Role of Thrombospondin-1 in sepsis-induced myocardial injury

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Abstract. Sepsis often causes myocardial injury with a high mortality. The aim of the present study was to investigate the effects of thrombospondin-1 (THBS1) on myocardial cell injury, oxi-dative stress and apoptosis in sepsis. The expression of THBS1 mRNA in lipopolysaccharide (LPS)-induced mouse primary cardiomyocytes was detected by reverse transcription-quantitative PCR (RT-qPCR). A eukaryotic small interfering (si)RNA expression vector was constructed and transfected into myocardial cells to knockdown THBS1 mRNA expression, which was confirmed by RT-qPCR. Four *in vitro* experimental groups were used: i) Control, ii) LPS, iii) THBS1 siRNA (siTHBS1) and iv) siTHBS + LPS. ELISA was used to detect cardiac troponin I (cTnI), pro-brain natriuretic peptide (proBNP), reactive oxygen species (ROS), caspase-3, IL-6 and TNF-α. *In vivo* mouse sepsis models were also established, and H&E, TUNEL and caspase-3 staining were used to evaluate myocardial cell injury and apoptosis. Clinical samples were collected to analyze the serum THBS1 levels and to associate this with the prognosis of patients with sepsis-induced myocardial injury. The expression level of THBS1 mRNA in myocardial cells induced by LPS was increased, and the serum THBS1 level in patients with myocardial injury in sepsis was also significantly increased compared with patients without sepsis-induced myocardial injury. In the siTHBS1-treated mice with myocardial injury, the levels of cTnI and proBNP were significantly decreased, the levels of the inflammatory cytokines IL-6 and TNF-α were significantly decreased, ROS were significantly decreased, caspase-3 was significantly decreased, and myocardial cell apoptosis was also reduced, compared with the LPS group. Data from the present study suggested that THBS1 may be closely related to the biological behavior of myocardial cells and may be a therapeutic target for myocardial injury in sepsis.

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Introduction

Sepsis is a life-threatening organ dysfunction caused by the host's dysfunctional response to infection (1). Myocardial injury is one of the most common complications of sepsis, which mostly occurs in the middle and late stages of the disease (2). It was shown that ~50% of patients with sepsis also experience complications with myocardial depression (2), which is manifested by left and right ventricular cardiac dysfunction and reduced left ventricular ejection fraction; the mortality rate is as high as 70% (3). Cardiac dysfunction may complicate the course of sepsis and septic shock, and cardiac dysfunction caused by myocardial injury is an important causes of death in patients with sepsis (3,4). At present, the specific mechanism of septic cardiomyopathy is not clear, but activation of the apoptosis pathway has been reported to serve an important role in septic myocardial injury (5). A previous study confirmed that activation of caspases and release of mitochondrial cytochrome *c* can be found in septic cardiomyocytes (6). Inhibition of apoptosis can reverse the occurrence of septic myocardial injury (5). Maintaining myocardial mitochondrial membrane potential and inhibiting the activation of the apoptotic proteins caspase-3 and caspase-7 can reduce the apop-totic rate of myocardial cells (7). Therefore, methods to improve myocardial injury in sepsis and to reduce the apoptosis of myocardial cells were explored in the present study.

Thrombospondin (THBS; also known as TSP) is a secretory glycoprotein that serves a role in embryonic development, wound healing, angiogenesis and the inflammatory response (8,9). THBS has a multimodular structure, with each region performing different functions through specific binding of different factors; high-resolution determination revealed its unique and interesting pro-tein structure (8). Thrombospondins (THBSs) are divided into two groups, A and B. Group A includes THBS1 and THBS2, which can form trimers; group B includes THBS3, THBS4 and THBS5, which can form pentamers (10). Based on the THBS molecular structure, THBSs can be divided into two groups, the THBS1 type and the THBS2 I repetitive sequence TSRs (group A), whereas the other members do not contain this sequence (group B) (10). TSRs, also known as lysine repeat sequences, are involved in cell attachment, inhibition of angiogenesis, protein-protein and

protein-mucopolysaccharide interactions (11); thus, THBS1 can regulate the adhesion, migration, proliferation and neovascularization of vascular endothelial cells. Group B members lack the TSR sequence and therefore have no antiangiogenic effect in the tumor microenvironment (12).

Among the THBS family, THBS1 can regulate tumor growth, cell migration and vascular formation (13). Research on THBS1 in septic cardiomyocytes has rarely been reported (11-13). The mechanism of organ damage induced by sepsis, especially myocardial injury, has been examined previously (14); however, the specific effects of THBS1 on myocardial cell injury, oxidative stress and apoptosis in sepsis are not entirely understood. In the present study, THBS1 small interfering (si)RNA was transfected into primary cardiac cells, which are known to have relatively high THBS1 protein expression, and the effects of THBS1 gene silencing on myocardial cell injury, oxidative stress, the inflammatory response and apoptosis were observed.

Materials and methods

Animals, cells and main reagents. Male C57dx newborn mice and male C57BL/6 mice were pur-chased from the Experimental Animal Center of Shanghai Jiao Tong University (Shanghai, China). All of the experimental procedures were performed after obtaining the approval of the Ethical Committee for Animal Experiments of Shanghai General Hospital (Shanghai, China; 2020AWS006); all experimental procedures were carried out according with the guidelines of the Institutional Animal Care and Use Committee of Shanghai General Hospital. RPMI-1640 medium and fetal bovine serum were purchased from Gibco (Thermo Fisher Scientific, Inc.). Lipopolysaccharide (LPS). BCA protein quantitative kit was purchased from Vazyme Biotech Co., Ltd. siTHBS1 and control siRNA were purchased from Santa Cruz Biotechnology, Inc. Mouse anti-human THBS1 monoclonal antibody (cat. no. ab1823) was purchased from Abcam. Anti-β-actin (cat. no. ab8226) was purchased from Abcam and goat anti-rabbit polyclonal antibodies (HRP-conjugated) (cat. no. 31460; Thermo Fisher Scientific, Inc.).

