Original Research

Participation of ASK-1 in the cardiomyocyte-protective role of mechanical ventilation in a rat model of myocardial infarction

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Impact Statement

The present data demonstrated that NIPPV treatment could alleviate MI-induced cardiac damage, including infarction, neutrophil infiltration, myocardial hypertrophy, and fibrosis, by repressing the expression of ASK-1 and blocking the link between oxidative stress and extrinsic apoptosis. Our study suggests that NIPPV is an effective treatment for MI-induced HF; however, the mechanisms of NIPPV-dependent regulation of MI development require further research *in vitro* and *in vivo*.

Abstract

Non-invasive positive-pressure ventilation (NIPPV) has been demonstrated to exhibit a cardioprotective function in a rat model of myocardial infarction (MI). However, the mechanism underlying NIPPV-mediated MI progression requires further investigation. We aimed to investigate the effectiveness and corresponding mechanism of NIPPV in an acute MI-induced heart failure (HF) rat model. Thirty each of healthy wild type (WT) and apoptosis signal-regulating kinase 1 (ASK-1)-deficient rats were enrolled in this study. MI models were established via anterior descending branch ligation of the left coronary artery. The corresponding data indicated that NIPPV treatment reduced the heart infarct area, myocardial fibrosis degree, and cardiac function loss in MI rats, and ameliorated apoptosis and reactive oxygen species (ROS) levels in the heart tissue. Furthermore, the expression level of ASK-1 level, a key modulator of the ROS-induced extrinsic apoptosis pathway, was upregulated in the heart tissues of MI rats, but decreased after NIPPV treatment. Meanwhile, the downstream cleavage of caspase-3, caspase-9, and PARP, alongside p38 phosphorylation and FasL expression, exhibited a similar trend to that of ASK-1 expression. The involvement of ASK-1 in NIPPV-treated MI in ASK-1-deficient rats was examined. Although MI modeling indicated that cardiac function loss was alleviated in ASK-1-deficient rats, NIPPV treatment did not confer any clear efficiency in cardiac improvement in ASK-1-knockdown rats with MI modeling. Nonetheless, NIPPV inhibited ROS-induced extrinsic apoptosis in the heart tissues of rats with MI by regulating ASK-1 expression, and subsequently ameliorated cardiac function loss and MI-dependent pathogenic changes in the heart tissue.

Keywords: Heart failure, myocardial infarction, non-invasive positive-pressure ventilation, ASK-1, apoptosis, ROS

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Introduction

Heart failure (HF) is a progressive, multifactorial, and disabling syndrome that features various symptoms caused by ventricular dysfunction and systolic (injured contraction) or diastolic (injured relaxation) blood pressure. HF is the final clinical manifestation of various cardiovascular diseases. Ischemic heart disease is the main cause of death worldwide. Timely reperfusion is the most effective treatment strategy for ischemic heart disease; however, reperfusion itself can cause fatal cardiac damage, which is known as myocardial infarction (MI) injury.[1] There is an imbalance between antioxidant mechanisms and oxidative stress in MI; this imbalance is related to the excessive reactive oxygen species (ROS) generation promoted by reperfusion, which ultimately causes apoptosis in the heart tissues. Treatment strategies targeting oxidative stress-related apoptosis in MI injury have recently shown promise.

Recent studies have indicated that non-invasive positive-pressure ventilation (NIPPV) is an efficient therapy strategy for MI-induced HF that prevents microvascular embolism and suppresses the production of inflammatory factors.[2] NIPPV, which involves continuous positive airway pressure (PAP), bilevel PAP (BiPAP), and adaptive servo-ventilation, is a method of elevating alveolar ventilation without tracheal intubation. Some prior studies have demonstrated the economic and efficacious value of this therapy in reducing the complications that result from mechanical ventilation and decreasing overall mortality.[3,4] BiPAP ventilation has been successfully used for the treatment of long-term pulmonary

respiratory failure.[3,5] Corresponding clinical results indicated that the hypoxemia of patients with pulmonary edema and acute MI was evidently alleviated through BiPAP treatment; further, the success rate of rescuing left HF was significantly improved.[6]

Cardiac hypertrophy and fibrosis can serve as adaptive, compensatory, or maladaptive precursors of cardiac failure. Accumulating evidence has confirmed that ROS signaling is associated with the initiation of cardiac hypertrophy.[7–9] Various extracellular signals can elicit cardiomyocyte hypertrophy and fibrosis, and many diverse downstream pathways that mediate the hypertrophic growth response of these factors are capable of being activated by ROS, such as apoptosis signal-regulating kinase 1 (ASK-1).[10] ASK-1 belongs to the MAP3K family, existing upstream of p38 and JNK. ASK-1 can activate JNK and p38 by phosphorylating intermediate kinases; it also plays a role in linking the apoptotic cell death and cellular oxidative stress responses. ASK-1 also contributes to various systemic diseases, such as acute ischemia/reperfusion (IR) injury and HF,[11] by decreasing the functional and structural integrity of the mitochondria in cardiac cells.[12] ASK-1-deficient mice exhibit reduced levels of cardiomyocyte apoptosis, fibrosis, and hypertrophy.[13] Therefore, ASK-1 suppression may be a promising strategy to slow or ameliorate pathogenic changes that are relevant across different forms of HF.

To investigate the mechanism of NIPPV in the treatment of acute MI-induced HF, a rat MI model was established. The infarct area, pathogenic changes, myocardial apoptosis, and ROS generation were detected in rat MI model hearts; these were analyzed to study the efficiency of noninvasive ventilation as a therapy strategy for MI-induced HF. Meanwhile, by implementing an MI ASK-1-deficient rat model, the involvement of ASK-1 and its downstream signal transduction in the cardioprotective function of NIPPV was evaluated. Therefore, this study aimed to explore the role of NIPPV in a rat MI model by mediating the ASK-1-associated pathway.

