Article

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The Clinical Significance of iNOS/NO Signaling Pathway in Traumatic Shock and the Mechanism under the Promotion on the Development of Traumatic Shock via Endoplasmic Reticulum Stress

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Objective: This study aims to clarify the clinical significance of the inducible nitric oxide synthase (iNOs)/nitric oxide (NO) signaling pathway and endoplasmic reticulum stress (ERS) in traumatic shock (TS) and the mechanism of action, so as to offer a novel direction for the emergency treatment of TS in the future.

Methods: The clinical data of 90 patients with TS treated in our hospital between June 2019 and January 2021 were retrospectively analyzed. Patients were divided into mild (n = 30), moderate (n = 30), and severe group (n = 30) based on their disease severity. Furthermore, patients were assigned into Groups A and B for fluid resuscitation based on a pulse index continuous cardiac output (PICCO) monitor and fluid resuscitation based on monitoring results of central venous pressure (CVP) and mean arterial pressure (MAP), respectively. Additionally, the 18 purchased Sprague Dawley (SD) rats were randomized into model (TS model), control (normal rats) and intervention (TS model injected with iNOS inhibitor) groups, with 6 rats each. iNOs and NO levels were measured by colorimetry, and the concentrations of inflammatory factors were quantified using enzyme-linked immunosorbent assays (ELISAs). Polymerase chain reaction (PCR) and western blot were adopted for the quantification of ERS markers (*glucose-related protein 78* (*GRP78*), *GRP94* and *C/EBP homologous protein* (*CHOP*)), and hematoxylin-eosin (HE) staining of rat cardiac tissue was carried out to observe the pathological state of myocardial tissue.

Results: The moderate group showed higher levels of iNOS, NO, *GRP78*, *GRP94* and *CHOP* than the mild group and lower levels of them than the severe group (all $p < 0.05$). MAP, extravascular lung water index (EVLWI), pulmonary vascular permeability index (PVPI), and locus control region (LCR) increased in both Groups A and B after resuscitation, with more significant increases of these parameters in Group A. The application of PICCO technique lowered the levels of iNOS, NO, inflammatory factors, *GRP78*, *GRP94* and *CHOP* in TS patients. In addition, the intervention group had lower levels of iNOS, NO, inflammatory factors, *GRP78*, *GRP94*, and *CHOP* than the model group and higher levels of them than the control group. According to the results of HE staining of myocardial tissue, the intervention group had significantly alleviated myocardial necrosis than the model group, with slightly stained cytoplasm, visible contraction bands in most myocardium, and significantly reduced neutrophil infiltration.

Conclusions: iNOS/NO and ERS increase with the severity of TS, and PICCO can effectively lower their levels. The results of animal experiments suggest that the inhibition of iNOS/NO can relieve inflammation and ERS intensification, thus alleviating the progression of TS.

Keywords: iNOS/NO; traumatic shock; endoplasmic reticulum stress; inflammatory factors

Introduction

Traumatic shock (TS) is a severe condition due to organ damage, bleeding and circulatory disturbance following external violence, with an extremely high mortality [1]. Patients with TS primarily die from internal circulation and organ dysfunction, as well as severe post-trauma pain and negative psychology of fear and panic [2]. Clinical investigation reveals that approximately 65% of TS cases worldwide are attributed to traffic accidents [3]. Mechanical and fall injuries are also the primary causes of TS [4]. On average, there are over 3 million new TS cases each year, six to eight times as many as two decades ago [5]. As a critical manifestation of compensatory syndrome, TS is also the leading cause of death [6]. According to statistics, TS causes an estimated 1.5 million deaths worldwide each year, with rising mortality [7]. For TS patients, timely and accurate rescue measures are the only feasible means to ensure their life safety. However, given the uncharacterized pathological mechanism of TS, the rescue still depends on timely rescue and cardiopulmonary resuscitation that re-

quire high professional skills and physical strength of emergency physicians, with a depressing success rate at present [8]. Accordingly, it is urgent to search for more effective clinical TS rescue schemes to improve patient survival.

As research deepens, the study of the pathogenesis of TS from the molecular perspective has received extensive recognition and attention in clinical practice [9,10]. Inducible nitric oxide synthase (iNOs)/nitric oxide (NO), abnormally expressed in ischemia-reperfusion injury, is a signaling pathway confirmed to be essential in the pathogenesis of organ injury and dysfunction [11–13]. iNOS is a subtype of NO that is induced to be expressed after injury, which is strongly oxidized in the body and promotes the conversion of NO into peroxynitrite anion, triggering a series of downstream cascade reactions, including the activation of pro-apoptotic factors, the medication of inflammation activation, and the stimulation of malignant tissue lesions [14]. With obvious neurotoxicity, iNOS has been deemed as the key to the pathogenesis of diseases including acute ischemic stroke [15]. Moreover, significantly abnormally expressed iNOS/NO has been found in diseases such as gastric ulcer and intestinal ischemia-reperfusion injury [16,17]. Günther *et al.* [18] have reported the strong correlation between iNOS/NO and TS, but the specific mechanism is still under exploration. Similarly, endoplasmic reticulum (ER) stress (ERS), a pathological reaction strongly linked to organ function damage, is a hotspot in exploring the mechanism of TS over the past few years [19]. ERS is a reaction process in which the aggregation of misfolded and unfolded proteins in the ER cavity and the calcium ion balance disturbance activate unfolded protein response (UPR), ER overload reaction, and apoptosis pathway, with obvious activation of oxidative stress and inflammatory responses and massive programmed cell deaths in tissues as the most direct manifestations [20]. Currently, *glucose-related protein 78* (*GRP78*), *GRP94* and *C/EBP homologous protein* (*CHOP*) are clinically recognized markers of ERS, and the elevated expression levels of the three indicate an increase in the severity of ERS [21]. Jiang *et al.* [22] have indicated NLR family pyrin domain containing 3 (NLRP3) inflammasome and iNOS/NO as the crucial influential factors for inflammation and apoptosis in ERS. Accordingly, we inferred a certain potential association between the mechanism underlying the involvement of iNOS/NO in TS and ERS.

Given the high incidence and mortality of TS worldwide, it is crucial to search for novel rescue protocols as soon as possible to protect patient safety. As a hotspot of modern medical research, targeted intervention therapy from a molecular science perspective has also been hailed as a novel direction for the future treatment of various diseases. Accordingly, this study probed into the clinical significance of the iNOS/NO signaling pathway and ERS in TS and the mechanism of action, with a view to guiding future emergency treatment of TS and laying a reliable foundation for subsequent research.