Cell culture. A total of 12 male C57dx mice (age, 3 days; weight 1.5-2 g) were randomly selected. After cervical dislocation, the hearts were extracted and the atria were cut off; the ventricles were rinsed with 1 ml collagenase, and the heart tissue was cut into ~1 mm^3 pieces. The pieces of heart tissue were mixed with collagenase and transferred into a 15-ml centrifuge tube and were repeated-ly shaken and mixed at 37˚C for 1 min. After allowing the tubes to stand and for the solid tissue to settle, the supernatant was removed, and this was repeated until the tissue mass was dissolved completely. The obtained supernatant was centrifuged at 2,000 x g for 10 min (37˚C), the supernatant was discarded and the precipitate was mixed with 2 ml of medium, which was inoculated into a 75 ml culture bottle. After incubating at 37˚C for 90 min, the non-myocardial cells had adhered to the bottle, and the cell suspension ($5x10^5$-$6x10^5$ cells/ml), which contained the myocardial primary cells, was subsequently inoculated into another culture bottle and incubated at 37˚C for 24 h.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the cardiomyocytes ($5x10^5$-$6x10^5$ cells/ml) using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The concentration of RNA was measured by a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Inc.) and its purity was determined. Total RNA was reverse transcribed into cDNA using a TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (Agrisera) for 30 min at 42˚C, then 5 sec at 85˚C. qPCR was subsequently performed using Unique Aptamer qPCR SYBR Green Master Mix (MedChemExpress), according to the manufacturer's protocol. After the reaction, the amplification and dissolution curves were verified, and the results were interpreted strictly in accordance with the instructions of the kit. The $2^{-\Delta\Delta Cq}$ method (15) was used to calculate the level of THBS1 mRNA expression. The primer sequences were as follows: THBS1, forward 5'-GGAAAGAUU UCACUGCAUATT-3', reverse 5'-UAUGCAGUGAAAUCUU UCCAG-3'; and GAPDH, forward 5'-GTCAAGGCTGAGAAC GGGAA-3', reverse, 5'-AAATGAGCCCCAGCCTTCTC-3'. The thermocycling conditions used for qPCR were as follows: Initial denaturation at 94˚C for 5 min; followed by 30 cycles of 5 sec at 94˚C and annealing and extension at 65˚C for 30 sec.

Western blotting. The cells ($5x10^5$-$6x10^5$ cells/ml) were placed in RIPA lysis buffer (Beyotime Institute of Biotechnology). The total protein concentration was detected using a BCA kit. The protein samples (60 μg/well) were separated by 8% SDS-PAGE and then transferred to a PVDF membrane. The membrane was blocked with 5% skimmed milk powder (37˚C for 1 h) and then incubated with the indicated primary antibodies, anti-THBS1 (1:200; cat. no. ab1823) or β-actin (1:200; cat. no. ab8226) at 4˚C for 12 h. Following the primary antibody incubation, the cells were washed with TBS-Tween 20 and incubated with the secondary antibody (1:2,000) at 37˚C for 1 h. Protein bands were visualized with an ECL system (Abcam) using a western blot gel imager (ChemiDoc; Bio-Rad Laboratories, Inc.). Protein expression levels were calculated using ImageJ V1.8.0.112 software (National Institutes of Health) using actin as the internal reference.

Effects of siTHBS1 on myocardial cell injury, oxidative stress, inflammatory response and apoptosis

Cell grouping. Primary cells ($5x10^5$-$6x10^5$ cells/ml) were inoculated into a 25 cm^2 culture flask containing RPMI-1640 medium supplemented with 10% fetal bovine serum by volume. Then, cells were digested with trypsin and inoculated into 6-well plates. Four experimental groups were prepared: i) Control group; ii) LPS group; iii) siTHBS1 group; and iv) siTHBS1 + LPS group. There was no intervention in the Control group. Cells were treated at 37˚C for 24 h with 1 mg/l LPS to stimulate the cardiomyocytes. siTHBS1 was transfected into the cardiomyocytes using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C at 24 h with or without previously stimulating with LPS, aforementioned.

Detection of THBS1 mRNA in cells after transfection for 48 h. The expression of THBS1 mRNA in the cells was

Table I. Clinicopathological characteristics of the patients with sepsis-induced myocardial injury.

Clinicopathological characteristic	Total (n=84)
Males (%)	51 (60.7)
Age, years	58.68±14.94
Location: Shanghai, China	84 (100)
Dates of admission	Jan 2018-Dec 2020
Comorbidities, n (%)	
Hypertension	37 (44.0)
Diabetes	14 (16.7)
Immune diseases	8 (9.5)
CKD	10 (11.9)
Liver disease	7 (8.3)
COPD	11 (13.1)
Days (every 24 h) of noradrenaline use	2.226±3.507
Days (every 24 h) of mechanical ventilation	6.333±7.630
SOFA	7.643±3.998
APACHE II	16.90±7.108
Mortality (%)	23 (27.4)
Need for dialysis, day	1.464±3.276

Values are expressed as the mean ± standard deviation. APACHE II, Acute Physiology and Chronic Health Evaluation II; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; SOFA, sequential organ failure assessment.

detected by RT-qPCR, aforementioned. Each group was tested with three biological replicates twice.

Detection index. Cell injury, oxidative stress, inflammatory response and apoptosis were detected 48 h after transfection. The cells were inoculated into a 96-well plate. After routine culture for 48 h, expression of cardiac troponin I (cTNI) (mouse TNNI3/cTn-I ELISA kit; cat. no. D721149-0048; Sangon Biotech Co., Ltd.), pro-brain natriuretic peptide (proBNP; mouse NT-proBNP ELISA kit; cat. no. E-EL-M0834; Elabscience Biotechnology, Inc.), reactive oxygen species (ROS; ROS ELISA kit; cat. no. ZK-M5156; ZIKER), IL-6 (IL-6 ELISA kit; cat. no. ab46100; Abcam), TNF-α (TNF-α ELISA kit; cat. no. SEKM-0034; Beijing Solarbio Science & Technology Co., Ltd.) and caspase-3 (caspase-3 ELISA kit; cat. no. BH-E0764; Thermo Fisher Scientific, Inc.) were detected by ELISA. Supernatant was used in the ELISA, which was obtained by centrifugation of the cell culture at 4˚C at 6,000 x g for 5-10 min.

