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Original Research Article

Sevoflurane alleviates liver ischemia reperfusion injury through inactivation of the TRAF6/NF-κB signaling pathway

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Abstract

Purpose: *To evaluate the role and mechanism of action of sevoflurane in liver ischemia reperfusion injury.*
Methods: *Rats were pretreated with sevoflurane and then underwent liver ischemia followed by reperfusion to establish an animal model of liver ischemia reperfusion injury. Pathological changes in liver tissues were investigated by hematoxylin and eosin (H & E) staining, and serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using a chemistry analyzer. ELISA was used to determine the levels of myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), IL-6, superoxide (SOD), malonaldehyde (MDA), catalase (CAT), and glutathione (GSH).*
Results: *Pathological changes in liver tissue, including sinusoidal congestion, vacuole formation, and infiltration of inflammatory cells and lymphocytes, were identified in rats post-ischemia reperfusion injury. In addition, serum ALT and AST levels increased following ischemia reperfusion injury. However, administration of sevoflurane ameliorated the pathological liver damage and decreased the serum ALT and AST levels induced by ischemia reperfusion. Pro-inflammatory cytokines, such as MPO, TNF-α, IL-1β, and IL-6 were upregulated in rats following ischemia reperfusion injury, and this upregulation was reversed by sevoflurane administration. Sevoflurane administration also attenuated the ischemia reperfusion-induced increase in MDA and decrease in SOD, CAT, and GSH. Ischemia reperfusion repressed IκBα protein expression and promoted protein expression of TNF receptor associated factor 6 (TRAF6), phospho (p)-IκBα, and p-p65 in liver tissue. However, sevoflurane reversed the effect of ischemia reperfusion on IκBα, TRAF6, p-IκBα, and p-65 expression.*
Conclusion: *Sevoflurane administration reduced pathological liver injury post-ischemia reperfusion by suppressing the inflammatory response and oxidative stress through inactivation of the TRAF6/NF-κB pathway.*

Keywords: *Sevoflurane, Liver ischemia reperfusion, Pathological injury, Inflammation, Oxidative stress, TRAF, NF-κB*

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INTRODUCTION

Liver ischemia reperfusion injury is a complicated pathophysiological process that occurs during liver transplantation and tissue resection surgery [1]. It is a major cause of the high mortality during liver and gallbladder surgery [2]. Hepatocellular swelling, sinusoidal congestion, and inflammatory cell infiltration in the portal area are the main manifestations of liver ischemia reperfusion injury [3]. Liver ischemia reperfusion injury leads to severe liver damage and can even result in death [4]. Inflammation and oxidative stress have been found to be associated with liver ischemia reperfusion injury pathogenesis [5]. Strategies to repress inflammation and oxidative stress showed promise in ameliorating ischemia reperfusion-induced liver injury [6].

Although the molecular mechanism of ischemia reperfusion has been explored widely, the pathophysiological process is not fully understood. Interestingly, use of volatile anesthetics has been shown to prevent tissue damage after liver ischemia by regulating blood flow [7]. Sevoflurane is an inhaled anesthetic that functions as a bronchodilator by regulating calcium homeostasis [8,9]. Sevoflurane protects against lung [10] and myocardial [11] ischemia reperfusion injury. Sevoflurane also prevents cerebral ischemia reperfusion injury [12]. The protective effect of sevoflurane against liver ischemia reperfusion injury has been investigated in animal models. Sevoflurane downregulated NFKB3 expression by enhancing miR-9-5p expression and protected the liver from ischemia reperfusion injury [13]. Sevoflurane administration reduced glucose regulatory protein 78 expression, thereby suppressing ischemia reperfusion-induced liver damage and cell apoptosis [14]. However, the mechanism by which sevoflurane exerts its protective effect on liver ischemia reperfusion injury remains unclear and requires further study. In this study, the effects and mechanism of sevoflurane treatment on liver inflammation and oxidative stress induced by ischemia/reperfusion were investigated.

