Am J Transl Res 2023;15(3):1693-1703
www.ajtr.org /ISSN:1943-8141/AJTR0145566

*Original Article*

# Mechanical ventilation reduces myocardial injury in rats with acute heart failure by inhibiting cardiomyocyte apoptosis

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Received July 26, 2022; Accepted February 1, 2023; Epub March 15, 2023; Published March 30, 2023

**Abstract:** Objective: To explore the mechanism by which mechanical ventilation improves myocardial injury in rats with acute heart failure (AHF). Methods: Thirty-six male Sprague Dawley rats were randomized into a sham group, heart failure (HF) group, and mechanical ventilation (MV) group. The AHF rat model was established by pentobarbital perfusion under right internal jugular vein monitoring. The symptoms of heart failure, changes in hemodynamic parameters, cardiac function, N-terminal pro-B-type natriuretic peptide (NT-proBNP), oxidative stress-related indicators, myocardial apoptosis index, and expression of apoptosis-related proteins were compared in an AHF rat model with or without mechanical ventilation. Results: Compared to the sham group, the hemodynamics and cardiac function of MV and HF groups were markedly reduced ($P<0.05$), and the serum levels of NT-proBNP of MV and HF groups were elevated ($P<0.05$). The levels of malondialdehyde (MDA) were lowest in the sham group, followed by the MV group, and highest in the HF group. Glutathione (GSH) and superoxide dismutase (SOD) were lowest in the HF group, inermediate in MV group, and highest in the sham group ($P<0.05$). Mechanical ventilation improved myocardial injury and reduced apoptosis of myocardial cells in a rat model of AHF. Conclusion: Mechanical ventilation in the early stage of heart failure can significantly reduce the excessive occurrence of oxidative stress in rats and significantly improve apoptosis in myocardial cells in AHF rats, so as to effectively improve the symptoms of AHF and reduce the mortality of AHF rats.

**Keywords:** Acute heart failure, apoptotic cardiomyocytes, oxidative stress, mechanical ventilation

## Introduction

Acute heart failure (AHF) is an acute clinical syndrome caused by multiple factors, that is mainly manifested by the acute aggravation or rapid onset of heart failure symptoms [1]. AHF has a poor prognosis. The in-hospital mortality has been low in recent years, but the long-term mortality remains high. The 5-year mortality can reach 60% [2]. When AHF occurs, the cardiac output (CO) drops sharply, and the left ventricular (LV) end diastolic pressure and pulmonary venous pressure increase rapidly, resulting in pulmonary interstitial and alveolar edema. The patient's compensatory forced inhalation increases the fluctuation in the intrathoracic negative pressure difference, increases the LV transmural pressure and afterload, further reduces the CO, leads to insufficient myocardial oxygen supply, increases the cardiac load, and aggravates heart failure, which forms a vicious cycle [3].

After AHF, the imbalance in the oxidative stress response further aggravates the damage of myocardial cells [4]. As an important metabolic substance in cells, glutathione (GSH) participates in the tricarboxylic acid cycle and glucose metabolism in the body, protects the sulfhydryl groups of important enzyme proteins in the body from oxidation and inactivation, and ensures cell utilization and energy metabolism. Circu et al. discovered that a decline in GSH level was a underlying early activation signal of apoptosis, and the subsequent oxygen free radicals promoted cell apoptosis [5]. Superoxide dismutase (SOD) is an antioxidant metalloenzyme in the body. It catalyzes the disproportion-

ation of superoxide anion free radical to produce oxygen and hydrogen peroxide, which exerts an important role in balancing oxidation and antioxidation [6, 7]. MDA is a crucial index reflecting the body's antioxidant ability, that can reflect the rate and intensity of lipid peroxidation, thus indirectly reflecting the level of tissue peroxidation damage [8]. It has been reported that the level of oxygen free radicals (OFR) increases in the myocardium of rats with hypoxic myocardial injury [9]. OFR acts on unsaturated fatty acids on cell membranes, causing membrane lipid peroxidation, leading to cardiomyocyte injury and the formation of lipid peroxides. MDA is a vital metabolite of OFR in vivo, that effectively reflects the level of tissue peroxidation [10]. Therefore, we analyzed whether mechanical ventilation could improve the level of oxidative stress in rats after AHF by detecting the indicators related to oxidative stress.