Sepsis model. Healthy specific pathogen free male C57BL/6 mice (n=18; age, 8 weeks; weight ~20±2 g) were used. Before the experiment, the mice were provided free access to food and water, and maintained at an ambient temperature of 20˚C and 40-60% humidity under a 14/10-h light/dark cycle. Intraperitoneal injection of LPS 15 mg/kg was used to make the model, and successful model establishment was determined based on a previous study (16); sepsis modelling was confirmed successful by histopathological changes in cardiac muscle 24 h after modelling, as well as determination of cTNI, proBNP, Il-6, and TNF-α levels by ELISA using serum which was collected following euthanasia. Three groups (n=6 mice/group) were used in the *in vivo* experiments: i) Control group; ii) LPS group; and iii) siTHBS1 + LPS group (15 nmol/20 g weight siTHBS1 was injected through the caudal vein). Euthanasia was performed in a CO_2 chamber with a flow rate of 50% volume displacement/min. The means used to verify mouse death were whether the heart stopped completely and whether the pupils were dilated.

H&E, TUNEL and caspase-3 staining. Upright fluorescence Metallurgical Microscope was NIKON ECLIPSE C1 and inverted fluorescence microscope was NIKON ECLIPSE TI-SR (magnifications, x200 and 400). H&E staining and TUNEL analysis were performed to assess the histopathological changes in myocardial tissue. Mouse myocardial tissues were fixed with 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd.) for 12 h at 4˚C. The nuclei were counterstained in the TUNEL and caspase-3 staining assay with DAPI for 10 min at 37˚C. Paraffin embedding, tissue sections and H&E staining were performed (the width of the sections used was 0.5-mm and H&E staining was performed at 37˚C for 30 min). A TUNEL assay kit (Roche Diagnostics; cat. no. 11684817910) was used to measure the rate of apoptosis of the cardiomyocytes, according to the manufacturer's specifications. A caspase-3 fluorometry kit (Wuhan Servicebio Technology Co., Ltd.) was used for detection of caspase-3 levels. LDH activity, which is a marker of cell injury, was not examined because LDH is greatly affected by sepsis (17).

Collection of clinical samples. A total of 2 ml serum was collected from patients with sepsis-induced myocardial injury (n=84), those without sepsis-induced myocardial injury (n=84) and healthy individuals (n=10) and stored at -80˚C. The inclusion criteria included patients who were diagnosed with sepsis and were 18-80 years old. The exclusion criteria included the presence of malignant tumors and post-transplant patients. THBS1 was detected by ELISA, aforementioned. The prognosis of the patients was recorded during the 28-day follow-up. Ethics approval was obtained for the use of humans/human tissues prior to the start of the study from the ethics committee based at Shanghai General Hospital (approval no. 2020sq049). Informed consent was obtained from each patient prior to participation. Basic clinicopathological features, including age and sex, of the patients with sepsis-induced myocardial injury (n=84) are shown in Table I.

Statistical analysis. SPSS v17.0 (SPSS, Inc.) was used to process the data. The expression levels of THBS1 mRNA and cTNI, proBNP, ROS, IL-6, TNF- α and caspase-3 levels in the different groups were compared by one-way ANOVA; Fisher's LSD test was used for pairwise comparisons. Kaplan-Meier survival curves were analyzed by log-rank; the cut-off value used to separate high vs. low THBS1 was the mean value of expression 271.179 ng/ml. The optimal cutoff values were determined by the highest values of sensitivity

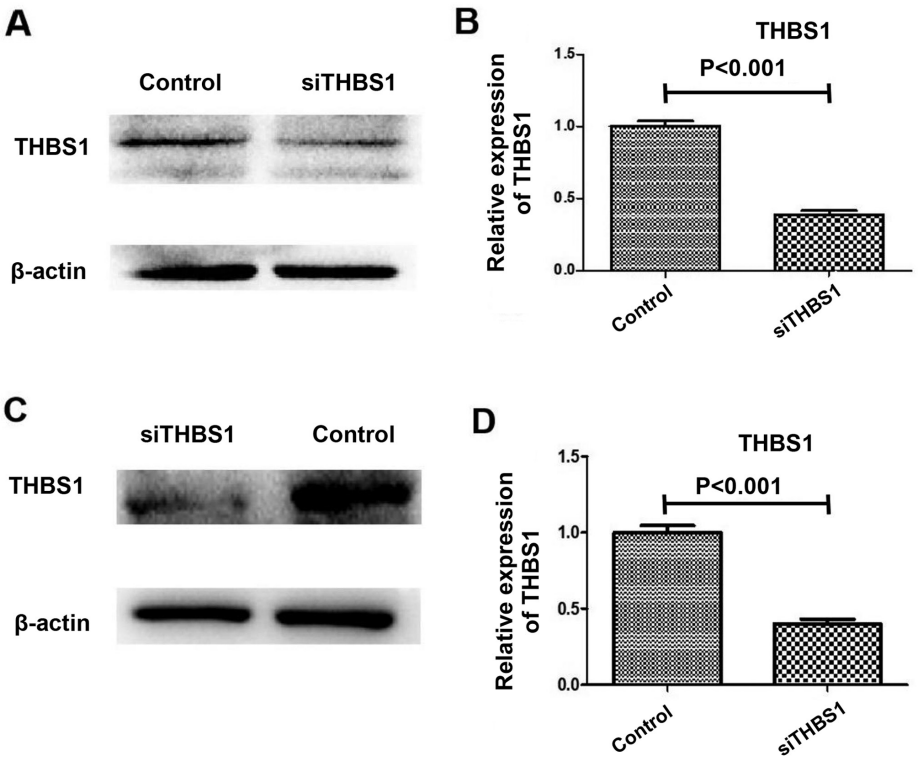

Figure 1. Successful knockdown of THBS1 by siRNA *in vivo* and *in vitro*. THBS1 expression was reduced in the *in vivo* model mice injected with si-THBS1, as determined using (A) western blotting and (B) RT-qPCR (significance was found in parts B between the groups). THBS1 ex-pression was reduced in the *in vitro* primary myocardial cell cultures following siTHBS1 transfec-tion, as determined using (C) western blotting and (D) RT-qPCR (significance was found in parts D between the groups). RT-qPCR, reverse transcription-quantitative PCR; si, small interfering RNA; THBS1, thrombospondin-1.

and specificity indicated in the area under the receiver operatic characteristic (ROC) curve (AUC) analysis. P≤0.05 was used to indicate a statistically significant difference.