EXPERIMENTAL

Animal models

Thirty Wistar rats (6–8 weeks old, weighing 200–240 g) were purchased from the Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China) and divided randomly into three groups: sham, ischemia reperfusion (I/R), and I/R+sevoflurane. The animal study protocol was approved by the Ethics Committee of The Ninth People's Hospital of Chongqing (approval no. 2019033) and was performed in accordance with National Institutes of Health Laboratory Animal Care and Use Guidelines [15].

For establishment of the rat model of ischemia reperfusion injury, rats were injected with 1 % pentobarbital sodium and placed on the operating table. The liver was isolated from the surrounding ligaments by an incision in the middle of the abdomen. The pedicles of the left and middle lobes of the liver were blocked with vessel clips. After 2 h and when the lobes turned white, the ischemia was complete. Reperfusion was performed for 2 h, blood samples were collected with a syringe, and plasma was obtained by centrifugation at 12000 *g* for 10 min. After washing with 0.9 % saline, liver tissue was collected. Rats in the sham group underwent laparotomy without the ischemia and reperfusion processes. For rats in the I/R+sevoflurane group, rats were placed in a container connected to an anesthesia machine and pretreated with 2.4 % sevoflurane (1 minimum alveolar anesthetic concentration) for 30 min before the ischemia and reperfusion procedures were performed.

Hematoxylin and eosin (H & E) staining

Isolated liver tissues were fixed with 4 % formalin, embedded in paraffin, and cut into 4-μm sections. The sections were deparaffinized in xylene and rehydrated with a graded ethanol series. After immersing in hematoxylin for 2 min and washing with 0.9 % saline, the sections were stained with eosin (Solarbio, Beijing, China) for 5 minutes. The sections were examined under an optical microscope (Zeiss, Oberkochen, Germany).

Biochemical assays

Serum levels of aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using an automated chemistry analyzer (IDEXX Laboratories, Westbrook, ME, USA). Myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), and IL-6 levels in liver tissues were measured using commercial assay kits (Sigma Aldrich, St. Louis, MO, USA). Malonaldehyde (MDA), superoxide (SOD), catalase (CAT), and glutathione (GSH) levels were measured using commercial assay kits (Sigma Aldrich).

Western blot assay

Proteins were extracted from liver tissues using the ProteoPrep Total Extraction Sample Kit (Sigma-Aldrich) and protein concentrations were

determined using a BCA protein assay kit (Beyotime, Shanghai, China). Samples were separated by SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were blocked in 5 % skim milk and then incubated with primary antibodies against TRAF6 (1:2000; Cell Signaling Technology, Danvers, MA, USA), IκBα and p-IκBα (1:2500; Cell Signaling Technology), p65 and p-p65 (1:3000; Cell Signaling Technology), and GAPDH (1:3500; Abcam, Cambridge, MA, USA). The membranes were then incubated with horseradish peroxidase-linked secondary antibody (1:5000; Abcam) and detection was performed using an enhanced chemiluminescence detection kit (Beyotime).

Statistical analysis

Data were presented as mean ± standard error of mean and analyzed using SPSS 16.0 software. Student's t-test and one-way analysis of variance (ANOVA) were used to analyze differences between groups. $P < 0.05$ was considered significant.

RESULTS

Sevoflurane alleviated ischemia reperfusion-induced liver injury

Rats underwent liver ischemia followed by reperfusion, and then liver tissues were analyzed using H & E staining. The results showed that ischemia reperfusion induced pathological changes in liver tissue as demonstrated by hepatocellular swelling, sinusoidal congestion, and infiltration of inflammatory cells and lymphocytes (Figure 1 A). However, pretreatment with sevoflurane ameliorated pathological liver damage (Figure 1 A). Indicators of liver damage, such as ALT (Figure 1 B) and AST (Figure 1 C) levels, increased post-ischemia reperfusion ($p < 0.01$); however, sevoflurane administration decreased ALT (Figure 1 B) and AST (Figure 1 C) levels and alleviated ischemia reperfusion-induced liver injury.