Materials and Methods

Data about Patients

The clinical files of 90 patients with TS (age range: 20–61 years old, mean: 43.6 ± 7.4) treated in The Affiliated Suqian Hospital of Xuzhou Medical University between June 2019 and January 2021 were retrospectively analyzed. The patients suffered TS due to traffic accidents, mechanical injuries or falls from height. The study was performed in accordance with the Declaration of Helsinki on Biomedical Studies Involving Human Subjects. The study design was approved by the Ethics Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (No. 2021002). Due to the nature of the retrospective analysis, patient informed consent was waived.

Inclusion and Exclusion Criteria

Inclusion criteria: Patients (age >18) who met the diagnostic criteria for TS and were sent to hospital for emergency treatment within 1 h after trauma, with injury severity score (ISS) ≥16, survival time after resuscitation >72 h, and detailed case data, were included. Exclusion criteria: Patients with critical cardio-cerebrovascular diseases, tumors, organ dysfunction, autoimmune defects, coagulation dysfunction, long-term use of anticoagulants, drug allergies, severe craniocerebral injury, or incomplete clinical data were excluded, as well as those during pregnancy or lactation.

Patient Grouping

The patients were assigned to mild (n = 30), moderate (n = 30), or severe (n = 30) group according to their shock severity assessed as follows: mild shock: The patient was conscious but fidgety, pale, thirsty, and sweating, with heart rate (HR) >100 beats/minute, systolic blood pressure (SBP) ≥80 mmHg, pulse pressure <30 mmHg, and slight cyanosis and cold of extremities; moderate shock: The patient looked pale and indifferent, with cold and cyanosis of extremities, SBP of 60–80 mmHg, pulse pressure <20 mmHg, and significantly decreased urine output; severe shock: The patient was unconscious, with confusion, unresponsiveness, pale face, severe cold and cyanosis of extremities, marble-like changes in skin, weak pulse that disappeared under slight pressure, HR >120 beats/minute, SBP that dropped to 40–60 mmHg, and obviously decreased urine output or no urine. Then all patients were divided into Groups A and B for fluid resuscitation based on a pulse index continuous cardiac output (PICCO) monitor and fluid resuscitation based on monitoring results of central venous pressure (CVP) and mean arterial pressure (MAP), respectively.

Rescue Measures

All the patients were promptly rescued upon admission. To maintain airway patency, a double-lumen deep ve-

nous catheter was inserted through the subclavian and internal jugular veins to monitor vital signs in real time. Patients in Group B were treated with liquid resuscitation according to the CVP, with the CVP maintained at 8–12 mmHg; dopamine dosing was adjusted to maintain MAP at about 65 mmHg. Group B was given fluid resuscitation with PICCO. A 4F PV2014L16 catheter was inserted through the femoral artery and connected to a PICCO monitor that was connected to a temperature sensor and a CVP terminal to continuously monitor the patient's cardiac output. Additionally, the pulse curve analysis, arterial thermodilution method and pressure transducer were used to monitor the invasive arterial pressure. Normal saline (10–15 mL, temperature <8 °C) was injected from the temperature sensor end within 5 s. The dilution curves of the thermal dilution process were plotted for waveform feature analysis to obtain various PICCO parameters. Fluid resuscitation was carried out according to global end diastolic volume index (GEDVI), which was maintained at 680–800 mL/m^2. The dosage of dopamine was determined according to system vascular resistance index (SVRI) to keep SVRI at 1500–20,000 (dyn·s)/(cm^5·m^2). The dobutamine dosage was adjusted based on cardiac index (CI) to maintain CI at 3–5 L/min/m^2.

Collection of Patients' Test Samples

Fasting venous blood (4 mL) was drawn from each patient before and after fluid resuscitation and placed in coagulation-promoting tubes. Serum was centrifugally obtained (1505 ×g, 4 °C, 10 minutes) after a 30-minute still standing, followed by storage at –80 °C for subsequent experiments.

Animal Data

Eighteen healthy male Sprague Dawley (SD) rats, 8–12 weeks old with a weight of 280–320 g, were provided by Beijing Huayuan Times Technology Co., Ltd. (Animal License Number of SYXK [Beijing] 2021-0040). The rats were reared with five rats per cage at 20 °C–25 °C and humidity of 50%–65% under a 12-h/12-h light/dark cycle, with free access to food and water. This study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication no. 85-23, revised 1996). All animal experiments were approved by the Ethics Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (202101).

Modeling Methods

The rats were randomly assigned to a control group, a model group, and an intervention group of 6 rats each. With reference to the research by Deng *et al*. [10], TS models were established via femoral trauma. Specifically, the chosen rats were anesthetized through intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg), and their body temperature was kept at 37 °C by a rectal thermometer and a heating pad. Under aseptic conditions, a catheter was inserted into the right carotid artery of the rat and connected to a PICCO cardiopulmonary volume monitor, with which the MAP was recorded continuously. A 2.5 kg iron wheel was then released from a position 30 cm high to collide with the rat's femur once, triggering a traumatic injury. When MAP was decreased to 35 mmHg, the thigh wound was bandaged to reduce further bleeding. If the MAP drop continued, the MAP was maintained above 35 mmHg by injecting Ringer's lactate solution via the jugular vein cannula. The modeling was regarded as successful when the MAP was kept at 35–40 mmHg for 60 minutes. After that, a Ringer's lactate solution (20 mL/kg) was injected into rats at a rate of 20 mL/h to initiate resuscitation. Rats in the control group were given only a catheter, without TS or fluid resuscitation. Those in the intervention group were intravenously infused with 100 mg/kg aminoguanidine (AG, KL-0079, Shanghai Kanglang Biological Technology Co., Ltd. Shanghai, China), an iNOS inhibitor.

Collection of Animal Test Samples

At 3 h after modeling, all rats were executed by cervical dislocation under anesthesia. Heart blood (5 mL) was sampled from each rat and placed in a coagulation-promoting tube, followed by serum acquisition via the centrifugation method described above and storage for later testing.

Determination Methods

iNOS/NO Quantification

iNOS and NO levels in serum and liver tissue of rats were quantified by colorimetry with the corresponding kits provided by Shanghai Yaji Biotechnology Co., Ltd. (A014-1-1 and A014-1-9, Shanghai, China) under strict aseptic conditions and following the kit guidelines.

Polymerase Chain Reaction (PCR)

Total RNA from liver tissue was acquired using the TRIZOL method, followed by reverse transcription of it to cDNA with total RNA as template under the kit guidelines. Subsequently, a PCR was carried out with primers of *GRP78*, *GRP94*, *CHOP* and *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) (Genscript Biotechnology Co., Ltd., Nanjing, China, Table 1) and mixed solution of each reactant under 94 °C/60 s, followed by 40 cycles of 95 °C/20 s and 56 °C/30 s. $2^{-\triangle\triangle Ct}$ was used for relative mRNA expression calculation of *GRP78*, *GRP94* and *CHOP* (internal reference: *GAPDH*).