Materials and methods

Animal protocols

Adult male Wistar rats and ASK-1-deficient rats (220 ± 20 g) were provided by the Vital River Animal Experimental Center. All procedures were approved by the Animal Care and Use Committee of the First Affiliated Hospital of Gannan Medical University. MI injuries were performed as previously described by Yang *et al.*[14] Briefly, the mice were subjected to intraperitoneal anesthesia with 100 mg/kg pentobarbital sodium. During this procedure, temperature was maintained at 37°C by using a heating pad. After localization and display, the left coronary artery was ligated for 45 min. Reperfusion was subsequently initiated using a loosening ligature. Sham-operated control rats underwent similar treatment without left coronary artery ligation. After reperfusion, the muscle layer and skin were closed, and the rats were given 21 days to recover before hemodynamic determination. Postoperative pain was relieved by intramuscular injection of buprenorphine hydrochloride (0.65 mg/kg).

NIPPV treatment

Seven days after the left anterior descending artery (LAD) ligation procedure, the rats in the NIPPV group were continuously administered NIPPV daily using a SAR830-P CWE ventilator for 60 min. The respiratory rate was 60 breaths/min, and the tidal volume was 2 mL/100 g (body weight). Airway pressure was maintained at 8–10 cm H_2O containing 40% O_2. The rats in the control group did not undergo any treatment. All rats were euthanized 14 days after the operation.

Experimental grouping

Thirty healthy wild type (WT) rats and 30 ASK-1-deficient rats were randomly divided into six groups with 10 rats in each of the following groups: (1) sham operation WT rats (control group), (2) LAD operation WT rats (MI group), (3) NIPPV treatment and LAD operation WT rats (MI + NIPPV group), (4) sham operation in ASK-1-deficient rats (control + ASK-1 KD group), (5) LAD operation in ASK-1-deficient rats (MI + ASK-1 KD group), and (6) NIPPV treatment and LAD operation in ASK-1-deficient rats (MI + NIPPV + ASK-1 KD group). For the NIPPV treatment groups, NIPPV was applied after MI modeling, and the remaining procedures were conducted in the same manner as in the MI group.

Hematoxylin & eosin staining

After collection and embedding in paraffin, the hearts were sectioned (4 μm thickness) and subjected to hematoxylin & eosin (H&E) staining. In brief, following deparaffinization in xylene and rehydration with alcohol, all heart samples were treated with hematoxylin solution, differentiated with acid alcohol (1%), and subjected to eosin–phloxine solution counterstaining. Finally, these heart sections were exposed to a xylene-based mounting medium and visualized under a microscope to analyze histological alterations, including cardiomyocyte hydropic alterations, neutrophilic and lymphohistiocytic infiltration, hemorrhage, and acute myocardial necrosis.

Infarct area size measurement

Following reperfusion for 2 h, 2,3,5-triphenyltetrazolium chloride (TTC) staining was used to measure the infarct size. In brief, the rats were euthanized and 4 mL of 1% Evans blue dye was injected into the vena cava to delineate the non-infarcted portion of the heart. Then, hearts were removed, subjected to paraformaldehyde fixation overnight, and sectioned (five sections, 2 mm thickness); these 2 mm-thick sections were then flat embedded with paraffin, sectioned (4 μm), and subjected to TTC staining (incubated at 37°C for 2 h) to measure the infarct volume. Sections were placed on a light table and imaged on both sides. Different regions were subsequently delineated. Infarct size was expressed as the percentage of the ischemic area at risk. In myocardial slices, the non-infarcted area was defined as the Evans blue-stained area, whereas the infarcted area was identified as the TTC-unstained area.

Cardiac function assessment

Cardiac function was measured based on the protocol described in Gao's report.[15] Rats were anesthetized by intraperitoneal injection of chloral hydrate (300 mg/kg) 21 days after LAD surgery. The external right carotid artery was located, and a micro-tipped transducer catheter (1.4F) was inserted into the artery and was subsequently directed into the LV. The other end of this catheter was linked to the ES 2000 model. The left ventricular end-diastolic pressure (LVEDP), systolic pressure (LVSP), mean arterial pressure (MAP), and the rise and fall in the maximum rates of the left ventricle pressure (dP/dt max, dP/dt min) were determined and averaged across 10 consecutive beats.

ROS determination

ROS production was determined using a DCFH-DA fluorescence probe. The cells were initially incubated for the indicated times with *Propionibacterium acnes* and then with 10 μM DCFH-DA in the dark at 37°C for 30 min. Fluorescence intensity (Ex 488 nm/Em 525 nm) was determined using a fluorescence microscope.

Quantitative polymerase chain reaction

Total RNA was isolated from the cardiac tissues of H9C2 cells using TRIzol reagent. RNA was reverse-transcribed to obtain cDNA using a cDNA Reverse Transcription kit (Vazyme Biotech Co., Ltd.) at 42°C for 1 h and at 75°C for 5 min. Quantitative polymerase chain reaction (qPCR) was performed using the SYBR™ Green PCR Master Kit (Vazyme Biotech Co., Ltd.). The thermocycling conditions of qPCR were as follows: initial denaturation (95°C, 180 s) and 40 PCR cycles (95°C, 30 s; 56°C, 30 s; 72°C, 30 s). The fold-change in gene expression was determined using the $2^{-\Delta\Delta^{Cq}}$ method.[16] Messenger RNA (mRNA) expression was normalized to that of *Gadph* as the internal reference. All experiments were triplicated.

Western blotting

Proteins were isolated from heart tissue with RIPA lysis buffer, according to the relevant guidance (Thermo Fisher Scientific); the BCA protein assay kit was used to measure total protein concentration. The loaded proteins were subjected to SDS-PAGE and transferred to nitrocellulose membranes (Hybond-C, Amersham Biosciences), which were subsequently incubated overnight with the appropriate primary antibodies. The bound antibodies were then probed with horseradish peroxidase–conjugated secondary antibodies. The band intensity was quantified using BandScan 5.0.