At present, relevant studies have shown that the occurrence of heart failure promotes apoptosis of cardiomyocytes, and apoptosis also accelerates the progression of heart failure [11]. Apoptosis is one of the basic characteristics of cell life. It is a cell "suicide" phenomenon triggered by internal and external factors and closely regulated by the body. It is a prelude to DNA cleavage and necrosis of cardiomyocytes, causing contractile dysfunction of cardiomyocytes [12]. Ischemia, hypoxia, oxidative stress, and overload can induce and trigger apoptosis in heart failure [13]. Bcl-2 is an apoptosis suppressor gene that can directly regulate cell apoptosis. Bcl-2 family is an endocellular regulator. The family is divided into two proteins: anti-apoptotic and pro-apoptotic proteins. Anti-apoptotic proteins comprise Bcl-2 and Bcl-XL, while the pro-apoptotic protein is Bax. In the process of heart failure caused by continuous pressure overload, Bcl-2 protein is involved in inhibiting cardiomyocyte apoptosis, and Bax protein may only be involved in the initiation of cardiomyocyte apoptosis [14]. Caspase is a cysteine protease normally present in cells as a zymogen. After receiving an apoptotic stimulation signal, caspase is initiated to further activate the effector caspase. Effector caspase is involved in the cleavage of important substrates in apoptotic degradation [15]. As the final executor of apoptosis, Caspase3 normally exists in the form of inactive zymogen. Once the apoptotic cascade occurs, Caspase3 is cleaved and activated to cleaved Caspase3, so that inhibition of Caspase3 activation can also inhibit apoptosis [16].

Mechanical ventilation is one of the important means to rescue critically ill patients with respiratory failure and heart failure [17, 18]. At present, for patients with AHF, clinical use of mechanical ventilation can timely and effectively correct hypoxemia, stabilize the patient's condition, and plays an important application advantage in the rescue and treatment of AHF patients [19]. In addition, numerous studies have found that mechanical ventilation can effectively improve vital signs and blood gas indicators of patients with severe acute left heart failure, and improve their quality of life [20, 21].

At present, there are multiple studies on AHF and mechanical ventilation, but the related mechanisms have not been explored in detail. Therefore, in order to further explore mechanical ventilation on cardiomyocyte apoptosis in AHF rats, the effects of mechanical ventilation in treatment of AHF were explored at the cellular and molecular levels. The role and possible mechanism can provide a basis for the mechanical ventilation treatment of AHF.

## Materials and methods

### *Experiment design and grouping*

Thirty-six male specific pathogen free (SPF)-grade Sprague-Dawley (SD) rats (460±20 g) were provided by the animal experiment center of Chinese Academy of Medical Sciences (Certificate No.: 113700709652). The rats were randomly allocated to a sham group, heart failure (HF) group, and mechanical ventilation (MV) group, with 12 rats in each group. Rats in MV and HF groups were given pentobarbital (ThermoFisher, USA, 3%, 30 mg/kg) perfusion under right internal jugular vein monitoring. MV group rats were treated with mechanical ventilation immediately after operation. Mechanical ventilation was not given after successful modeling in the HF group. Sham group rats underwent only relevant invasive procedures without drug perfusion. All procedures were approved by the Animal Care and Use Committee of the First Affiliated Hospital of Gannan Medical

University. The operation of this study complied with the requirements of animal ethics. All the experimental animals were sacrificed after cervical dislocation.

The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital solution (30 mg/kg), and then the ventral side of the neck skin was incised longitudinally. The right common carotid artery was isolated, and a PE-50 cardiac catheter (Becton Dickinson, Franklin Lakes, NJ) was rapidly inserted backward. After recording the normal value, 1.0% sodium pentobarbital solution was infused at a constant rate of 0.1 ml/(kg·min) through the femoral vein catheter. Below 50% and maintaining no upward trend for more than 5 minutes, is the criterion for establishment of the AHF model [22]. Sham group rats were injected with the same amount of normal saline by the femoral vein. Another PE-50 catheter (Becton Dickinson, Franklin Lakes, NJ) was advanced from the left femoral artery to the descending aorta to measure arterial pressure and collect arterial blood. Right atrial pressure was measured with another PE-50 catheter inserted into the right atrium through the left external jugular vein. Routine lead II electrocardiogram (ECG) was monitored continuously. All the catheters were intermittently rinsed with normal saline containing 2.5 IU/mL bovine heparin.

Mechanical ventilation was continued until the rats died or survived for 180 min. Under sodium pentobarbital (3% sodium pentobarbital solution (30 mg/kg)) anesthesia, the cardiac function of the rats was observed by cardiac ultrasound, the blood of the rats was collected for the detection of relevant indicators, and the cardiac specimens were collected for pathologic examination.