Sevoflurane alleviated ischemia reperfusion-induced inflammation

ELISA data showed that ischemia reperfusion increased MPO activity (Figure 2 A) and increased levels of the proinflammatory cytokines TNF-α (Figure 2 B), IL-1β (Figure 2 C), and IL-6 (Figure 2 D) in liver tissue. However, pretreatment with sevoflurane suppressed the ischemia reperfusion-induced increase in MPO activity (Figure 2 A) and TNF-α (Figure 2 B), IL-1β (Figure 2 C), and IL-6 (Figure 2 D) levels.

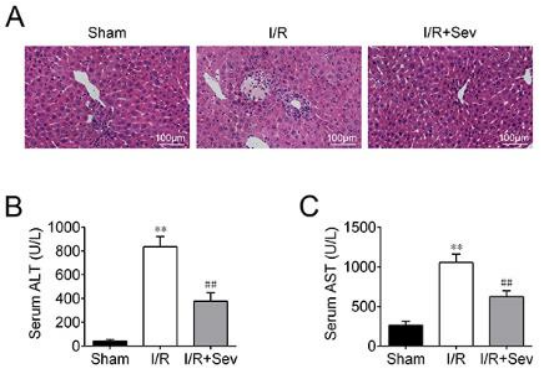

Figure 1: Sevoflurane alleviated ischemia reperfusion-induced liver injury. (A) Ischemia reperfusion induced pathological changes in liver tissue; however, pretreatment with sevoflurane ameliorated the pathological liver damage. (B) Ischemia reperfusion increased the serum ALT level; however, pretreatment with sevoflurane reversed this increase. (C) Ischemia reperfusion increased the serum AST level; however, pretreatment with sevoflurane reversed this increase. **, ## $P < 0.01$

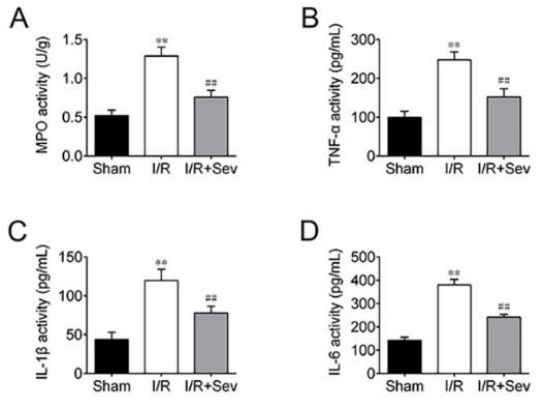

Figure 2: Sevoflurane alleviated ischemia reperfusion-induced inflammation. (A) Ischemia reperfusion increased MPO activity; however, pretreatment with sevoflurane reversed this increase. (B) Ischemia reperfusion increased the level of TNF-α; however, pretreatment with sevoflurane reversed this increase. (C) Ischemia reperfusion increased the level of IL-1β; however, pretreatment with sevoflurane reversed this increase. (D) Ischemia reperfusion increased the level of IL-6; however, pretreatment with sevoflurane reversed this increase. **, ##$P < 0.01$

Sevoflurane alleviated ischemia reperfusion-induced oxidative stress

ELISA data showed that SOD activity decreased in liver tissue following ischemia reperfusion (Figure 3 A). MDA levels increased (Figure 3 B), CAT activity decreased (Figure 3 C), and GSH levels decreased (Figure 3 D) in liver tissue following ischemia reperfusion. However, pretreatment with sevoflurane suppressed the

ischemia reperfusion-induced decrease in SOD (Figure 3 A) and CAT (Figure 3C) activities and suppressed the ischemia reperfusion-induced increase in MDA levels (Figure 3 B) and decrease in the GSH level (Figure 3 D).