Statistical analysis

The results of this study are displayed as mean ± standard deviation (SD). A one-way analysis of variation (ANOVA) followed by Tukey's post hoc test or a *t*-test were conducted to analyze the differences among multiple groups and two groups, respectively. Statistical significance was set at $P < 0\ 05$.

Results

ASK-1 expression was inhibited in the cardiac tissue of IR rats treated with NIPPV

To determine the effect of NIPPV on MI development and the involvement of ASK-1 in this process, we examined the expression and role of ASK-1 during MI development in WT and ASK-1-deficient rats. We established an LAD-induced MI rat model in both WT and ASK-1 knockdown (KD) rats; NIPPV treatment was performed for MI rats. qPCR data demonstrated that ASK-1 mRNA was upregulated in the heart tissue of WT rats with MI, whereas NIPPV treatment caused a reduction in ASK-1 mRNA (see Figure 1(A)). In ASK-1 KD rats, ASK-1 mRNA levels were maintained at a low level in the heart tissue compared to those in WT rats. However, it was also observed that MI modeling led to an upregulation of ASK-1 in the cardiac tissue (see Figure 1(A)). Furthermore, western blotting (WB) was performed to confirm the expression of ASK-1 protein in the heart tissue. ASK-1 expression was clearly increased in WT rats after modeling; however, the expression of ASK-1 decreased after NIPPV treatment. ASK-1 KD led to a significant reduction in ASK-1 expression level in heart homogenates and specimens (see Figure 1(B)). ASK-1 is positively associated with MI progression and could be negatively modulated by NIPPV.

NIPPV treatment alleviated cardiac infarction and improved cardiac function in MI rats via ASK-1 mediation

To evaluate the function of NIPPV in MI development, infarct size, pathogenic changes, and cardiac function were examined using TTC staining, H&E staining, and electrocardiogram (ECG determination. TTC staining showed that the infarct area in the MI group reached 30% after LAD surgery, whereas NIPPV treatment decreased the infarct area to 15%. As expected, MI ASK-1 KD rats also exhibited a similar infarct size to the NIPPV treatment group, with an infarct area of 14%. However, NIPPV treatment did not affect infarct proportion in ASK-1 KD rats (see Figure 2).

H&E staining was used to identify pathogenic changes in the MI model. The cardiomyocytes in the anterior wall region of the LV of the IR group were disordered and decreased in number; additionally, pathogenic changes, including hypertrophy and fibrosis, were observed in these groups. These changes caused by MI modeling were alleviated by NIPPV or ASK-1 KD. However, NIPPV did not exert a protective effect on the heart tissue of the MI ASK-1 KD rat model (Figure 3).

Next, we evaluated the influence of NIPPV and ASK-1 on cardiac function in a rat model of MI. Changes in all hemodynamic parameters after MI induction in rats were significant, including increasing LVEDP and decreasing LVSP, (dP/dt)max, -(dP/dt)min, and MAP, compared with those in the Sham group. NIPPV significantly affected LVEDP, LVSP, (dP/dt)max, -(dP/dt)min, and MAP in MI WT rats. In ASK-1 KD rats, LAD surgery caused a moderate increase in LVEDP and a decrease in LVSP, (dP/dt)max, -(dP/dt)min, and MAP. However, NIPPV treatment did not influence LVEDP, LVSP,

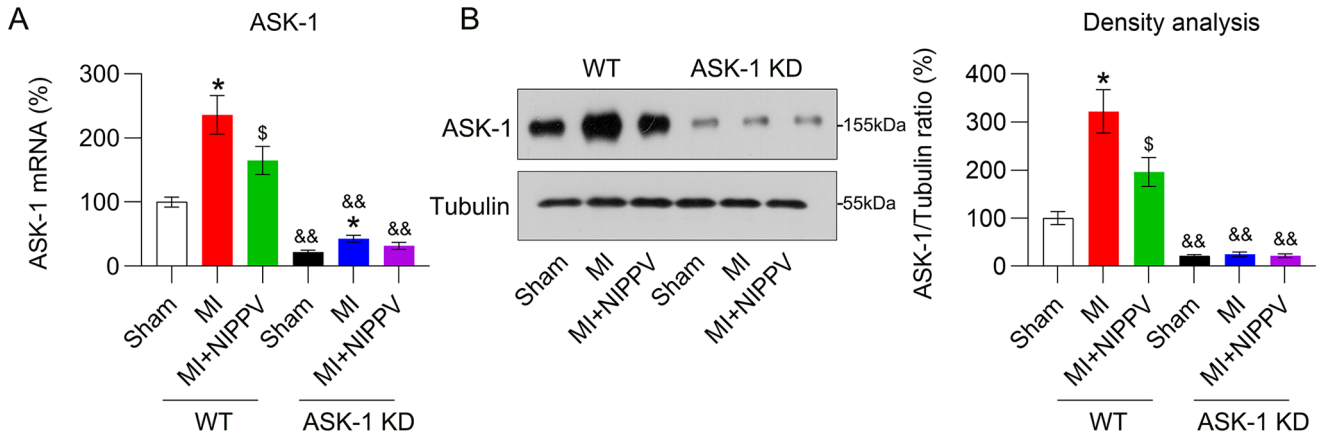

Figure 1. Expression of ASK-1 in NIPPV-treated MI rats.
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days post-surgery. (A) qPCR and (B) WB were performed to determine the expression of ASK-1 at the mRNA and protein level in the cardiac tissue from these rat models.
*$P < 0.05$ compared to the Sham group; $^{\$}P < 0.05$ compared to each MI group; $^{\&\&}P < 0.01$ compared to each WT group.