Western Blot

Total protein was acquired by lysis of liver tissues by radio immunoprecipitation assay (RIPA), and protein purity was verified via a bicinchoninic acid (BCA) assay. Total protein (20 μg) was taken for electrophoresis, which

Table 1. Primer sequence.

	F (5′-3′)	R (5′-3′)
GRP78	GATTGGACAAGAGAGAGGGT	CCATAACACGCTGGTCAAAGTC
GRP94	CAGTTGGATGGGTTAAACGCA	TTCCAGCGAGTGCATTTTCAT
CHOP	GAACGGCTCAAGCAGGAAATC	TTCACCATTCGGTCAATCAGAG
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG

GRP78, *glucose-related protein 78*; *CHOP*, *C/EBP homologous protein*; *GAPDH*, *Glyceraldehyde-3-phosphate dehydrogenase*; F, forward primer; R, reverse primer.

was then placed in a membrane that was subjected to a 1-hour immersion with 5% defatted milk. Subsequently, primary antibodies *GRP78* (1:2000, ab21685, Abcam, Shanghai, China), *GRP94* (1:2000, ab238126, Abcam, Shanghai, China), *CHOP* (1:2000, ab11419, Abcam, Shanghai, China) and β-actin (1:10,000, ab5694, Abcam, Shanghai, China) were added, followed by overnight incubation (4 °C). The horseradish peroxidase (HRP)-labeled secondary antibody (1:5000, ab20272, Abcam, Shanghai, China) was put in after tris buffer solution+tween (TBST) washing of the membrane the next day, followed by 1 h of incubation (37 °C). After incubation with enhanced chemiluminescence (ECL) luminescent solution, the target bands' gray values were analyzed via a gel ImageJ processing system V1.8.0.112 (National Institutes of Health, Bethesda, MD, USA), and the relative protein expression was calculated.

Hematoxylin-Eosin (HE) Staining

Rat cardiac tissue was acquired, followed by 24 h of immobilization with 10% formaldehyde, conventional dehydration, paraffin embedding, and cutting into 5-m slices. After hematoxylin-eosin (HE) staining and sealing, the slices were microscopically observed for pathological changes in cardiac tissue.

Statistical Analyses

This study adopted SPSS24.0 (IBM Corp., Armonk, NY, USA) for data analyses. Inter-group comparisons of enumeration data (expressed as n [%]) such as sex were conducted using the Chi-square test. Inter-group comparisons of measurement data (represented by $\bar{x} \pm$ s) such as iNOS/NO levels were conducted using the independent-samples *t*-test and paired *t*-test; multiple-group comparisons were performed using the one-way analysis of variance (ANOVA) and Least-Significant Difference (LSD) post-hoc test. $p < 0.05$ denotes a statistically significant difference.

Results

Association of iNOS/NO with Shock Severity

iNOS and NO levels were found to be the highest in the severe group, followed by the moderate group and the mild group (all $p < 0.05$, Fig. 1).

Association of the Levels of Inflammatory Factors with Shock Severity

In terms of inflammatory factors interleukin (IL)-1β, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β, their levels were also the highest in the severe group, followed in descending order by the moderate group and the mild group (all $p < 0.05$, Fig. 2).

Association of ERS with Shock Severity

GRP78 mRNA, *GRP94* mRNA, and *CHOP* mRNA were found to be the highest in the severe group and the lowest in the mild group, with those in the moderate group in between (all $p < 0.05$, Fig. 3). Consistently, Western blot also revealed the highest *GRP78*, *GRP94* and *CHOP* protein levels in the severe group, followed in descending order by the moderate group and mild group (all $p < 0.05$).

Impacts of PICCO on Vital Signs of TS Patients

As the state-of-art technology of modern clinical rescue, PICCO technology can effectively improve the rescue success rate and survival possibility of patients with critical diseases. At the current stage, the correlation of PICCO technology with iNOS/NO and ERS in TS patients remains uncharacterized, so the enrolled TS patients were further grouped into Groups A and B to observe the impact of PICCO on iNOS/NO and ERS. Before treatment, the two groups were similar in MAP, CVP, extravascular lung water index (EVLWI), pulmonary vascular permeability index (PVPI), and LCR levels (all $p > 0.05$). At 4 h and 24 h after treatment, Group A showed higher levels of MAP and EVLWI and lower levels of CVP, PVPR, and LCR than Group B (all $p < 0.05$). In both groups, MAP and EVLWI elevated after treatment, while CVP, PVPR, and LCR decreased (all $p < 0.05$) (Fig. 4).

Impacts of PICCO on iNOS/NO in TS Patients

Before treatment, the two groups were similar in iNOS and NO levels ($p > 0.05$); after treatment, iNOS and NO in both groups reduced ($p < 0.05$), with lower levels of them in Group A compared with Group B ($p < 0.05$, Fig. 5).

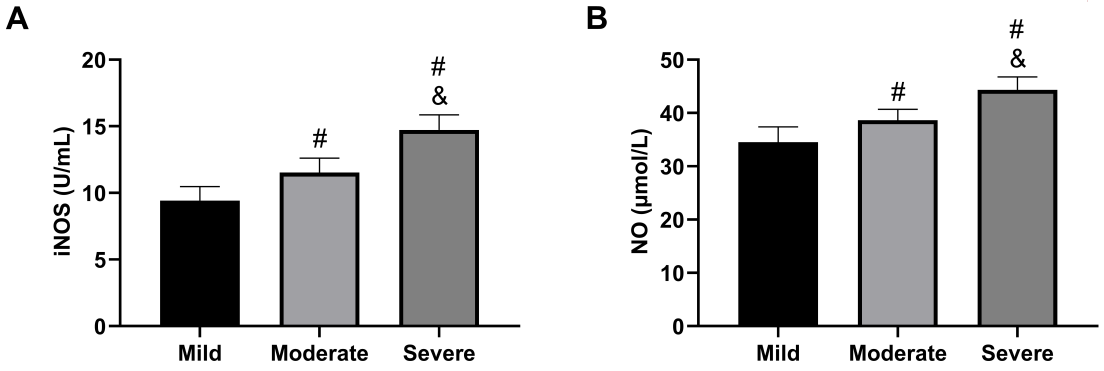

Fig. 1. Association of inducible nitric oxide synthase (iNOs)/nitric oxide (NO) with shock severity (n = 30). (A) Comparison of iNOS level. (B) Comparison of NO level. # *vs*. Mild group, $p < 0.05$. & *vs*. Moderate group, $p < 0.05$.