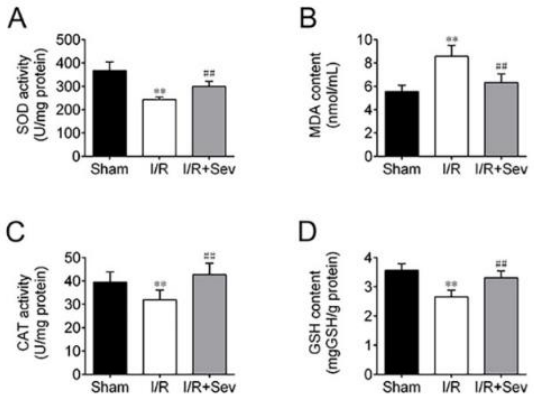

Figure 3: Sevoflurane alleviated ischemia reperfusion-induced oxidative stress. (A) Ischemia reperfusion decreased SOD activity; however, pretreatment with sevoflurane reversed this decrease. (B) Ischemia reperfusion increased the level of MDA; however, pretreatment with sevoflurane reversed this increase. (C) Ischemia reperfusion decreased CAT activity; however, pretreatment with sevoflurane reversed this decrease. (D) Ischemia reperfusion decreased the level of GSH; however, pretreatment with sevoflurane reversed this decrease. **, ##*P* < 0.01

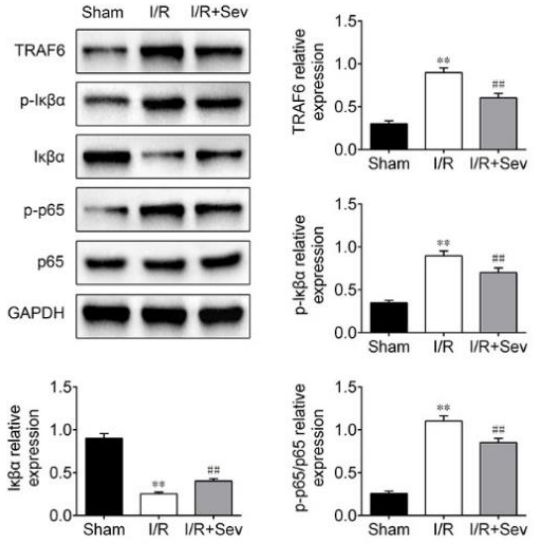

Figure 4: Sevoflurane reduced the TRAF6 expression induced upon liver ischemia reperfusion injury. Ischemia reperfusion decreased IκBα protein expression and increased TRAF6, p-p65, and p-IκBα expression; however, pretreatment with sevoflurane reversed the effects of ischemia reperfusion on IκBα, TRAF6, p-p65, and p-IκBα protein expression. **, ## *P* < 0.01

Sevoflurane reduced the TRAF 6 expression induced upon liver ischemia reperfusion injury

Western blot analysis showed that TRAF6 expression increased in liver tissue following ischemia reperfusion (Figure 4) and that pretreatment with sevoflurane reversed this increase (Figure 4). Ischemia reperfusion decreased protein expression of IκBα and increased the level of p-IκBα (Figure 4) in liver tissue; however, sevoflurane administration reversed the decrease in IκBα and reversed the increase in p-IκBα expression (Figure 4). Moreover, sevoflurane administration attenuated the ischemia reperfusion-induced increase in p-p65 in liver tissue (Figure 4) suggesting that sevoflurane reduced TRAF6 expression to ameliorate liver ischemia reperfusion injury.

DISCUSSION

Current strategies of reducing liver ischemia reperfusion injury, such as ischemic preconditioning or postconditioning, are either expensive or difficult to perform [16]. Treatment with drugs or small molecules to reduce production of reactive oxygen species [17] or activation of inflammatory networks [18] have been shown to be effectively reduce liver ischemia reperfusion injury. Because sevoflurane was shown to reduce production of reactive oxygen species and inflammatory cytokines, thereby attenuating acute lung injury [19], the role of sevoflurane on liver ischemia reperfusion injury was investigated in this study.