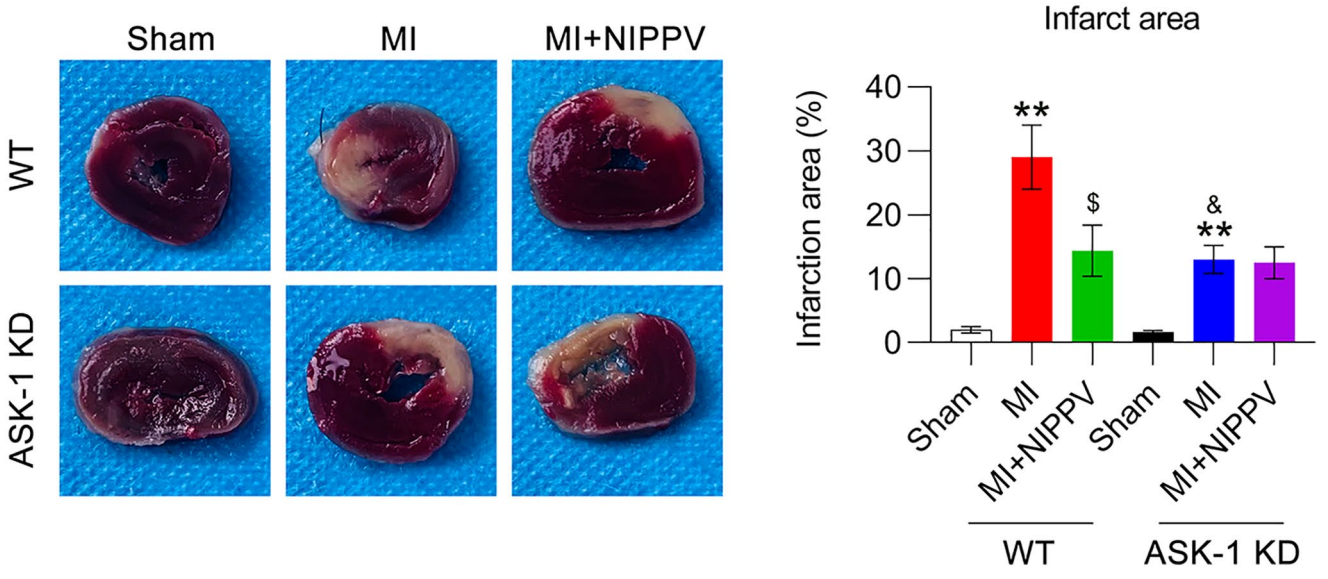

Figure 2. Role of ASK-1 deficiency in NIPPV-dependent reduction of infarct area in the cardiac tissue of MI rats.
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days post-surgery. TTC staining was performed on the heart tissues of the rat models. The right panel shows the quantification of the infarct area of the heart tissue from the rats.
**$P < 0.01$ compared to the Sham group; $^{\$}P < 0.05$ compared to each MI group; $^{\&}P < 0.05$ compared to each WT group.

(dP/dt)max, –(dP/dt)min, or MAP in ASK-1-KD rats (see Table 1). These data suggest that the cardioprotective role of NIPPV in MI rats is ASK-1 dependent.

NIPPV treatment alleviated cardiomyocyte apoptosis and oxidative stress in MI rats by regulating ASK-1 expression

Apoptotic cell death in the cardiac tissue is a major manifestation of acute MI. A TUNEL staining assay was performed to detect apoptotic dead cardiomyocytes in the heart tissues of MI rats. TUNEL staining demonstrated that TUNEL-positive cell number was upregulated in MI-treated WT and ASK-1 KD rats compared with that in non-treated rats. Nonetheless, NIPPV treatment reduced TUNEL cells number in MI-treated WT rats; in contrast, this treatment exhibited no significant effect in MI-treated ASK-1 KD rats (see Figure 4(A)). Pro-apoptotic BAX and anti-apoptotic BCL-2 levels were then assessed by WB. The corresponding data showed that the BAX protein levels were increased, whereas the BCL2 levels were reduced in MI-treated WT and ASK-1 KD rats. Moreover, both NIPPV treatment and ASK-1 KD reversed the effect of LAD surgery on BAX and BCL2 levels. However, in ASK-1 KD rats, NIPPV treatment exhibited no effect (see Figure 4(B)).

We next established that ROS generation increased in the heart tissue of rats after LAD surgery. However, MI-induced ROS levels were ameliorated in the ASK-1 KD rats. NIPPV treatment in WT rats downregulated ROS generation but did not influence ROS levels in ASK-1 KD rats (see Figure 5(A)). The Nrf2/HO-1 pathway is a well-documented antioxidative signal transduction pathway. Following LAD surgery,