Results

THBS1 knockdown. Successful knockdown of THBS1 by siRNA is shown in Fig. 1. THBS1 expression was reduced in the *in vivo* model mice injected with siTHBS1 compared with the control group, as determined using western blotting and RT-qPCR (Fig. 1A and B). Similarly, THBS1 expression was reduced in the *in vitro* primary myocardial cell cultures following siTHBS1 transfection compared with the control group, as determined using western blotting and RT-qPCR (Fig. 1C and D).

THBS1 expression in vitro. In the LPS-stimulated primary cardiomyocytes, RT-qPCR analysis demonstrated a significant increase in THBS1 mRNA expression compared with the Control group (Fig. 2B); a similar increase was observed for THBS1 protein expression detected by western blotting (Fig. 2A).

Caspase-3 and ROS levels determined by ELISA in vitro. In LPS-stimulated primary cardiomyocytes, ELISA demonstrated a significant increase in caspase-3 and ROS levels in the LPS group compared with the control group. Caspase-3 was significantly decreased compared with the LPS group, indicating that myocardial cell apoptosis was decreased (Fig. 2C), and ROS levels were significantly decreased compared with the LPS group, indicating that the oxidative stress response was decreased (Fig. 2D).

cTNI, proBNP, Il-6, and TNF-α levels detected by ELISA in vitro. In the LPS-stimulated primary myocardial cells, ELISA results demonstrated that the cTNI, proBNP, Il-6 and TNF-α levels were significantly increased compared with the control group (Fig. 3A-D, respectively). cTNI, proBNP, IL-6 and TNF-α levels were significantly reduced in the siTHBS1 + LPS group compared with the LPS group.

Histopathological changes in cardiac muscle 24 h after sepsis modelling in vivo. In the mouse cardiac pathology sections from mice intraperitoneally injected with LPS (15 mg/kg), myocardial histopathological changes were observed 24 h after mouse modeling under a light microscope. In the Control group (Fig. 4A and D), the myocardial fibers were arranged neatly with clear horizontal stripes; the cardiomyocytes displayed normal morphology, the cytoplasm was pink, the nuclear membrane was intact, the chromatin was dense and deeply stained, and there were no significant changes in the myocardial interstitium. In the LPS group (Fig. 4B and E) the myocardial fibers were irregular; there was interstitial edema, hemorrhage, and inflammatory cell infiltration. In the siTHBS1 + LPS group (Fig. 4C and F), there was interstitial edema, but inflammatory cell infiltration was reduced compared with the control. Successful knockdown of THBS1 by siRNA is shown in Fig. 1A and B.

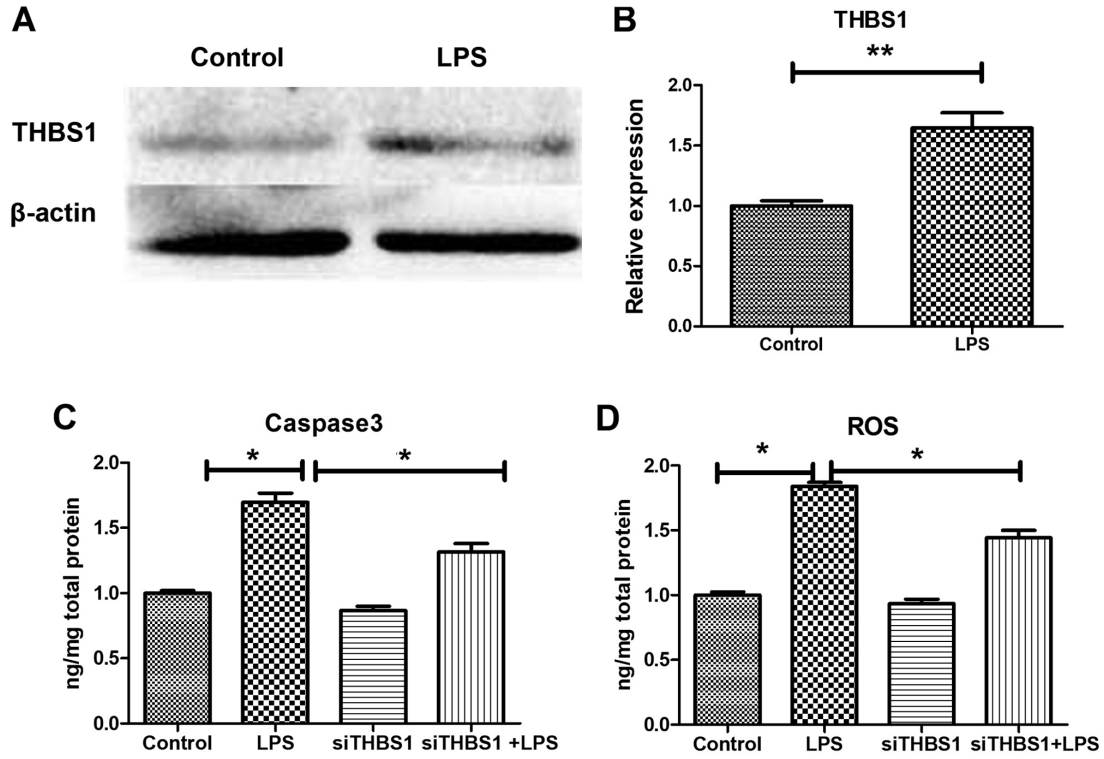

Figure 2. Levels of THBS1, caspase-3 and ROS in LPS-stimulated primary cardiomyocytes *in vitro*. (A) Western blotting of THBS1 protein expression. (B) Reverse transcription-quantitative PCR results of THBS1 mRNA expression. ELISA results of (C) caspase-3 and (D) ROS levels. *P<0.05, **P<0.001. LPS, lipopolysaccharide; ROS, reactive oxygen species; si, small interfering RNA; THBS1, thrombospondin-1.

