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(RESEARCH ARTICLE)

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The effects and mechanisms of sivelestat in alleviating post-resuscitation brain injury

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Abstract

Objective: To investigate the neuroprotective effects of sivelestat (SV) in a porcine cardiac arrest-resuscitation model while exploring its association with the NF-κB/NLRP3 pathway.

Methods: Fifteen healthy male pigs were randomly assigned to the sham operation group (Sham, n=5), the cardiopulmonary resuscitation group (CPR, n=5), and the CPR with SV treatment group (CPR+SV, n=5). Cardiac arrest was induced by ventricular fibrillation for 9 minutes in the CPR and CPR+SV groups, followed by 6 minutes of cardiopulmonary resuscitation to establish the experimental model. To assess the potential protective effects of SV, five minutes after successful resuscitation, the CPR+SV group received SV at 10 mg/kg via femoral vein infusion for 1 hour using a micro-infusion pump. Blood samples were collected from the femoral vein at baseline and at 0.5, 1, 2, and 4 hours post-resuscitation for serum levels of neuron-specific enolase (NSE) and S100β protein, recognized as markers of brain injury, via ELISA. Neurological function was assessed 24 hours post-resuscitation using neurological deficit scores (NDS). The animals were euthanized 24 hours post-resuscitation, and left ventricular apical myocardial and frontal lobe cortex tissues were harvested. Brain tissue levels of inflammatory markers, IL-1β and IL-18, were analyzed using Western blotting. Western blot was used to evaluate the effect of SV in reducing the expression levels of NF-κB, NLRP3, cleaved caspase-1, and GSDMD, key markers of pyroptosis, in the brain tissues of pigs after CPR.

Results: Post-resuscitation, the CPR and CPR+SV groups exhibited significantly higher serum levels of NSE and S100β and elevated NDS scores compared to the Sham group (all $P<0.05$). Nevertheless, the CPR+SV group showed significantly improved neurological scores and reduced levels of brain injury markers at all time points post-resuscitation versus the CPR group ($P<0.05$). At 24 hours post-resuscitation, IL-1β and IL-18 levels in the brain tissues of CPR and CPR+SV groups were markedly higher than those in the Sham group ($P<0.05$). Conversely, the levels of inflammatory factors in the CPR+SV group were significantly reduced compared to the CPR group ($P<0.05$). At 24 hours post-resuscitation, brain tissue expression of NF-κB, NLRP3, cleaved caspase-1, and GSDMD was markedly elevated in the CPR group ($P<0.05$). SV markedly suppressed the expression of these pyroptosis-associated proteins ($P<0.05$), highlighting its potential role in neuroprotective via inhibition of the NF-κB/NLRP3 pathway.

Conclusion: SV effectively mitigates post-resuscitation brain injury and enhances neurological outcomes, likely through its regulatory effects on the NF-κB/NLRP3 pathway.

Keywords: Cardiac Arrest; Cardiopulmonary Resuscitation; Brain Injury; Sivelestat Sodium; NF-κB/NLRP3

1. Introduction

Cardiac arrest is a frequently encountered critical condition in clinical settings, with a global incidence rate of 1 per 1,000 annually (0.1%), making it the third leading cause of death after cancer and cardiovascular diseases[1, 2]. Despite

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advancements in modern medicine and the widespread adoption of cardiopulmonary resuscitation (CPR) techniques, the rate of return of spontaneous circulation (ROSC) ranges from 30% to 48%, but only about 10% of patients achieve normal functional recovery. Additionally, nearly 70% of patients succumb to ischemic brain injury or experience long-term neurological deficits[3, 4]. Thus, the severity of post-resuscitation brain injury is a key indicator of the success of cardiac arrest treatment[5]. This underscores the urgent need for effective interventions that can mitigate these devastating outcomes.