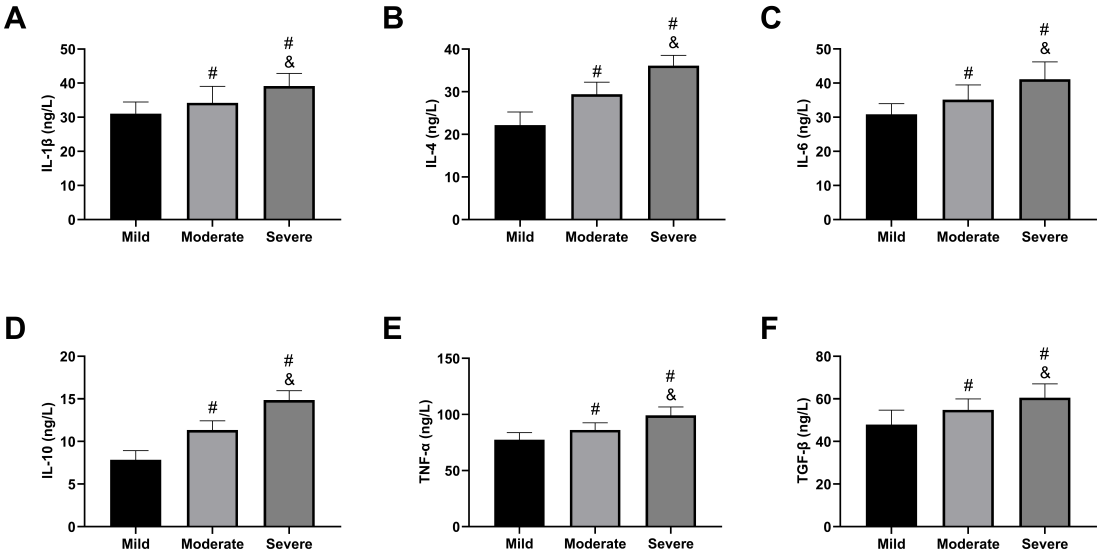

Fig. 2. Association of inflammatory factors with shock severity (n = 30). (A) Comparison of interleukin (IL)-1β. (B) Comparison of IL-4. (C) Comparison of IL-6. (D) Comparison of IL-10. (E) Comparison of tumor necrosis factor (TNF)-α. (F) Comparison of transforming growth factor (TGF)-β. # *vs*. Mild group, $p < 0.05$. & *vs*. Moderate group, $p < 0.05$.

Impacts of PICCO on Inflammatory Factors in TS Patients

Groups A and B showed no significant difference in IL-1β, IL-4, IL-6, IL-10, TNF-α and TGF-β before treatment (all $p > 0.05$); these inflammatory factors showed reduced levels after treatment in both groups, with even lower levels in Group A (all $p < 0.05$) (Fig. 6).

Impacts of PICCO on ERS in TS Patients

Before treatment, the two groups presented no significant differences in *GRP78* mRNA, *GRP94* mRNA, and *CHOP* mRNA (all $p > 0.05$); after treatment, the levels of them in both groups decreased (all $p < 0.05$), with lower levels in Group A as compared to Group B (all $p < 0.05$, Fig. 7). Further validation using Western blot showed consistent results.

Impacts of Inhibiting iNOS/NO on TS

TS models were established to clarify the mechanism through which iNOS/NO affects TS, and iNOS and NO levels in blood specimens of rats were quantified. As indicated by Fig. 8, the model group had statistically higher iNOS and NO levels than the control and intervention groups, and the intervention group presented higher iNOS and NO levels than the control group (both $p < 0.05$). Additionally, according to the quantification results of inflammatory factors, the highest levels of IL-1β, IL-4, IL-6, IL-10, TNF-α and TGF-β were found in the model group, followed by the intervention group and control group from high to low (all $p < 0.05$). Moreover, according to the HE staining results, the myocardial cells in the control group were clearly visible and neatly arranged; in the model group, a large number of infarct focus, neutrophil infiltration, dissolved cytoplasm, and disappeared striations were observed; the intervention group showed substantially alleviated myocardial necrosis,

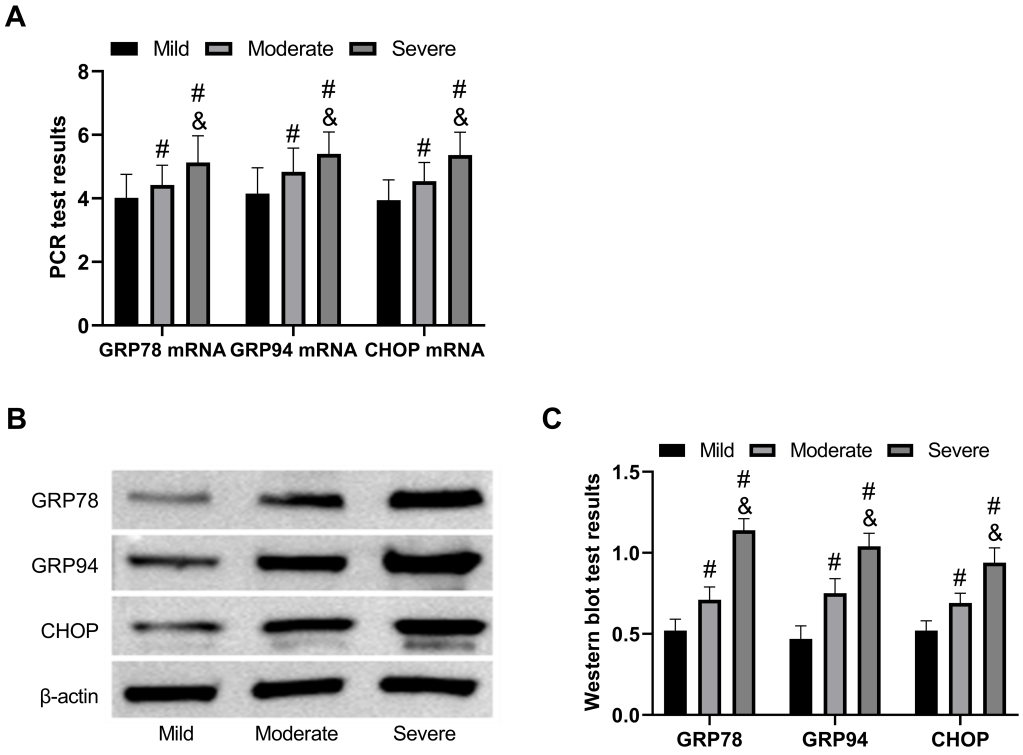

Fig. 3. Association of endoplasmic reticulum stress (ERS) with shock severity (n = 30). (A) Levels of *glucose-related protein 78* (*GRP78*) mRNA, *GRP94* mRNA and *C/EBP homologous protein* (*CHOP*) mRNA according to polymerase chain reaction (PCR) assay. (B) Western blot. (C) Levels of *GRP78*, *GRP94* and *CHOP* proteins according to Western blot. # *vs.* Mild group, $p < 0.05$. & *vs.* Moderate group, $p < 0.05$.

slightly stained cytoplasm, contraction bands in most of the myocardium, and significantly reduced neutrophil infiltration compared to the model group.