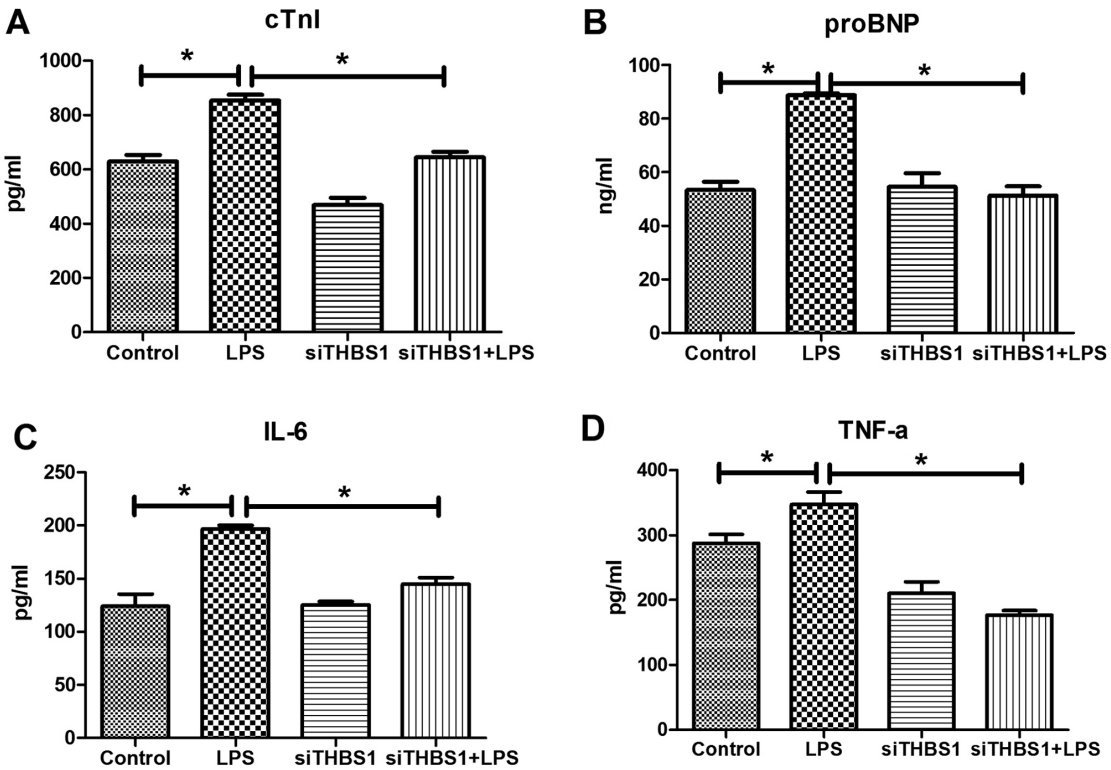

Figure 3. Levels of cTNI, proBNP, IL-6, and TNF-α in LPS-stimulated primary cardiomyocytes *in vitro*. ELISA was used to determine the levels of (A) cTNI, (B) proBNP, (C) IL-6 and (D) TNF-α in the various groups. *P<0.05, **P<0.001. cTnI, cardiac troponin I; LPS, lipopolysaccharide; pro-BNP, pro-brain natri-uretic peptide; si, small interfering RNA; THBS1, thrombospondin-1.

TUNEL analysis and caspase-3 immunofluorescence for myocardial injury in sepsis model mice. TUNEL staining was used to observe the changes of the myocardial cells in mice 24 h after modeling. LPS (15 mg/kg) treatment (Fig. 5B)

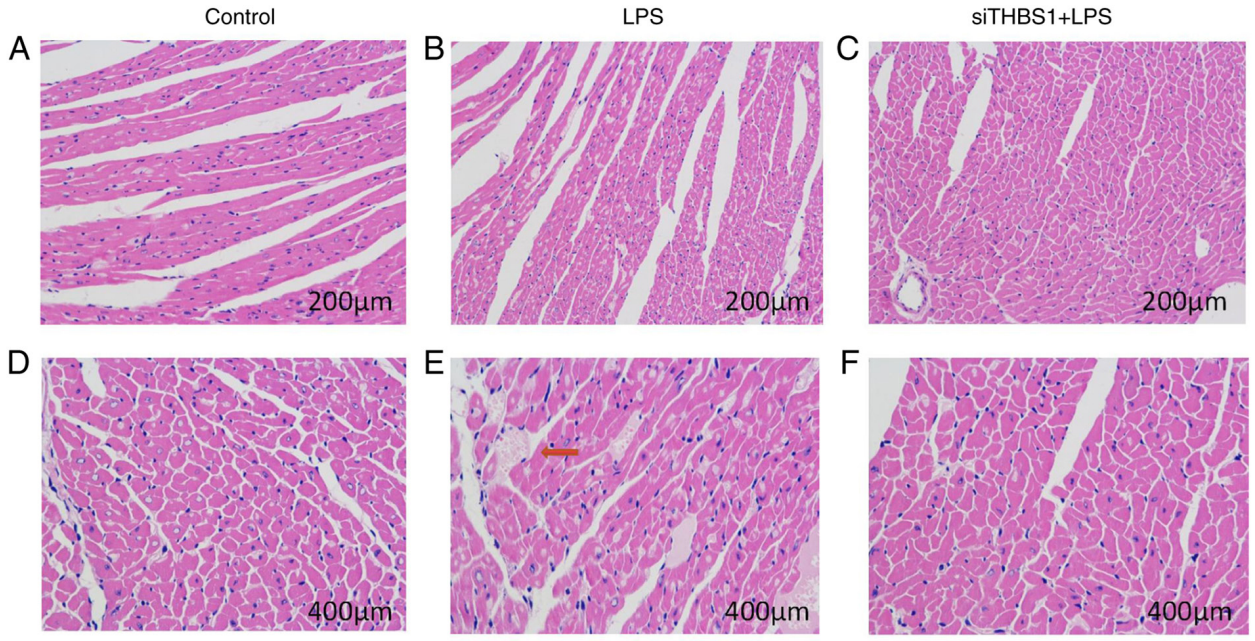

Figure 4.Histopathological changes in cardiac muscle 24 h after sepsis modelling *in vivo*. (A) Control, (B) LPS and (C) siTHBS1 + LPS. Parts A, B and C present representative images captured at x200 magnification. (D) Control, (E) LPS and (F) siTHBS1 + LPS. Images in parts D, E and F were captured at x400 magnification. LPS, lipopolysaccharide; si, small interfering RNA; THBS1, thrombospondin-1.