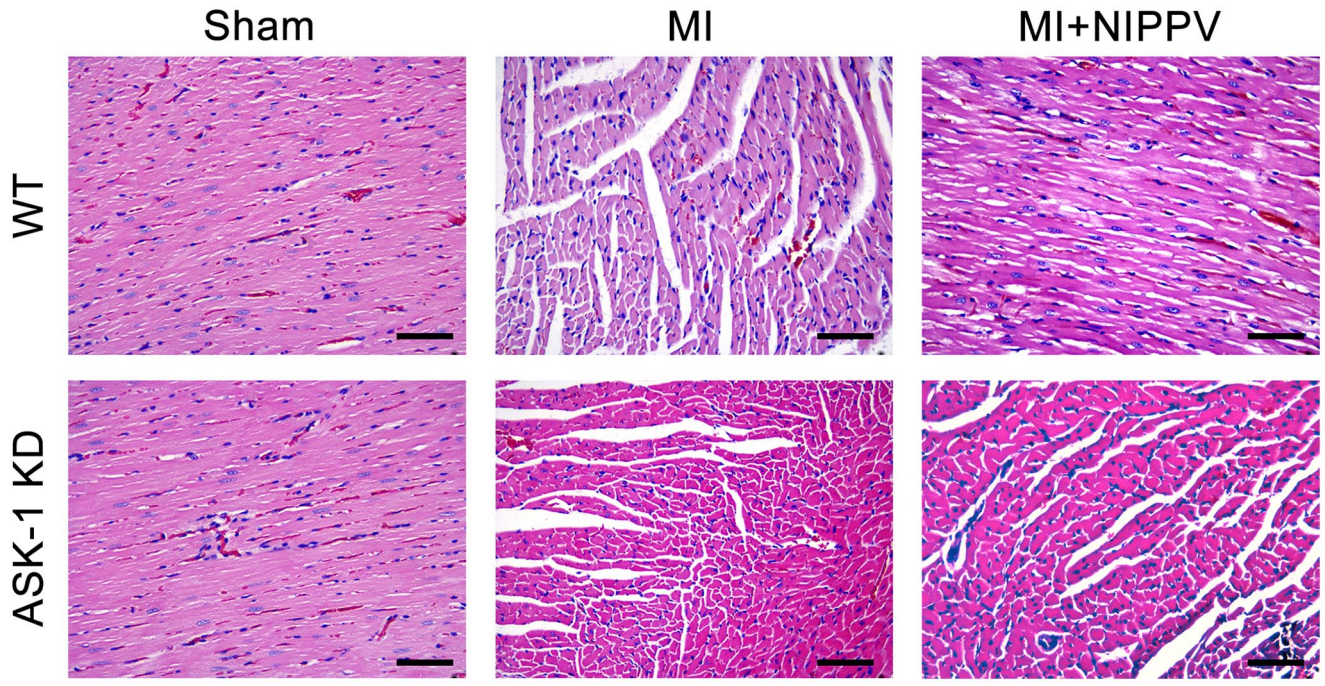

Figure 3. Role of ASK-1 KD in NIPPV-dependent reduction of pathological changes in the cardiac tissues of MI rats.
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days postoperatively. H&E staining shows the corresponding rat myocardial tissue. Scale bar, 200 μm.

Table 1. Effect of NIPPV on cardiac function in WT and ASK-1-deficient mice with or without MI modeling.

	WT			ASK-1 deficient		
	Control	MI	MI + NIPPV	Control	MI	MI + NIPPV
LVEDP (mmHg)	3.2 ± 0.6	7.5 ± 0.7**	4.1 ± 0.5$	3.3 ± 0.7	5.5 ± 0.9*&	5.3 ± 0.6&
LVSP (mmHg)	110.0 ± 9.0	63.9 ± 8.1**	88.5 ± 8.3$	113.3 ± 8.0	74.2 ± 7.8*	72.9 ± 6.6&
(dP/dt)max (mmHg/s)	3861.2 ± 311.8	2170.1 ± 209.9**	2883.3 ± 224.3$	3855.2 ± 307.7	2898.2 ± 275.1*&	2835.8 ± 292.7
–(dP/dt)min (mmHg/s)	3676.5 ± 307.2	2323.2 ± 301.2*	2953.1 ± 322.7$	3628.7 ± 327.1	3067.7 ± 288.5*&	3103.1 ± 276.5
MAP(mmHg)	81.5 ± 8.1	56.7 ± 8.0*	70.2 ± 8.0$	79.8 ± 6.8	67.2 ± 7.9*	69.6 ± 9.6

ASK-1: apoptosis signal-regulating kinase 1; NIPPV: non-invasive positive-pressure ventilation; WT: wild type; MI: myocardial infarction; LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; dP/dtmax, dP/dtmin: maximal rates of rise and fall in LV pressure; MAP: mean arterial pressure.
Values are expressed as mean ± SD.
*$P < 0.05$, **$P < 0.01$ compared to the Sham group; $$P < 0.05$ compared to each MI group; &$P < 0.05$ compared to each WT group.

Nrf2 and HO-1 levels were reduced in the heart tissues of rats. In addition, Nrf2 and HO-1 levels were higher in the MI-treated ASK-1 KD rats than those in the WT rats. NIPPV treatment restored Nrf2 and HO-1 levels in MI-treated WT rats but not in ASK-1 KD rats. These data clearly demonstrated that MI induced apoptosis and oxidative stress in the cardiac tissue, which was alleviated by NIPPV treatment via the regulation of ASK-1 expression.

Signal transduction of ASK-1-associated ROS-dependent extrinsic apoptosis was blocked by NIPPV treatment in MI rats

ROS are capable of activating ASK-1 through the reduction of thioredoxin expression. Once activated, ASK-1 further activates MAPK proteins, such as p38, through phosphorylation. Activated p38 initiates extrinsic apoptosis by inducing the transcription of apoptosis-associated genes, such as *FasL*, *Bim*, and *c-Jun*, which leads to caspase-3 and -9 activation. Apoptotic cell death is subsequently executed by caspase-3 through PARP cleavage. Here, to probe the ROS-initiated ASK-1-associated extrinsic apoptosis in NIPPV-treated MI rats, we examined the protein levels of FasL; phosphorylated p38; and cleaved caspase-3, caspase-9, and PARP. LAD surgery induced p38 phosphorylation; FasL expression; and cleavage of caspase-3, caspase-9, and PARP, whereas blockage of this ROS-apoptosis axis was observed after NIPPV treatment in WT rats. In ASK-1-deficient rats, MI modeling caused a moderate upregulation of these signals compared with that observed in the WT group with the same treatment. Notably, NIPPV did not affect the cascade transduction of the p38-FasL-caspase-PARP pathway (see Figure 6). These results demonstrated that NIPPV treatment could block the signal transduction of the ASK-1-associated ROS-apoptosis axis in the cardiac tissue of MI rats.