Impacts of Suppressing iNOS/NO on ERS

According to PCR and Western blot results (Fig. 9), the model and intervention groups had higher levels of *GRP78*, *GRP94* and *CHOP* than the control group (all $p < 0.05$), and the intervention group showed lower levels of them than the model group (all $p < 0.05$).

Discussion

TS, as one clinical high-risk pathological manifestation, directly endangers patients' life safety [23]. The current clinical rescue measures still have great limitations, with a success rate far from satisfactory. Therefore, the search for novel TS rescue schemes is a clinical focus and difficulty at the current stage. Pathogenesis research from a molecular perspective has a great edge in disease diagnosis and treatment, enabling the assessment of disease progression based on changes at the molecular level. In addition, molecular targeted therapies may be more effective than the current clinical treatments [24]. Moreover, the fast molecular reaction carries profound potential significance for the rescue and treatment of critical diseases [25]. Accordingly, this study preliminarily investigates the clinical implications and mechanisms of iNOS/NO and ERS in TS, which has far-reaching reference value for the future diagnosis and treatment of TS.

The iNOS/NO pathway has been shown to play a crucial role in organ injury, but its specific mechanisms and pathways of action in TS need further study. In the present study, patients with severe TS showed obvious increases in iNOS and NO, which preliminarily confirms the association of iNOS/NO with TS progression. Subsequent quantification of inflammatory factors showed consistent findings, that is, patients with a more severe shock showed a higher inflammation level. Evidence has linked inflammatory responses to organ function damage: the massive release and activation of inflammatory factors accelerate the malignant changes and necrosis of tissue cells while enhancing normal cell apoptosis [26]. Therefore, the detection results of inflammatory factors in our study can be expected. Later, the ERS quantification results revealed that ERS also increased with the increase in TS severity. ERS is known to be critical in the process of tissue injury. ERS, as aforementioned, is a reaction process in which cells activate UPR, ER overload reaction, and signal pathways including apoptosis pathway to cope with aggregation of misfolded or unfolded proteins

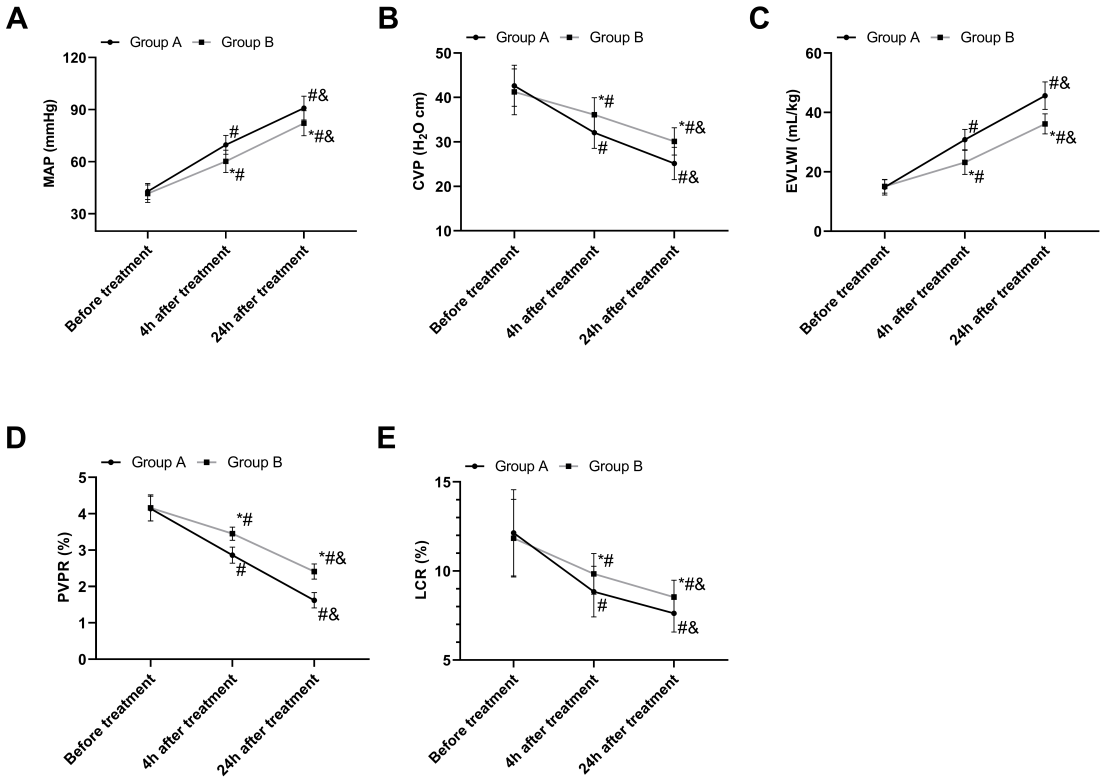

Fig. 4. Impacts of pulse index continuous cardiac output (PICCO) on vital signs of TS patients (n = 30). (A) Comparison of mean arterial pressure (MAP). (B) Comparison of central venous pressure (CVP). (C) Comparison of extravascular lung water index (EVLWI). (D) Comparison of vascular permeability index (PVPI). (E) Comparison of locus control region (LCR). # *vs.* Before therapy, $p < 0.05$. & *vs.* 4 h after treatment, $p < 0.05$. * *vs.* Group A, $p < 0.05$.