Previous studies showed that ischemia reperfusion-induced hepatic injury associates with hepatocyte balloon degeneration and irregular cell arrangement in liver tissue [14], as well as increased serum AST and ALT levels [20]. This study confirmed that ischemia reperfusion induced pathological damage of liver tissue and increased levels of the liver damage indicators ALT and AST. In consistent with a previous report that sevoflurane protected against hepatic ischemia reperfusion injury [21], pathological damage of liver tissue post-ischemia reperfusion was ameliorated by sevoflurane treatment. Serum ALT and AST levels in rats post-ischemia reperfusion injury were also downregulated followed sevoflurane treatment.

Ischemia reperfusion induced infiltration of inflammatory cells and lymphocytes in liver tissue and promoted secretion of cytokines to aggravate the tissue damage [18]. Inflammatory markers, such as TNF-α, IL-1β, and IL-6 were significantly upregulated during liver ischemia

reperfusion injury [22]. Sevoflurane administration suppressed secretion of inflammatory cytokines during ischemia reperfusion injury [22]. Levels of MPO, TNF-α, IL-1β, and IL-6 in ischemia reperfusion-induced rats were decreased by sevoflurane administration suggesting the anti-inflammatory role of sevoflurane in liver ischemia reperfusion injury.

Ischemia reperfusion induced lipid peroxidation, mitochondria dysfunction, and cell apoptosis causing oxidative stress-induced live tissue damage [17]. Pretreatment with the antioxidant GSH, exogenous catalase, or SOD were used as antioxidant interventions to reduce ischemic injury [17]. Sevoflurane administration attenuated the ischemia reperfusion-induced increase in MDA and decrease in SOD, CAT, and GSH demonstrating the antioxidant effect of sevoflurane against liver ischemia reperfusion injury.

Toll-like receptor 4 mediates liver injury induced by ischemia reperfusion via MyD88-dependent or independent pathways [18]. MyD88 recruits TRAF6 to promote IκBα kinase-mediated phosphorylation of IκBα and to activate NF-κB for production of inflammatory cytokines during liver damage [18]. The suppressive effect of sevoflurane on the Toll-like receptor 4-NF-κB pathway was validated in rats with cerebral ischemia reperfusion injury [23] or acute lung injury [24]. This study showed that sevoflurane administration enhanced IκBα expression and reduced TRAF6 and p-IκBα expression. Moreover, sevoflurane administration attenuated the ischemia reperfusion-induced increase in p-p65 expression in liver tissue demonstrating that sevoflurane reduced TRAF6 expression, thereby inactivating NF-κB and ameliorating liver ischemia reperfusion injury. In addition, MyD88 recruits TRAF6 to mediate the inflammatory response during liver injury through the extracellular signal-regulated kinase/c-Jun NH2-terminal kinase/activator protein 1 pathway or My88 recruits AKT to promote the accumulation of reactive oxygen species through NAPDH oxidase [18]. Sevoflurane administration reduced expression of p-c-Jun NH2-terminal kinase, thereby suppressed cell apoptosis during hepatic ischemia reperfusion injury [14]. Thus, other pathways involved in sevoflurane-mediated alleviation of hepatic ischemia reperfusion injury should be investigated in the future studies.

CONCLUSION

This study demonstrates that sevoflurane functions as a protective molecule in liver ischemia reperfusion injury. The TRAF6/NF-κB pathway has been implicated in the ameliorative effect of sevoflurane on ischemia reperfusion injury. Thus, sevoflurane is a potential therapeutic strategy for the management of liver ischemia reperfusion injury.

DECLARATIONS

Conflict of Interest

There are no conflicts of interest to disclose with regard to this work.

Availability of data and materials

All data generated or analyzed during this study are included.

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. Hongqiang Liu and Ying Yuan contributed equally to the work. They both designed the study and supervised data collection. Dan Rao analyzed and interpreted the data. Hongqiang Liu, Ying Yuan, and Dan Rao prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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