Studies have shown that pyroptosis, a novel form of inflammatory cell death, exacerbates target organ damage through plasma membrane rupture and the release of intracellular toxins and inflammatory mediators. Importantly, pyroptosis also plays a significant role in the progression of post-cardiac arrest brain injury[6, 7]. Research has demonstrated that NOD-like receptor pyrin domain 3 (NLRP3) acts as a key upstream protein in initiating pyroptosis, while nuclear factor-κB (NF-κB) serves as a critical upstream regulator of NLRP3 activity[8, 9]. In this context, targeting the NF-κB/NLRP3-mediated pyroptosis pathway could offer novel therapeutic targets for mitigating post-resuscitation brain injury. Additionally, sivelestat (SV), a specific inhibitor of neutrophil elastase, exerts multiple biological effects that are primarily attributed to its anti-inflammatory properties. It has shown efficacy in alleviating brain injury following local ischemia-reperfusion[10, 11], yet its specific effects on global cerebral ischemia-reperfusion injury and the underlying mechanisms involving the NF-κB/NLRP3 pathway remain unclear.

This study aims to validate the therapeutic effects of SV on post-resuscitation brain injury in a porcine model and to explore its potential mechanism of inhibiting pyroptosis via the NF-κB/NLRP3 pathway, providing new insights into treatment strategies.

2. Materials and Methods

2.1. Experimental Animal Sources and Grouping Interventions

A total of 15 healthy male domestic pigs (32-40 kg) were supplied by Shanghai Jiagan Biotechnology Co., Ltd. (Animal Certificate No. SCXK (Shanghai) 2020-0006). The animals were randomly allocated to the Sham group, the CPR group, and the CPR+SV group. The Sham group underwent surgical preparation without ventricular fibrillation induction or resuscitation, serving as a control group. In both the CPR and CPR+SV groups, cardiac arrest was induced by ventricular fibrillation. The CPR+SV group received an infusion of SV at 10 mg/kg through the femoral vein, initiated 5 minutes after successful resuscitation and continued for 1 hour.

After a 12-hour fast, anesthesia was induced via intramuscular injection of tiletamine/zolazepam (5 mg/kg) and xylazine (1 mg/kg) into the gluteal muscles. Following initial anesthesia, the animals were placed on a triangular surgical board connected to an ECG and oxygen saturation monitors, and intravenously anesthetized with propofol (2 mg/kg) through an auricular vein cannula, followed by maintenance infusion at 4-6 mg/kg/h. Endotracheal intubation was performed using a veterinary laryngoscope, followed by mechanical ventilation with volume-controlled mode, set to tidal volume 8-10 mL/kg, respiratory rate 12 breaths/min, and oxygen concentration 21%. Surgical incisions were made for catheter insertion in the right femoral artery and vein to monitor blood pressure, atrial pressure, and for blood sampling, drug delivery, and ventricular fibrillation induction by inserting an electrode into the right external jugular vein through a surgical incision.

After stabilization, 1 mA alternating current was delivered via the electrode to induce ventricular fibrillation. Cardiac arrest was confirmed by the appearance of ventricular fibrillation waves on the ECG and a drop in blood pressure to a flatline. The ventilator was immediately disconnected, and the animals were observed without intervention for 9 minutes. CPR was performed following international guidelines. Two professional rescuers alternated chest compressions, monitored by a feedback device to ensure a depth of 5-6 cm and a frequency of 100-120 compressions/min. Manual compressions and bag-valve ventilation were maintained at a 30:2 ratio. Epinephrine (20 μg/kg) was injected intravenously starting 2 minutes after initiating CPR, with the same dose repeated every 3 minutes thereafter. After 6 minutes of CPR, defibrillation was performed using a biphasic shock at 150 J. Restoration of supraventricular rhythm and maintenance of mean arterial pressure above 50 mmHg were evaluated immediately. If spontaneous rhythm and blood pressure were not restored, CPR was resumed for 2 minutes followed by defibrillation, repeated up to 5 cycles. Resuscitated animals were reconnected to mechanical ventilation and observed under continuous anesthesia monitoring for 6 hours. After this period, mechanical ventilation was discontinued, and the endotracheal tube and vascular catheters were carefully removed. The surgical incisions were disinfected and sutured, and the animals were returned to the pen for 24 hours of further observation.

2.2. Experimental Observation Indicators

Neurological function evaluation: The animals' neurological status was assessed at baseline and 24 hours post-modeling using the NDS, which evaluates seven parameters: respiratory status, consciousness, standing/walking/motor ability, muscle tone, and restraint responses. The scoring system ranges from 0 to 400, with higher scores indicating more severe neurological impairment.