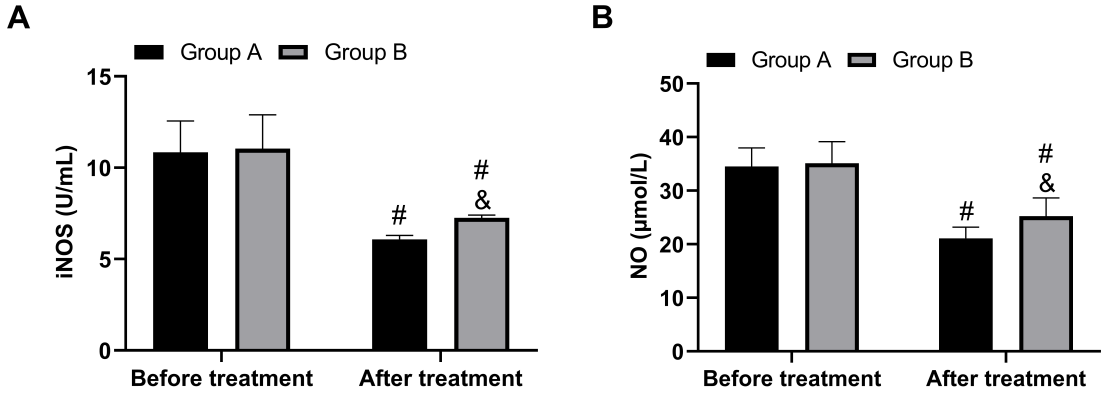

Fig. 5. Impacts of PICCO on iNOS/NO in TS patients (n = 30). (A) Comparison of iNOS level. (B) Comparison of NO level. # *vs.* Before therapy, $p < 0.05$. & *vs.* Group A, $p < 0.05$.

in the ER cavity and calcium ion balance disturbance. Its most direct manifestations are obvious oxidative stress and inflammation activation, as well as a large number of programmed cell deaths in tissues [27]. Currently, ERS has been found to be strongly correlated with various organ dysfunction such as nonalcoholic fatty liver, renal failure, and liver failure [28,29]. It is also a crucial process that directly mediates the cell life cycle and has been confirmed to be implicated in the development of hemorrhagic shock [30]. Although not fully validated, a strong association of ERS with TS has been indicated. The results of our study have also demonstrated a strong link between ERS and TS development, that is, when TS occurs, the patient's iNOS/NO will be activated and the level of inflammatory response will increase, causing severe damage to organs and tissues. At this time, organs and tissues eventually develop ERS due to various reactions such as metabolic disorders, ER overload reactions, and inflammatory transduction, which aggravates TS and endangers the patient's life.

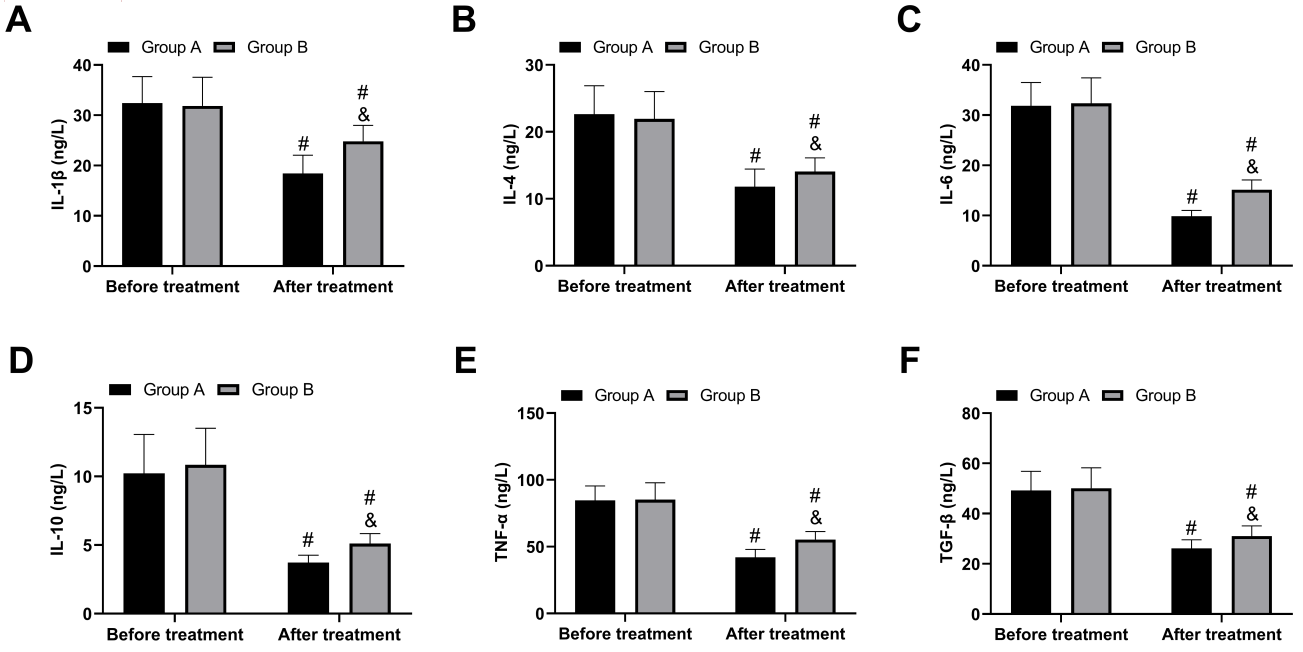

Fig. 6. Impacts of PICCO on inflammatory factors in TS patients (n = 30). (A) Comparison of IL-1β. (B) Comparison of IL-4. (C) Comparison of IL-6. (D) Comparison of IL-10. (E) Comparison of TNF-α. (F) Comparison of TGF-β. # *vs*. Before therapy, $p < 0.05$. & *vs*. Group A, $p < 0.05$.

