation, AMPK/SIRT1 pathway

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## INTRODUCTION

Emergent orthopedic surgery is more common in elderly patients, who are at a higher risk of subsequent complications and reduced neurocognitive function following surgery. This reduced neurocognitive function is also known as postoperative cognitive dysfunction (POCD), which refers to damage of certain

neurophysiological regions of the brain [1]. Sevoflurane is often used as a volatile anesthetic agent during orthopedic surgery. Sevoflurane inhalation anesthesia has been shown to induce increased apoptosis of hippocampal neurons in aging rats, leading to cognitive dysfunction [2]. However, the pathogenesis and mechanism of POCD in response to sevoflurane has not been established. Understanding the mechanism that

leads to POCD following orthopedic surgery with sevoflurane will be beneficial to improve postoperative patients' quality of life and reduce medical costs.

C1q/Tumor necrosis factor (TNF)-associated protein 6 (CTRP6) is a member of the adiponectin paralog homologous protein family [3]. Although CTRPs share the same structural features as adiponectin, each CTRP has a unique tissue distribution and provide a variety of functions [4]. CTRP6 is present in adipose tissue, the placenta, heart, and brain. CTRP6 plays a protective role in cerebral ischemia-reperfusion injury by reducing inflammation, oxidative stress, and apoptosis in PC12 cells [3]. Additionally, CTRP6 has been linked to obesity, adipose tissue inflammation, and insulin resistance [5]. CTRP6 can also activate adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1) to reduce apoptosis [6]. However, its possible effects on POCD are still unclear.

AMPK is a key regulator that maintains the stability of energy metabolism by regulating various regulatory signaling pathways at the cellular level [6]. Studies have shown that phosphorylation of AMPK activates SIRT1, and AMPK and SIRT1 are associated with cellular aging and neurological diseases [7]. Another study reported that the activation of AMPK/SIRT1 improved cognitive dysfunction caused by sevoflurane [5]. Interestingly, the regulatory role of CTRP6 on the AMPK pathway has been previously established. However, this paper is the first to our knowledge to address the role of CTRP6 in POCD.

## EXPERIMENTAL

### Animals and grouping

Male Sprague-Dawley rats (n=20, 200–250 g) were purchased from the Animal Laboratory Center of China Medical University. All animal experiments were approved by the Ethics Committee of Zhejiang Taizhou Hospital of Traditional Chinese Medicine (approval no. 2019041) for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [8].

Rats were housed in sterile condition at a temperature of 22±2°C with 50% humidity and free access to standard pelleted chow and drinking water. The rats were randomly divided into four groups: control group; SEV group: rats were anesthetized with 2% sevoflurane for 5 h;

SEV+AAV-NC: rats were injected with AAV negative control before anesthesia for 5 h in 2% sevoflurane; SEV+AAV-CTRP6: rats were pretreated with CTRP6 AAV before anesthesia for 5 h in 2% sevoflurane.

### Immunoblot assay

Immunoblot assays were performed similar to a previous study [9]. Brains were extracted, and proteins were lysed with RIPA buffer (Beyotime Biotechnology, Shanghai, China), isolated using a 10% SDS-PAGE, transferred onto PVDF membranes, and blocked with 5% fat-free milk in TBST buffer. Subsequently, the membranes were incubated with primary antibodies targeting CTRP6 (1:1000, Abcam, Cambridge, UK), p-AMPK (1:1000, Abcam), AMPK (1:1000, Abcam), SIRT1 (1:1000, Abcam), and  $\beta$ -actin (1:10000, Abcam) overnight at 4°C. Membranes were then incubated with specific secondary antibodies at room temperature for 1 h. Blots were analyzed with a Pierce™ ECL kit (ThermoFisher Scientific, Waltham, MA USA).

### Morris water maze (MWM) test

MWM tests were carried out in a swimming pool with four quadrants. Before the experiment, the rats were trained to find the location of the platform over four days. The time to find the platform for each rat was recorded. After the final training, the platform was removed, and the escape latency, time of crossing targets, and time in the target quadrant were recorded.

### Nissl staining

Rat brains were extracted at the conclusion of the experiment. After sectioning in a freezing microtome, slices were immediately immersed in 0.2% thionine at 37°C for 10 min, photographed with an Axiolmager M1 microscope (Zeiss, Inc., Jena, Germany), and transferred in 4% paraformaldehyde (PFA) for immersion fixation over 24 h.

### Enzyme-linked immunosorbent assay (ELISA)

The serum concentrations of interleukin (IL)-1 $\beta$ , TNF- $\alpha$ , and IL-6 were determined using an ELISA kit according to the manufacturer's protocol. Briefly, rat serum samples were added to plates followed by a biotin-conjugated specific antibody and avidin-conjugated horseradish peroxidase (HRP), followed lastly by an enzyme substrate. The intensity of the color in each well was measured with an ELISA reader (R&D systems, Minneapolis, MN, USA).