- **Detection of Brain Injury Biomarkers:** Venous blood was drawn at baseline and 1, 2, 4, and 24 hours post-modeling, centrifuged to obtain serum, and stored at -80°C. Serum levels of NSE and S100β, indicative of brain injury, were subsequently measured by enzyme-linked immunosorbent assay (ELISA).
- **Detection of NF-κB/NLRP3 Signaling in Brain Tissue:** At 24 hours post-modeling, the animals were euthanized, and the cerebral cortex was rapidly excised, chopped into small pieces, and frozen in liquid nitrogen. Protein expression levels of NF-κB and NLRP3 signaling molecules were then analyzed by Western blotting.
- **Detection of Key Pyroptotic Proteins and Products in Brain Tissue:** At 24 hours post-modeling, the animals were euthanized, and the cerebral cortex was excised and immediately frozen**.** Western blotting was used to quantify protein levels of cleaved caspase-1 and the N-terminal domain of GSDMD**,** as well as the tissue concentrations of IL-1β and IL-18.

2.3. Statistical Analysis Methods

Data analysis was performed with SPSS 22.0 statistical software (IBM, USA). The normality of the distribution of measurement data was first tested. Once confirmed to follow a normal distribution, data were expressed as mean ± standard deviation (x±s). A one-way analysis of variance (ANOVA) was used for comparisons among three groups, and pairwise comparisons were conducted using Bonferroni post hoc tests. For categorical data, results were expressed as percentages. Comparisons between two groups were performed using Fisher's exact test when appropriate. A P-value of <0.05 was considered statistically significant.

3. Result

3.1. Neurological Function and Brain Injury Markers

After resuscitation, the serum concentrations of NSE and S100β were significantly higher in the CPR and CPR+SV groups compared to the Sham group, with an increase in NDS scores (all P<0.05). In contrast, the neurological function scores and brain injury biomarker levels in the CPR+SV group were significantly lower at all time points post-resuscitation compared to the CPR group (P<0.05), indicating that SV has a protective effect against brain injury after resuscitation.

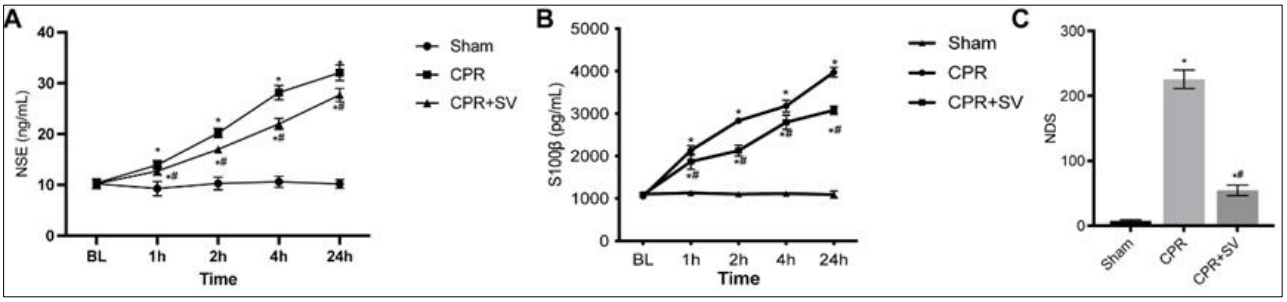

Figure 1 Comparison of neurological function and brain injury biomarkers in experimental pigs across groups. *P<0.05 compared to Sham group; *#P<0.05 compared to CPR group

3.2. Inflammatory Cytokine Levels

After 24 hours of resuscitation, the levels of IL-1β and IL-18 in the brain tissue of animals in the CPR and CPR+SV groups were significantly higher than those in the Sham group (P<0.05). However, the levels of inflammatory factors in the CPR+SV group were significantly lower than in the CPR group (P<0.05), indicating that SV can alleviate brain tissue inflammation.