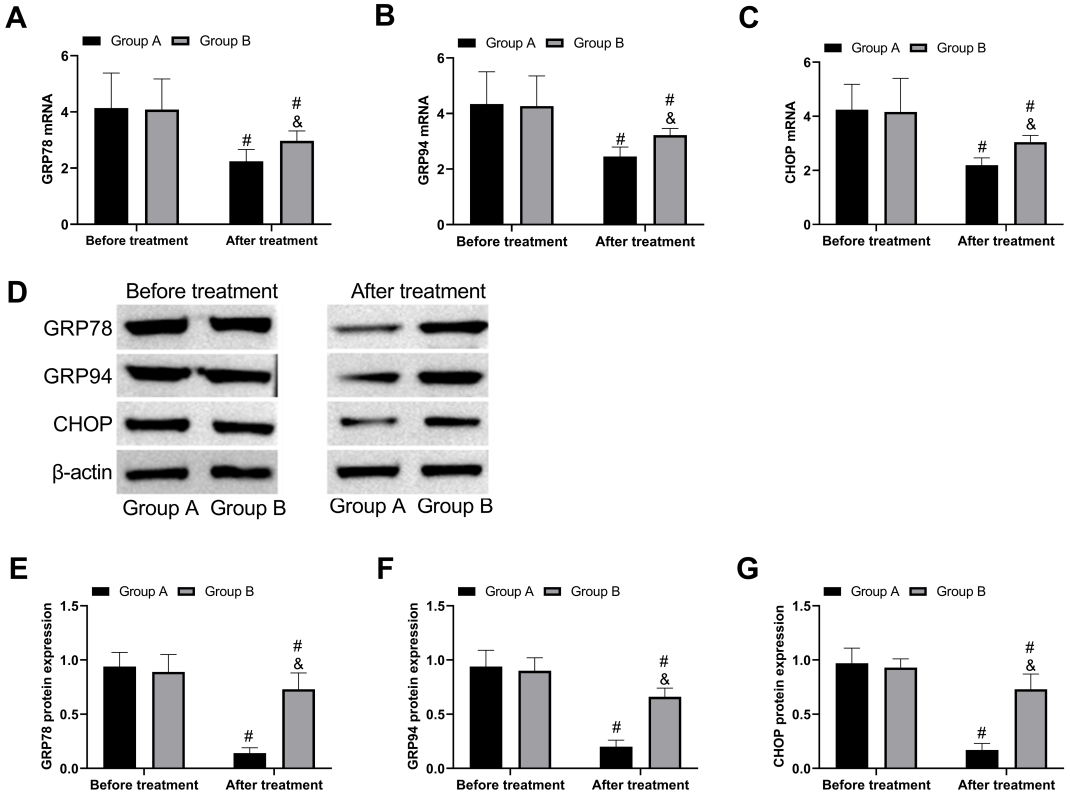

Fig. 7. Impacts of PICCO on ERS in TS patients (n = 30). (A) Comparison of *GRP78* mRNA level. (B) Comparison of *GRP94* mRNA level. (C) Comparison of *CHOP* mRNA level. (D) Western blot. (E) Comparison of *GRP78* protein level. (F) Comparison of *GRP94* protein level. (G) Comparison of *CHOP* protein level. # *vs*. Before therapy, $p < 0.05$. & *vs*. Group A, $p < 0.05$.

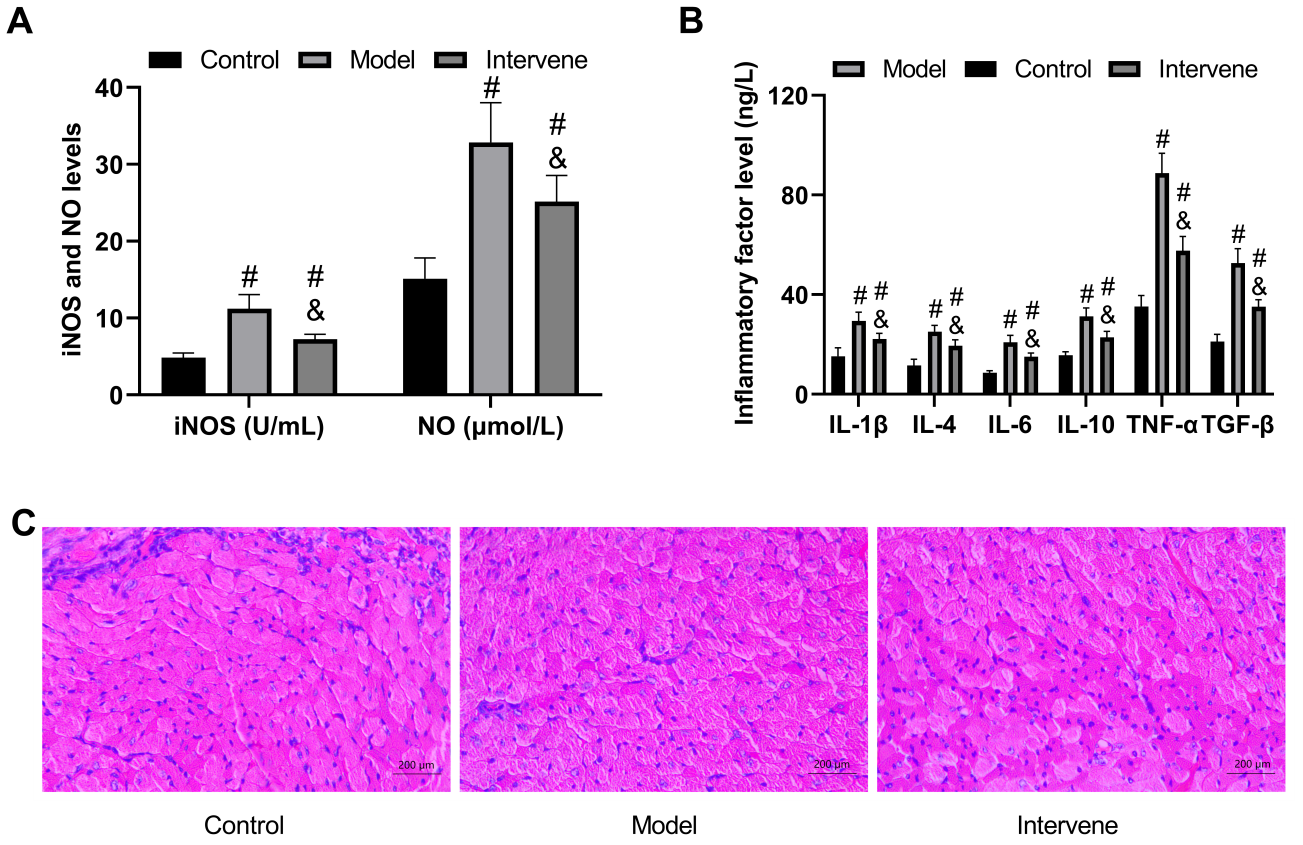

Fig. 8. Impacts of inhibiting iNOS/NO on TS (n = 6). (A) Comparison of iNOS and NO. (B) Comparison of inflammatory factors. (C) HE staining results of cardiac tissue. # *vs*. Control group, $p < 0.05$. & *vs*. Model group, $p < 0.05$.