## Quantitative PCR

Trizol reagent (Invitrogen, Waltham, MA, USA) was used to extract total RNA. Then, RNA was reverse-transcribed into cDNA using Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI, USA). FastStart Universal SYBR Green Master kit (Roche, Basel, Switzerland) was used for quantitative mRNA detection using an ABI StepOne system (Applied BioSystems, Foster City, CA, USA). The levels of targeted genes were determined using the  $2^{-\Delta\Delta Ct}$  method. The primers used are listed in Table 1.

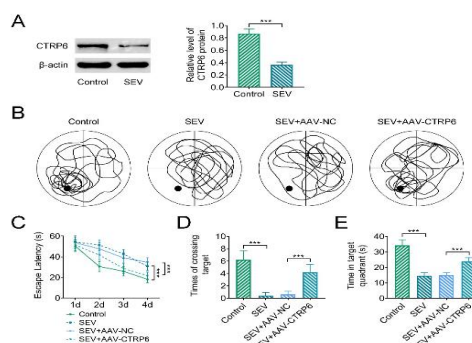
## Statistical analysis

Data are displayed as mean  $\pm$  SD. Statistical analysis was performed using GraphPad Prism (San Diego, CA, USA). Statistical differences between groups were identified using an analysis of variance (ANOVA).  $P < 0.05$  was considered statistically significant.

## RESULTS

### CTRP6 reduced sevoflurane-induced cognitive impairment in rats

To detect the effect of CTRP6 in SEV-induced cognitive impaired rats, the level of CTRP6 was first measured in a cognitive impairment rat model. Significant downregulation of CTRP6 was observed in the hippocampus of SEV-induced rats (Figure 1A). As displayed in Figure 1B, the cognitive ability in SEV group was impaired significantly. However, CTRP6 overexpression improved the performance of rats in MWM test (Figure 1B–E). The time spent in the target quadrant and the time of crossing target were significantly reduced in SEV group. However, the escape latency increased in SEV group. The overexpression of CTRP6 altered these parameters (Figure 1C–E). These results suggest the impaired cognitive ability from sevoflurane was resolved by CTRP6 overexpression.



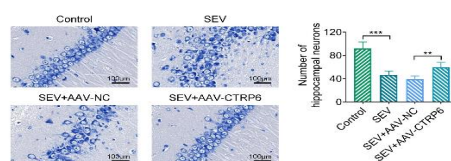
**Figure 1:** CTRP6 reduces sevoflurane-induced cognitive impairment in rats. A. Expression level of CTRP6 in the control and SEV group. B. MWM assay in different groups. C. Escape latency (s). D. Time of crossing target (unit), E. Time in target quadrant in different groups (s). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### CTRP6 alleviates SEV-induced neuron injury

Rats were then subjected to Nissl staining. The number of hippocampal neurons in SEV-induced cognitive impaired rats was significantly reduced compared with the control group. The hippocampal neuron population was higher in the CTRP6 overexpression group (Figure 2). This suggests that overexpression of CTRP6 alleviates neuron death caused by SEV.

### CTRP6 reduced SEV-induced inflammatory response

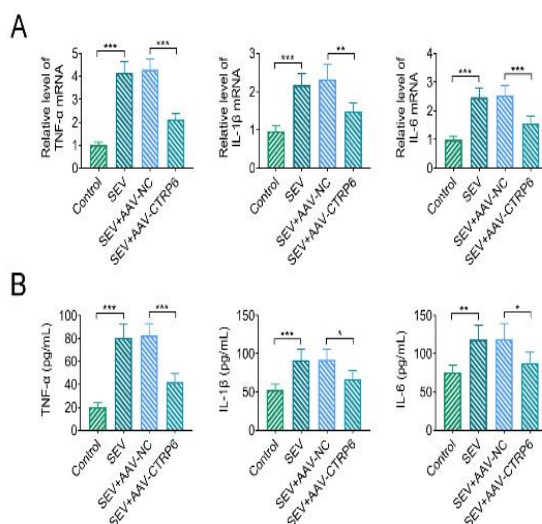
The serum level and mRNA of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were quantified using ELISA or qPCR. We observed SEV-induced rats had higher levels of these inflammatory markers compared to the control and CTRP6 overexpression groups (Figure 3A, B). These data suggest that CTRP6 could relieve the SEV-induced inflammation response.