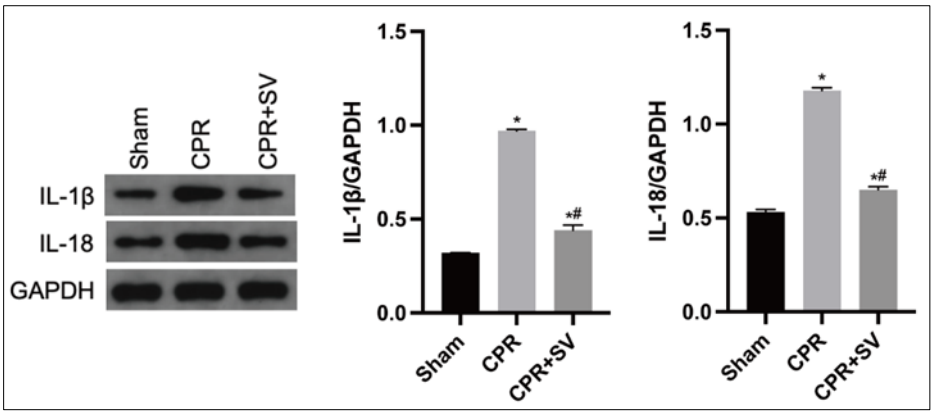

Figure 2 Comparison of brain tissue inflammatory response damage in experimental pigs post-resuscitation. *P<0.05 compared to Sham group; *#P<0.05 compared to CPR group

3.3. Regulation of pyroptosis pathway

After 24 hours of resuscitation, the expression of NF-κB, NLRP3, Cleaved Caspase-1, and GSDMD in the brain tissue of the CPR group was significantly elevated (P<0.05). However, SV significantly inhibited the expression of these pyroptosis-related proteins (P<0.05), suggesting that SV exerts neuroprotective effects by inhibiting the NF-κB/NLRP3 pathway.

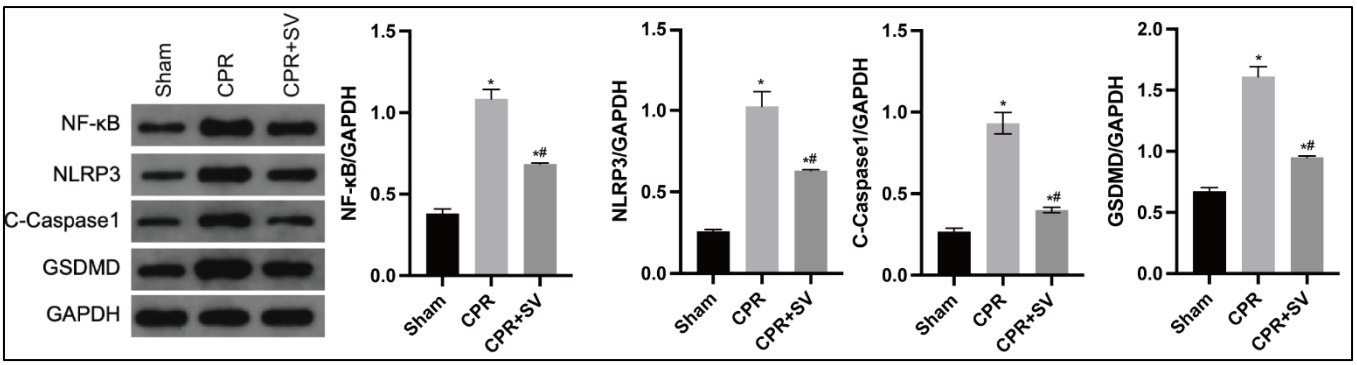

Figure 3 Effect of SV on the NF-κB/NLRP3 pyroptosis signaling pathway in brain tissue post-resuscitation. *P<0.05 compared to Sham group; *#P<0.05 compared to CPR group

4. Discussion

This study successfully established a large animal pig CPR model and used it to preliminarily investigate the protective effect of SV in reducing brain injury after resuscitation. The results showed that, compared to the CPR group, SV treatment significantly reduced brain dysfunction and brain injury markers levels in experimental pigs post-resuscitation. Further analysis suggests that its protective effect may be related to the inhibition of brain tissue inflammation and the regulation of the NF-κB/NLRP3 pyroptosis signaling pathway.

SV, a specific neutrophil elastase inhibitor that alleviates target organ damage by inhibiting the inflammatory response mediated by this enzyme. It has been shown to have various organ-protective effects, including anti-inflammatory, antioxidant, and anti-apoptotic properties[12]. In the context of ischemia-reperfusion injury, studies have shown that SV effectively alleviates local ischemia-reperfusion damage in multiple organs, showing significant therapeutic potential. Aune et al[13] demonstrated in an ex vivo rat heart ischemia-reperfusion injury model that SV reduced myocardial infarction area and improved left ventricular contraction function. Its protective mechanism may be related to reducing the production of reactive oxygen species and maintaining nitric oxide levels. In systemic ischemia-reperfusion events, existing studies have only reported that SV mitigates hemorrhagic shock-induced lung injury by inhibiting the NF-κB-mediated inflammatory response pathway.