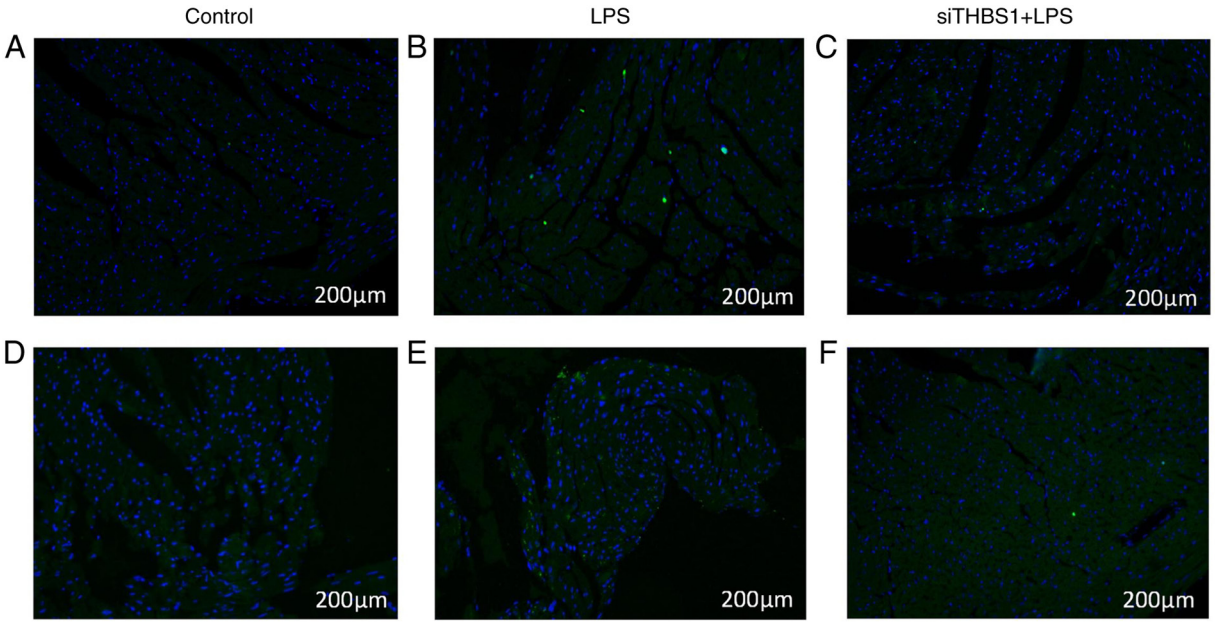

Figure 5. TUNEL analysis and caspase-3 immunofluorescence for myocardial injury in sepsis mice. TUNEL staining was used to observe the changes of the myocardial cells in mice 24 h after model-ing. (A) Control group, (B) LPS group (LPS15 mg/kg) treatment and (C) siTHBS1 + LPS group. Magnification, x200. Caspase-3 immunostaining was used to observe the changes of myocardial cells in mice 24 h after modeling. (D) Control group, (E) LPS group and (F) siTHBS1 + LPS group. Magnification, x200. LPS, lipopolysaccharide; si, small interfering RNA; THBS1, throm-bospondin-1.

led to increased apoptosis compared with the Control group (Fig. 5A), whereas the siTHBS1 + LPS group (Fig. 5C) showed decreased apoptosis compared with the LPS group.

Caspase-3 immunostaining was used to observe the changes of myocardial cells in mice 24 h after modeling. The LPS group (Fig. 5E) displayed increased caspase-3 expression compared with the Control group (Fig. 5D), and there was decreased caspase-3 expression in the siTHBS1 + LPS group compared with the LPS group (Fig. 5F).

THBS1 expression in patients with sepsis-induced myocardial injury. THBS1 serum levels were significantly increased in patients with sepsis accompanied by myocardial injury compared with the control group (Fig. 6A), and THBS1 was also higher in deceased patients compared with those who survived (Fig. 6B). The survival rate of patients with high THBS1 was lower compared with that of patients with low THBS1 over the 28-day follow-up (Fig. 6C). THBS1 expression can be used to predict myocardial