**Figure 2:** CTRP6 alleviates SEV-induced neuron injury. Nissl staining in different groups. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

**Table 1:** Primers for quantitative PCR

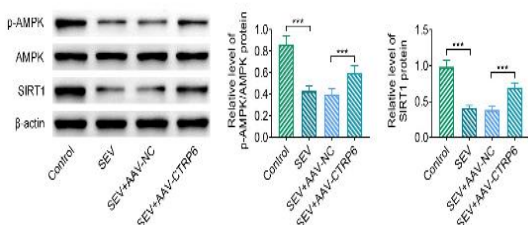
Gene	Forward primers (5'-3')	Reverse primer (5'-3')
TNF- $\alpha$	GAAGTGGCAGAAGAGGCACT	GGTCTGGGCCATAGAAGTGA
IL-6	CTGATGCTGGTGACAACCAC	CAGAATTGCCATTGCACAAC
IL-1 $\beta$	TGGACCTTCCAGGATGAGGAC	GTTTCATCTCGGAGCCTGTAGTG
GAPDH	AGTATGACTCCACTCACGGC	CACCAGTAGACTCCACGACA



**Figure 3:** CTRP6 relieves SEV-induced inflammation response. A and B: mRNA levels and levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in different groups. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

### CTRP6 ameliorates SEV-induced cognitive injury by AMPK/SIRT1 pathway

The levels of p-AMPK and SIRT1 were inhibited in the SEV group. CTRP6 overexpression relieved the inhibition effect of SEV (Figure 4). These data suggest that CTRP6 relieved the cognitive dysfunction induced by sevoflurane via AMPK/SIRT1.



**Figure 4:** CTRP6 improves SEV-induced cognitive injury by the AMPK/SIRT1 pathway. The level of p-AMPK, AMPK, and SIRT1 in each group. \* $P < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

## DISCUSSION

Central nervous system complications following surgery in older adults are characterized by confusion, anxiety, personality changes, and impaired memory [9,10]. Such changes in personality, social skills, and cognitive abilities and skills are referred to as POCD [11]. POCD has been suggested to be a deterioration of mental function, manifested in decreased memory and concentration after surgery. Cognitive impairment after anesthesia treatment

is common, especially in the elderly. Depending on the patient age, time of onset, and the type of surgery (cardiac and non-cardiac), the incidence of POCD ranges from 10 – 62 %.

POCD is potentially involved in central nervous, endocrine, and immune systems disorders, but its mechanism is still unclear [12]. To improve the outcome of POCD, we aimed to clarify the regulatory mechanism and identify key regulatory proteins. In this study, a key protein in CTRP family, CTRP6, was found to be associated with the progression of POCD. Data in this study suggest that CTRP6 could alleviate cognitive dysfunction induced by sevoflurane in rats and could serve as a promising target to alleviate POCD.

The various effects of CTRP6 in different cellular processes and the pathology of diseases has been studied extensively. For example, miRNA-29 modulated CTRP6 and subsequent porcine adipogenesis via the AKT/MAPK pathway [13]. Another study indicated that CTRP6 attenuated apoptosis via AMPK/SIRT1-modulated miR-34a-5p expression in salivary acinar cells [14]. In this study, CTRP6 improved the cognitive dysfunction induced by sevoflurane via mediating this pathway. In addition, CTRP6 played a key role in kidney fibrosis through the promotion of fatty acid oxidation via the AMPK pathway [15].

The depletion of CTRP6 can reduce intramuscular and subcutaneous fat in pigs. In addition, CTRP6 depletion also suppressed high glucose-induced oxidative stress, inflammation, and extracellular matrix accumulation in mesangial cells via the Akt/NF- $\kappa$ B pathway [16]. Here, the effects of CTRP6 on the progression of POCD via AMPK-SIRT1 axis were also explored. These findings indicate that CTRP6 could serve as a promising target in multiple diseases.

In this study, CTRP6 affected POCD via the AMPK/SIRT1 axis. AMPK is known as a key regulator that maintains energy metabolism. It consists of a catalytic subunit ( $\alpha$ ) and two regulatory subunits ( $\beta$  and  $\gamma$ ), which are activated by phosphorylation of threonine 172 (Thr172) [17]. SIRT1 is a nicotinamide adenine dinucleotide (NAD)<sup>+</sup> dependent protein deacetylase that plays an important role in regulating physiological and pathological processes such as apoptosis, metabolism, and differentiation through deacetylation of intracellular signal transducers. Phosphorylation of AMPK activates SIRT1. AMPK/SIRT1 is associated with cellular senescence and neurological diseases; activation of AMPK/SIRT1 improves cognitive dysfunction caused by

sevoflurane [18]. Therefore, the effects of CTRP6 on AMPK/SIRT1 pathway in a POCD rat model could be a promising therapeutic target.

## CONCLUSION

This study suggests that CTRP6 alleviates sevoflurane-induced cognitive dysfunction in rats. It also ameliorates sevoflurane-induced nerve injury and inflammation in rats and improves cognitive dysfunction via the AMPK/SIRT1 pathway. Together, these data suggest CTRP6 could be a novel target for POCD treatment.

## DECLARATIONS

### 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. Yunming Nie and Yimin Zhou designed the study and supervised data collection. Yanyue Wu and Xuefen Huang analyzed and interpreted the data. Zhengzheng Chen and Yimin Zhou prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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