As the latest technology in modern clinical rescue, PICCO technology can effectively increase the success rate of rescuing patients from many critical diseases and increase their survival possibility. PICCO technology has also demonstrated excellent performance in TS. However, at the current stage, the association of PICCO technology with iNOS/NO and ERS in TS patients remains unclear. Our above assays have tentatively confirmed the association of iNOS/NO and ERS with TS. Therefore, understanding the impacts of PICCO on iNOS/NO and ERS can provide a more accurate reference for the first aid of TS in the future. Further, TS patients were grouped into Group A given PICCO technique for resuscitation and Group B given conventional resuscitation. iNOS, NO, inflammatory factors, and ERS were found to be statistically lower in Group A, indicating the ability of PICCO to more effectively alleviate the pathological process of TS and its higher clinical significance for the treatment of TS patients. The reasons for the difference can be interpreted as follows: PICCO, as a commonly used hemodynamic monitoring technique, can help continuously monitor hemodynamic indexes via pulmonary thermodilution and arterial pulse waveform techniques to provide guidance for fluid management, diuretic use, vasoactive drug use, and volume resuscitation, thus helping accurately evaluate the treatment effect and patient outcomes [31]. Traditional monitoring of MAP and CVP, on the contrary, is of obvious uncertainty for clinical treatment. In addition, CVP may not accurately reflect the patient's cardiac preload due to changes in abdominal and thoracic cavity pressure, which may also contribute to treatment errors to some extent [32]. Moreover, it is likely that some patients with chest injury suffer from circulatory disorders due to the impact of cardiopulmonary function, leading to large fluctuations in the monitoring results of MAP and CVP that cannot reflect patients' pathological state objectively and directly. This is also verified by comparing indexes such as EVLWI, PVPI, and LCR between the two groups.

Finally, TS animal models were established to probe into the mechanism of iNOS/NO and ERS in TS, and the success of the modeling was confirmed by the elevation of iNOS/NO, ERS and inflammatory factors in the model group. The intervention group was found to have lower iNOS/NO, ERS and inflammatory factors than the model group, with significantly alleviated injury severity of cardiac tissue, suggesting the ability of AG, an iNOS inhibitor, to inhibit the pathological procession of TS. According to our study results, iNOS/NO obviously activated in TS cases mediated the release of massive downstream inflammatory factors, the damage of free radicals in tissues, and the dis-

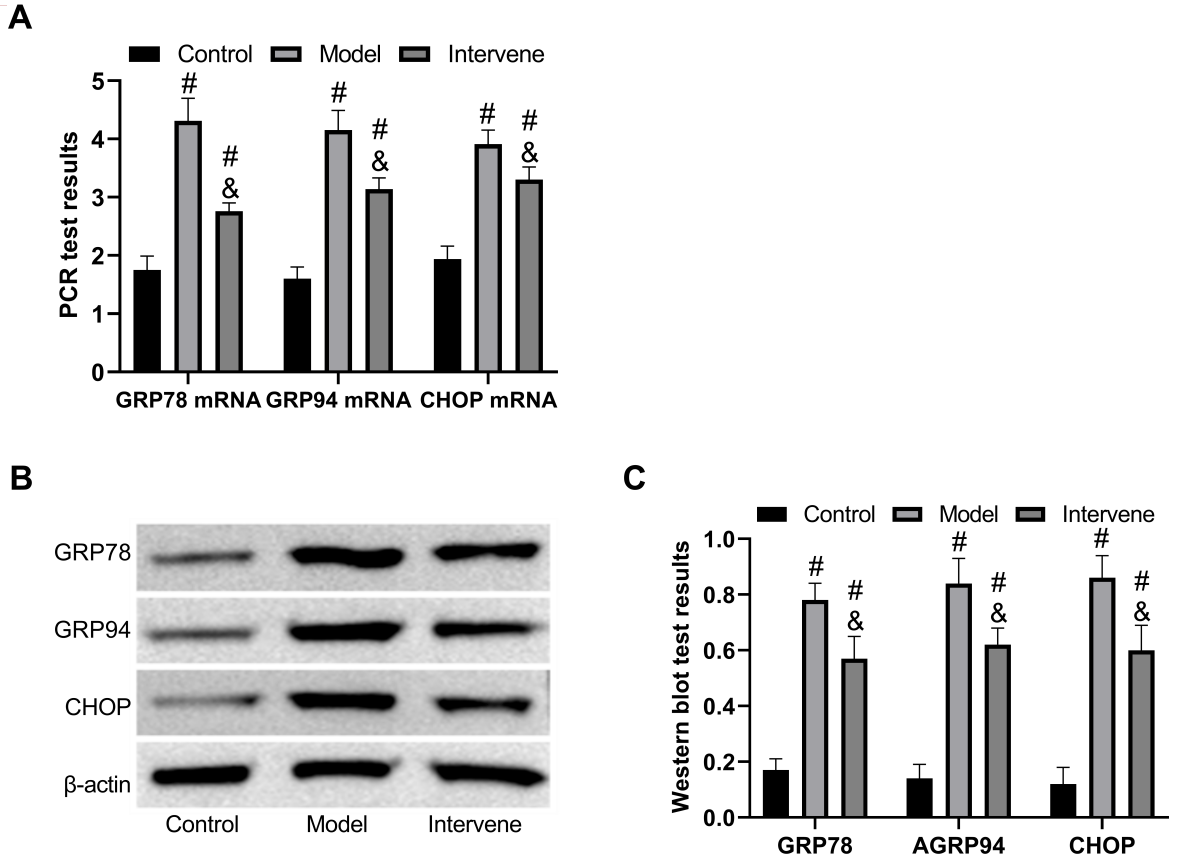

Fig. 9. Impacts of suppressing iNOS/NO on ERS (n = 6). (A) Levels of *GRP78* mRNA, *GRP94* mRNA and *CHOP* mRNA according to PCR assay. (B) Western blot. (C) Levels of *GRP78*, *GRP94* and *CHOP* proteins according to Western blot. # *vs*. Control group, $p < 0.05$. & *vs*. Model group, $p < 0.05$.

turbance of calcium balance, which accelerated ERS and thus promoted the pathological development of TS.

In the future, we will conduct a more in-depth and comprehensive analysis of the mechanism of action of iNOS/NO and ERS in TS, so as to provide a more accurate reference for molecular targeted therapy of TS. In addition, the sample size will be expanded to improve the reliability of experimental results and the follow-up period will be extended to better and more comprehensively understand the impacts of iNOS/NO on the prognosis of TS patients.

Conclusions

iNOS/NO and ERS levels increase with the increasing severity of TS, and PICCO can effectively lower their levels. The animal experiments suggest that the inhibition of iNOS/NO can alleviate inflammatory responses and ERS development, thus inhibiting the pathological process of TS.

Availability of Data and Materials

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

AHL and XN—designed the research study; XN—performed the research; AHL and XN—provided help and advice on the data acquisition; XN—analyzed the data. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study design was approved by the Ethics Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (No. 2021002). Due to the nature of the retrospective analysis, patient informed consent was waived. All animal experiments were approved by the Ethics Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (202101).

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Conflict of Interest

The authors declare no conflict of interest.

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