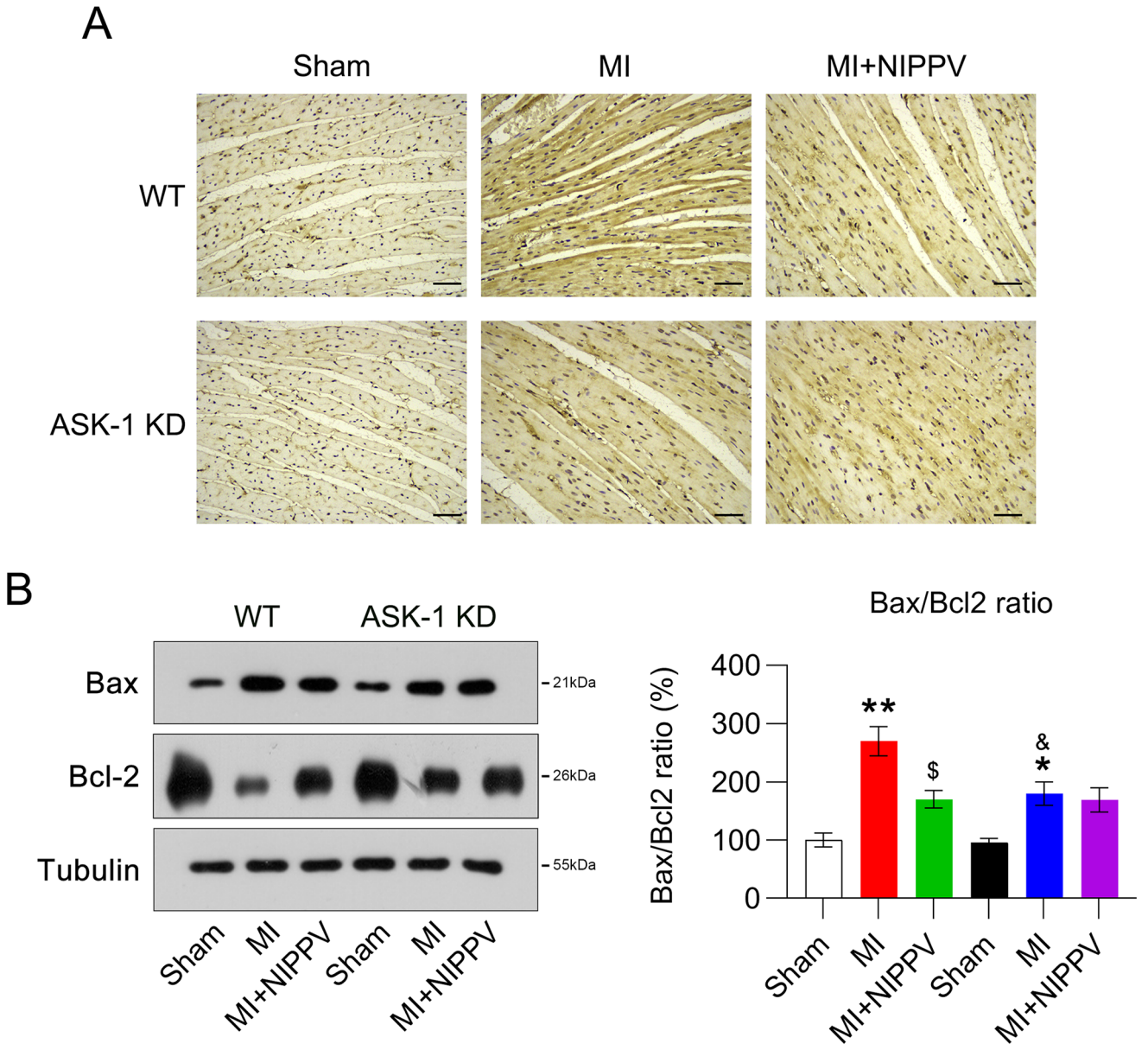

Figure 4. Effect of ASK-1 KD in NIPPV-dependent alleviation of apoptosis in the cardiac tissue of MI rats.
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days postoperatively. (A) TUNEL staining indicating the presence of apoptotic cells. Scale bar, 100 μm. (B) The left panel shows the WB analysis of BAX and BCL2 protein levels in the heart tissue of rats. The right panel represents the ratio of BAX/BCL2 protein expression.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared to the Sham group; $^{\$}P < 0.05$ compared to each MI group; $^{\&}P < 0.05$ compared to each WT group.

Discussion

MI is a major cause of death worldwide. The current standardized therapy involves reperfusion; however, reperfusion itself can paradoxically result in severe injury to the myocardium, involving ROS production, calcium overload, neutrophil infiltration, and cytokine generation. Accumulating evidence and clinical trial results have suggested that there are currently no efficacious therapies that effectively shield the myocardium from reperfusion injury. This study aimed to identify a novel treatment, NIPPV, which may, at least, relieve myocardial IR injury symptoms. The corresponding data showed that the implementation of NIPPV treatment 7 days post-MI modeling could protect cardiac functions, histological alterations, apoptosis, and oxidative stress in the cardiac tissue of rats induced by LAD surgery. In addition, we elucidated a new signaling mechanism in which NIPPV protects the heart tissue against MI injury via the ASK-1-associated ROS-apoptosis signaling pathway.