Brain injury biomarkers (NSE and S100β) are crucial indicators that reflect the extent of neuronal damage, and their elevated levels are often associated with severe neurological dysfunction. This study aims to explore the potential protective effect of SV in alleviating brain injury following resuscitation. According to relevant literature, SV was administered at a dose of 10 mg/kg, 5 minutes after successful resuscitation[13, 14]. The results showed that both the neurofunctional scores (NDS) and serum concentrations of brain injury markers (NSE and S100β) in the CPR and CPR+SV groups were significantly higher than those in the Sham group, demonstrating significant brain dysfunction and injury post-resuscitation. However, after SV treatment, the neurofunctional indicators in the CPR+SV group improved overall, while the concentrations of brain injury markers decreased significantly at specific time points. Ikegame et al[10] found in a mouse focal cerebral ischemia model that SV significantly reduced brain edema and capillary leakage, thereby improving neurological function after cerebral ischemia. This suggests that SV may exhibit a neuroprotective effect by alleviating neurological dysfunction and brain injury after resuscitation.

Inflammation is a pivotal mechanism of brain injury following resuscitation. The substantial rise in pro-inflammatory factors such as IL-1β and IL-18 is a major contributor to the exacerbation of brain injury. This study demonstrated that treatment with SV markedly decreased the levels of these inflammatory markers, likely due to the inhibition of the NF-κB signaling pathway and the subsequent suppression of NLRP3 activity. This mechanism has also been confirmed in studies of ischemia-reperfusion injury in other organs. For instance, Hu et al[15] observed in a mouse model of liver ischemia-reperfusion and in patients undergoing liver resection, that SV significantly mitigated liver ischemia-reperfusion injury by inhibiting the Ninj1/Dusp1 axis, thereby curtailing inflammatory signaling and enabling rapid recovery of liver function after surgery. These findings reinforce the critical role of the NF-κB/NLRP3 pathway in brain injury post-resuscitation and highlight the potential of SV as a therapeutic agent for managing inflammation.

This study confirms that SV effectively mitigates brain injury after resuscitation by reducing the expression of NLRP3, cleaved caspase-1, and GSDMD. This finding aligns with the critical role of pyroptosis in brain injury as observed by Diao et al in their research on cardiopulmonary resuscitation, further validating that pyroptosis is a critical therapeutic target for post-resuscitation brain injury. The innovation of this study lies in demonstrating SV's role in regulating the pyroptosis pathway, thereby opening new avenues for treating brain injury after resuscitation[6].

This study has some limitations. Short observation time: This study focused solely on acute brain injury and inflammatory responses within 24 hours post-resuscitation and did not evaluate long-term neurological recovery and chronic inflammatory changes. Future research should extend the observation period to assess the role of SV in long-term neuroprotection. Mechanism diversity: While this study concentrated on the NF-κB/NLRP3 pathway, SV may also confer protective effects through other mechanisms, such as antioxidative stress and enhancement of vascular function. These potential mechanisms warrant further exploration via multi-omics analysis and cellular-level experiments. Dose and administration strategy: This study employed a single dose (10 mg/kg) of SV without examining the dose-response relationship or optimal timing for administration. Future research should systematically explore the impact of different doses and administration routes on the protective effects of SV.

5. Conclusion

In conclusion, SV significantly reduces brain injury and improves neurological function post-resuscitation. Its efficacy likely due to the inhibition of the NF-κB/NLRP3 pathway. This study advances our understanding of brain injury treatment following resuscitation and highlights the potential clinical application of targeting this pathway.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

The work was complied with the Declaration of Helsinki and was approved by the Institutional Animal Care and Use Committee (ZJCLA-20010639).

Author Contributions

- Jiuzhou Lin: Research Design, Experimental Operation, Thesis Writing and editing;
- Min Tang, Yaling Jin, Weiting Chen: Experimental operation;

- Lihui Chen, Jiuzhou Lin: Data collection and organization, statistical analysis

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Availability of data and materials

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

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