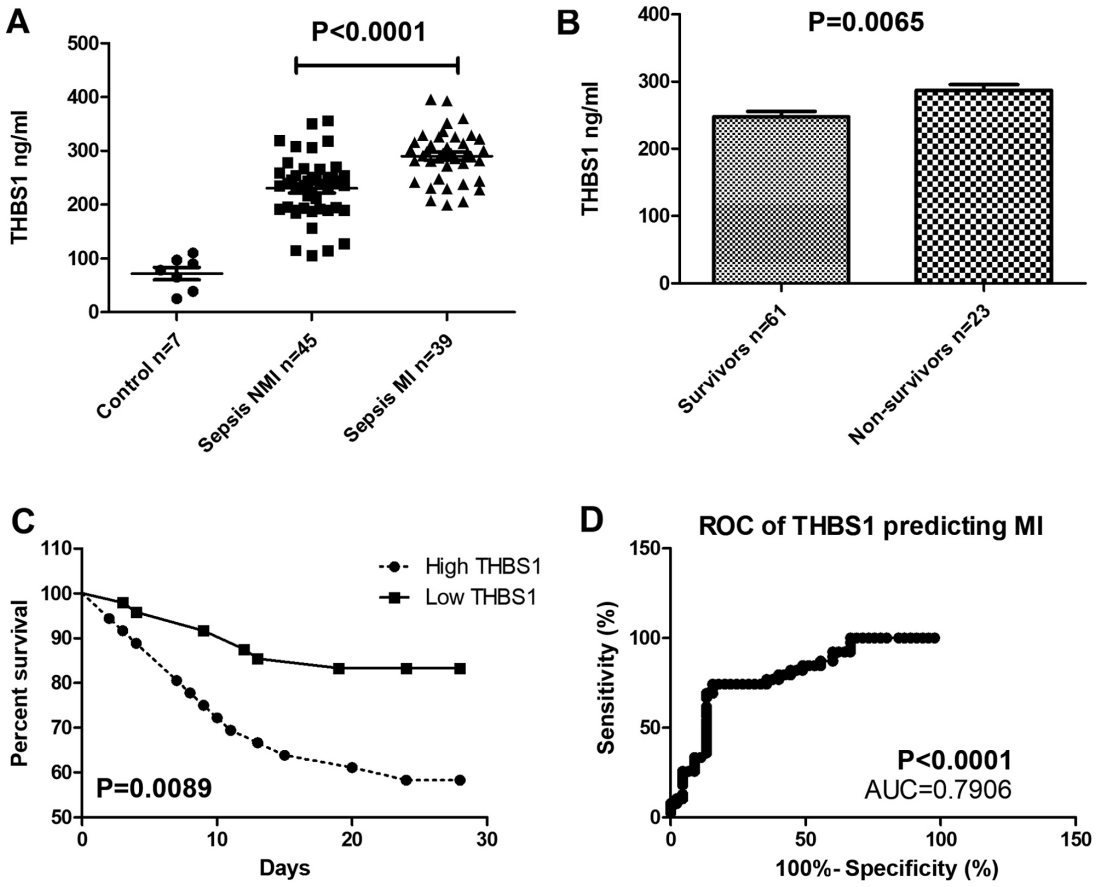

Figure 6. THBS1 expression in patients with myocardial injury due to sepsis. (A) Levels of THBS1 in patients with sepsis, patients with sepsis accompanied by myocardial injury and control patients. (B) Expression of THBS1in survivors and non-survivors. (C) Kaplan-Meier survival curve of the high and the low THBS1 expression groups. (D) AUC curve of THBS1 in predicting myocardial injury. AUC, area under the ROC curve; MI, myocardial injury; NMI, no myocardial injury; ROC, receiver operatingcharacteristic; THBS1, thrombospondin-1.

injury, the cutoff value of THBS1 is 271.2 ng/ml. When THBS1>271.2 ng/ml, myocardial injury may happen (AUC =0.7906; P<0.001; Fig. 6D).

Discussion

Although THBS1 has been shown to have positive roles on various biological functions (18), the effects of THBS1 for the treatment of cardiac injury are unclear. THBS1 promotes the over production of free radicals and ROS (18). Free radicals attack the cell membrane, increase cell death and result in the production of caspase-3 (18). Results from the present study demonstrated that decreased THBS1 expression by siRNA effectively reducing caspase-3 levels and decreasing ROS concentration and cTnI activity following LPS treatment. These results indicated that decreased THBS1 may decrease oxidative damage in LPS-induced cardiac injury. Thus, whether THBS1 takes part in the process of LPS-induced cardiac injury requires further investigation.

Apoptosis is a type of programmed cell death that can be triggered by environmental or chemical irritants (1,16,17). In the early stage of apoptosis, caspase-3 is activated. The activated caspase-3 consists of two large subunits (17 kDa) and two small subunits (12 kDa), which eventually leads to apoptosis (4). The present study demonstrated that decreased THBS1 expression leads to decreased levels of caspase-3. Therefore, the results suggested that THBS1 may promote LPS-induced myocardial apoptosis.

As a potent inflammatory mediator, TNF-α can be activated by oxidative stress (5). Under the stimulation of pro-inflammatory cytokines, TNF-α is activated, it accumulates and subsequently accelerates the production of IL-6, which is a pro-inflammatory cytokine (5). Owing to the adverse impact of oxidative stress, the cell upregulates genes involved in inflammation to activate or amplify the inflammatory response (17). The present study results showed that the LPS-induced increases in TNF-α and IL-6 levels were substantially suppressed by siTHBS1 co-treatment *in vitro*. Therefore, decrease THBS1 expression may protect against LPS-induced cardiac injury by reducing the expression of inflammatory cytokines to weaken the inflammatory response.

Studies on THBS1 in human sepsis are extremely limited, with a few studies showing no clear correlation between THBS1 expression and the prognosis of sepsis (19,20), and a single-center cohort study in intensive care showed no correlation between baseline THBS1 concentration and mortality in patients with sepsis (19). However, in the present study, THBS1 was found to be related to myocardial damage, and may be an important area for further multicenter, large sample confirmation.

Individual studies have confirmed that THBS1 may contribute to mortality in murine sepsis by affecting innate immunity (20).

Regarding inflammation, Xing *et al* (21) found that the production of THBS1 regulates the secretion of inflammatory cytokines in the THP-1 human monocyte cell line through the NF-κB signaling pathway (21). It has been shown that gingival stem cells can improve LPS-induced inflammation by secreting TGF-β3 and THBS1, which can induce M2-polarization of macrophages (22). In the present study, siTHBS1 led to reduced inflammation, reduced oxidative stress, reduced myocardial injury and reduced apoptosis of cardiomyocytes. The specific mechanism still needs further investigation.

Results from the present study demonstrated that the expression level of THBS1 mRNA in LPS-induced primary cardiomyocytes was higher compared with the expression levels in untreated cardiomyocytes. This study also confirmed in clinical samples that the expression level of THBS1 in sepsis patients with myocardial injury was also higher compared with that in the normal control group. An siRNA was constructed and transfected it into primary cardiomyocytes with the highest THBS1 expression. THBS1 mRNA expression in the cells was significantly downregulated compared with the control, which resulted in lower oxidative stress and apoptosis. To the best of our knowledge, this is the first study to report the effects of THBS1 expression on myocardial cell injury, oxidative stress and apoptosis in sepsis.