MI-induced HF always features a rapidly increasing LV end-diastolic pressure and pulmonary venous hypertension, which contribute to pulmonary interstitial and alveolar edema. MI is worsened by a lack of oxygen supply in the myocardium.[17] NIPPV was implemented as a treatment strategy in patients with HF who were experiencing respiratory distress, according to the 2018 Chinese protocol for HF diagnosis and

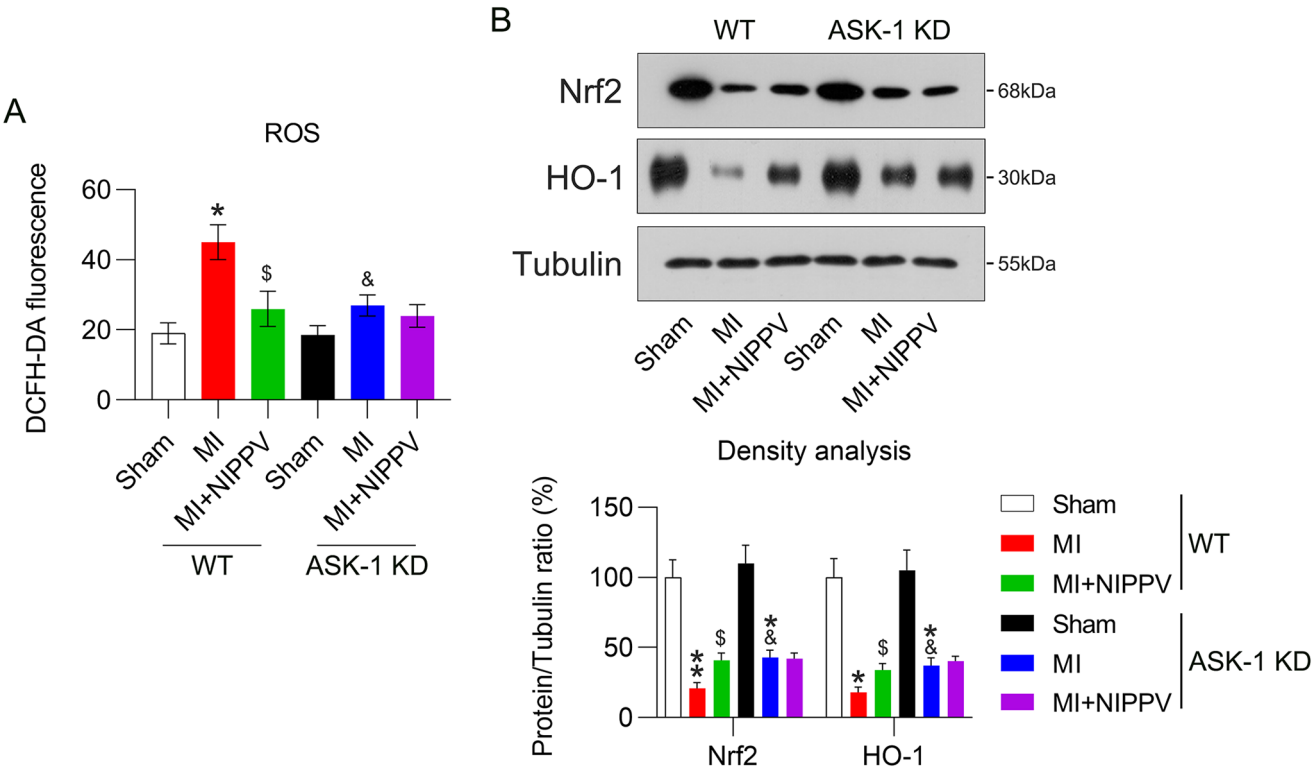

Figure 5. Effect of ASK-1 KD in NIPPV-dependent alleviation of oxidative stress in the heart tissue of MI rats.
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days postoperatively. (A) DCFH-DA staining to assess ROS production. (B) WB to detect the protein levels of antioxidative Nrf2 and HO-1 in the cardiac tissue of these rat models.
$^{*}P < 0.05$ compared to the Sham group; $^{\$}P < 0.05$ compared to each MI group; $^{\&}P < 0.05$ compared to each WT group.

treatment. Dyspnea can be improved and cardiac preload and pulmonary congestion can be eased via a BiPAP ventilator, which elevates intrathoracic pressure and decreases venous return so that the oxygen consumption of the cardiac muscle is reduced.[18] In addition, NIPPV treatment could lead to increased HSP70 levels, decreased TNF-α, MMP2, and MMP9 expressions, and decreased myocardial neutrophil infiltration and fibrosis in a rat model of MI.[2] Nonetheless, it is still unclear whether the improved effects on heart structure and function, followed by subsequent MI amelioration, are due to the mechanism of NIPPV. In this study, we established an MI rat model using LAD surgery. From day 7 postoperatively, NIPPV treatment was continuously performed with a small animal ventilator for 1 h/day. Our *in vivo* experiment clearly demonstrated that NIPPV possessed the capacity to alleviate LAD surgery-initiated MI and pathological changes, alongside reducing cellular apoptosis and oxidative stress; ultimately, this confirmed the cardioprotective efficiency of NIPPV during MI progression.

The imbalance between excessive free radical generation and antioxidant system following tissue damage contributes to biological membrane lipid peroxidation and severe cell damage. Antioxidative enzymes that function in an Nrf2/HO-1-dependent manner have been well-documented. Nrf2 transcriptionally upregulates antioxidative genes, such as HO-1, superoxide dismutase, and catalase, which activate cytoprotective pathways against inflammation and oxidative injury.[19] Furthermore, ROS can activate ROS-sensitive MAP3K ASK-1, which can activate the downstream MAP kinases JNK and p38.[20] Consequently, ROS activates the nuclear factor kappa B (NF-κB) pathway via the activation of ASK-1.[21] NF-κB activation can promote proinflammatory cytokine production, causing activation of the extrinsic apoptosis pathway via death receptors and the activation of *FasL* expression, caspase cleavage, and PARP cleavage.[22] In an MI animal model, apoptosis was repeatedly observed in the infarcted heart tissue.[23] A range of apoptotic cardiomyocytes in the border region between the MI areas contributes to cardiomyocyte loss, aggravates cardiac dysfunction, and even causes HF and mortality.[24,25] In this study, NIPPV treatment ameliorated ROS generation and myocardial apoptosis. As a key modulator of the ROS-induced extrinsic apoptotic pathway, ASK-1 was knocked down in our experimental rats to elucidate its involvement in NIPPV function. Meijles *et al.*[26] suggested that redox-sensitive ASK-1 can activate stress-regulated protein kinases, such as p38-MAPK and JNKs, and promote fibrosis in various tissues. In addition, they found that moderate levels of H_2O_2 activate ASK-1 in neonatal rat cardiomyocytes and perfused rat hearts. Specifically, ASK-1 was activated during ischemia in adult rat hearts, which aligned with the upregulation of ROS levels. Their data suggested that ASK-1 inhibitors protect the heart from hypertension-induced cardiac remodeling. Consistent with these previous findings, our results showed that ASK-1 KD itself inhibited MI-induced infarction and pathological changes, while leading to the downregulation of oxidative stress and extrinsic apoptosis in the cardiac tissue of MI

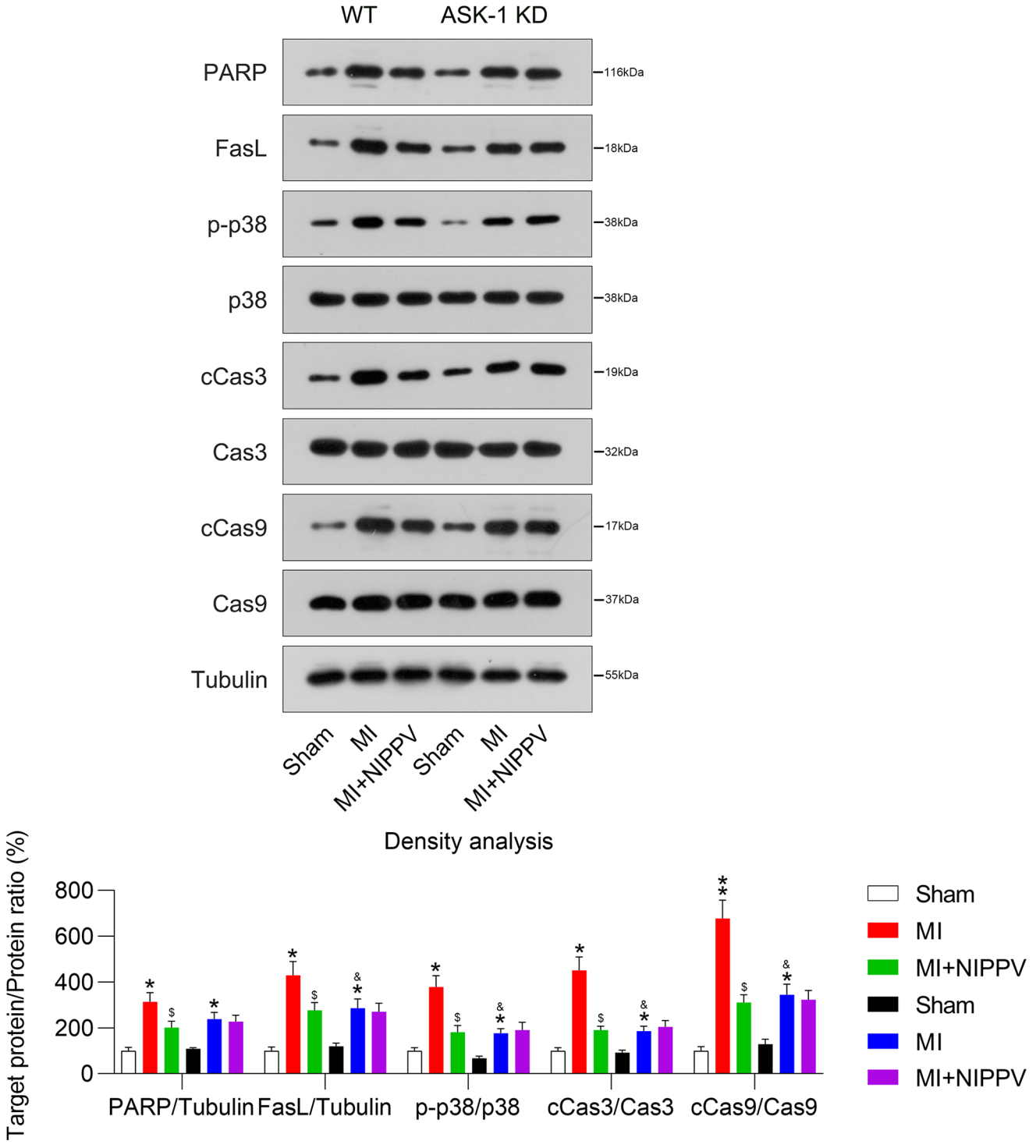

Figure 6. Effect of NIPPV on ASK-1-associated ROS-induced extrinsic apoptosis in the heart tissues of MI rats
LAD surgery was conducted on rats with or without (–/–) ASK-1 to establish an MI rat model; NIPPV treatment was performed 7 days postoperatively. WB showing the protein levels of FasL, phosphorylated p38, the cleaved forms of caspase-3 and -9, total caspase-3 and -9, and cleaved PARP in the cardiac tissue from these rat models.

rats. However, the treatment of ASK-1 KD rats with NIPPV did not confer any evident beneficial changes against MI characteristics or the ROS-triggered apoptotic pathway. Therefore, we hypothesized that ASK-1 may play an important role in cardiomyocyte injury by regulating oxidative stress and apoptosis. Meanwhile, NIPPV-induced cardioprotection was ASK-1 dependent.

Collectively, the present data demonstrated that NIPPV treatment could alleviate MI-induced cardiac damage, including infarct, neutrophil infiltration, myocardial hypertrophy, and fibrosis, by repressing the expression of ASK-1 and blocking the link between oxidative stress and extrinsic apoptosis. Therefore, our study suggests that NIPPV is an effective treatment for MI-induced HF; however, the

mechanisms of NIPPV-dependent regulation of MI development require further research *in vitro* and *in vivo*.

AUTHORS' CONTRIBUTIONS

All authors participated in the design and interpretation of the studies, analysis of the data, and review of the article; JL and AL conducted the experiments; SX and XL wrote and revised the article.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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