In conclusion, the results of the present study suggested that THBS1 may be closely related to the occurrence and development of sepsis-induced myocardial injury, which may lay a foundation for research into THBS1 as a therapeutic target for myocardial injury in sepsis.

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Availability of data and materials

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

Authors' contributions

YX and RW confirm the authenticity of all the raw data. YX, JZ, RT, WJ and RW contributed to the study conception and design, analysis and interpretation of data, drafting of the article and critical revision for important intellectual content. All of the authors have read and approved the final manuscript.

Ethics approval and consent to participate

All of the experimental procedures were performed after obtaining the approval of the Ethical Committee for Animal Experiments of Shanghai General Hospital (Shanghai, China; 2020AWS006); all experimental procedures were carried out according with the guidelines of the Institutional Animal Care and Use Committee of Shanghai General Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, *et al*: Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Crit Care Med 45: 486-552, 2017.
2. Frencken JF, Donker DW, Spitoni C, Koster-Brouwer ME, Soliman IW, Ong DSY, Horn J, van der Poll T, van Klei WA, Bonten MJM, *et al*: Myocardial Injury in Patients With Sepsis and Its Association With Long-Term Outcome. Circ Cardiovasc Qual Outcomes 11: e004040, 2018.
3. Aneman A and Vieillard-Baron A: Cardiac dysfunction in sepsis. Intensive Care Med 42: 2073-2076, 2016.
4. Xie Y, Tian R, Jin W, Xie H, Du J, Zhou Z and Wang R: Antithrombin III Predicts Acute Kidney Injury in Septic Elderly Patients. Exp Ther Med 19: 1024-1032, 2020.
5. Liu YC, Yu MM, Shou ST and Chai YF: Sepsis-induced cardiomyopathy: Mechanisms and treatments. Front Immunol 8: 1021, 2017.
6. Buerke U, Carter JM, Schlitt A, Russ M, Schmidt H, Sibelius U, Grandel U, Grimminger F, Seeger W, Mueller-Werdan U, *et al*: Apoptosis contributes to septic cardiomyopathy and is improved by simvastatin therapy. Shock 29: 497-503, 2008.
7. Peng S, Xu J, Ruan W, Li S and Xiao F: PPAR- γ activation prevents septic cardiac dysfunction via inhibition of apoptosis and necroptosis. Oxid Med Cell Longev 2017: 1-11, 2017.
8. Lopez-Dee Z, Pidcock K and Gutierrez LS: Thrombospondin-1: Multiple paths to inflammation. Mediators Inflamm 2011: 296069, 2011.
9. Bornstein P: Thrombospondins function as regulators of angiogenesis. J Cell Commun Signal 3: 189-200, 2009.
10. Stenina-Adognravi O: Thrombospondins: Old players, new games. Curr Opin Lipidol 24: 401-409, 2013.
11. Bigé N, Boffa JJ, Lepeytre F and Shweke N: Rôle de la thrombospondine-1 dans le développement des maladies rénales [Role of thrombospondin-1 in the development of kidney diseases. Med Sci (Paris) 29: 1131-1137, 2013 (In French).
12. Kuzmanov A, Wielockx B, Rezaei M, Kettelhake A and Breier G: Overexpression of factor inhibiting HIF-1 enhances vessel maturation and tumor growth via platelet-derived growth factor-C. Int J Cancer 131: E603-E613, 2012.
13. Weng TY, Wang CY, Hung YH, Chen WC, Chen YL and Lai MD: Differential Expression Pattern of THBS1 and THBS2 in Lung Cancer: Clinical Outcome and a Systematic-Analysis of Microarray Databases. PLoS One 11: e0161007, 2016.
14. Larche J, Lancel S, Hassoun SM, Favory R, Decoster B, Marchetti P, Chopin C and Neviere R: Inhibition of mitochondrial permeability transition prevents sepsis-induced myocardial dysfunction and mortality. J Am Coll Cardiol 48: 377-385, 2006.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.

16. Takehara K, Murakami T, Kuwahara-Arai K, Iba T, Nagaoka I and Sakamoto K: Evaluation of the effect of recombinant thrombomodulin on a lipopolysaccharide-induced murine sepsis model. Exp Ther Med 13: 2969-2974, 2017.
17. Lu J, Wei Z, Jiang H, Cheng L, Chen Q, Chen M, Yan J and Sun Z: Lactate dehydrogenase is associated with 28-day mortality in patients with sepsis: A retrospective observational study. J Surg Res 228: 314-321, 2018.
18. Gao JB, Tang WD, Wang HX and Xu Y: Predictive value of thrombospondin-1 for outcomes in patients with acute ischemic stroke. Clin Chim Acta 450: 176-180, 2015.
19. van der Wekken RJ, Kemperman H, Roest M and de Lange DW: Baseline thrombospondin-1 concentrations are not associated with mortality in septic patients: A single-center cohort study on the intensive care unit. Intensive Care Med Exp 5: 7, 2017.
20. McMaken S, Exline MC, Mehta P, Piper MC, Wang Y, Fischer SN, Newland CA, Schrader CA, Basler SR, Sarkar A, *et al*. Thrombospondin-1 Contributes to Mortality in Murine Sepsis through Effects on Innate Immunity.PLOS ONE 6: e19654,2011. https://doi.org/10.1371/journal.pone.0019654.
21. Xing T, Wang Y, Ding WJ, Li YL, Hu XD, Wang C, Ding A and Shen JL: Thrombospondin-1 Production Regulates the Inflammatory Cytokine Secretion in THP-1 Cells Through NF-κB Signaling Pathway. Inflammation 40: 1606-1621, 2017.
22. Chen X, Yang B, Tian J, Hong H, Du Y, Li K, Li X, Wang N, Yu X and Wei X: Dental Follicle Stem Cells Ameliorate Lipopolysaccharide-Induced Inflammation by Secreting TGF-β3 and TSP-1 to Elicit Macrophage M2 Polarization. Cell Physiol Biochem 51: 2290-2308, 2018.