*Hemodynamics and cardiac function monitoring*

Right atrial and aortic pressures as well as ECG were recorded continuously with a data acquisition system (Dataq, Akron, USA). Coronary perfusion pressure (CPP) was defined as the difference between aortic pressure and right atrial pressure.

Transthoracic echocardiography monitoring was conducted using an SSD-5500 ultrasound instrument (Aloka, Tokyo, Japan) equipped with 13 MHz linear array transducer for high frame rate imaging (102 Hz) and 7.5 MHz phased array probe for pulse wave, color and tissue Doppler imaging. Echocardiographic images were acquired in parasternal short and long axis and apical views. The end-diastolic and end-systolic wall thickness, systolic wall thickening, LV volume, and ejection fraction (EF) were measured. Aortic outflow velocity was measured by pulsed wave Doppler from five apical views, and stroke volume (SV) and LV cardiac output (CO) were calculated. All indicators were measured 3 times and averaged. The body surface area was used for correction. The body surface area (M2) = 0.09 × [body mass (kg)] 2/3. LA index = left atrial diameter/body surface area; LV index = LV diameter/body surface area; IVS index = interventricular septal thickness/body surface area, both in $mm/m^2$.

*Determination of serum N-terminal pro-B-type natriuretic peptide (NT-proBNP)*

After leaving the blood sample at room temperature for 30 min, the serum was gathered by centrifugation at 1500 g (4°C) for 10 min. The content of serum NT-proBNP was evaluated using electrochemiluminescence (ECLIA, Elecsys 2010 analyzer, Roche Diagnostics, Germany). According to the relevant kit instructions, the ELISA kit (Cayman Chemical, Ann Arbor, USA) was used for measurement of NT-proBNP content.

*Determination of related indices of oxidative stress*

According to the instructions of relevant kits, GSH, SOD, and malondialdehyde (MDA) were determined in rats.

*Hematoxylin and eosin (H&E) staining*

Myocardial tissue was immersed in 4% paraformaldehyde for 4 h, followed by transferring to 70% ethanol. It was then placed into a processing box, dehydrated in a series of alcohol gradients, and embedded in paraffin. Before immunostaining, 5 μm-thick myocardial tissue slices were dewaxed in xylene, rehydrated with reduced ethanol concentrations, and rinsed with phosphate buffer saline (PBS). After staining, the slices were dehydrated with increasing concentrations of ethanol and xylene. Pathologic damage score: according to the lesion area of

**Table 1.** Prism sequences of target genes

| Gene | Prism sequences |
|---|---|
| Bax | F: 5'-GATCAGCTCGGGCACTTTA-3' |
| | R: 5'-TGTTTGCTGATGGCAACTTC-3' |
| Bcl-2 | F: 5'-CCGGGAGAACAGGGTATGATAA-3' |
| | R: 5'-CCCACTCGTAGCCCCTCTG-3' |
| Caspase-3 | F: 5'-AACGGACCTGTGGACCTGAA-3' |
| | R: 5'-TCAATACCGCAGTCCAGCTCT-3' |
| cleaved-caspase3 | F: 5'-AAGCCGAAACTCTTCATC-3' |
| | R: 5'-TGAGCATTGACACAATACAC-3' |
| GAPDH | F: 5'-GGCACAGTCAAGGCTGAGAAT-3' |
| | R: 5'-ATGGTGGTGAAGACGCCAGTA-3' |

myocardial tissue, four aspects of hemorrhage, interstitial edema, neutrophil infiltration, degeneration, and necrosis were scored, with no lesion scored as 0 points, the proportion of lesion visual field less than or equal to 1/4 as 1 point, >1/4 as 2 points, and ≥1/2 as 3 points. The sum of four aspects was the injury score.

*TUNEL test*

The rat heart tissues were fixed with 4% paraformaldehyde, dehydrated, immersed in wax, embedded in paraffin, sliced, baked and fixed to make myocardial tissue sections. Afterwards, the tissue sections were dehydrated with xylene and gradient ethanol (as described above for H&E staining). After PBS rinsing, protease K solution was added to remove tissue protein, and then rinsed with distilled water. The procedure was performed according to the instructions of TUNEL kit, and cell apoptosis was observed using an optical microscope (× 400, Motic Corporation, Japan). The cell nucleus was brown-yellow and apoptotic. Ten visual fields were randomly taken for each slice, and the total number of cells and apoptotic cells were recorded. The apoptotic index = number of apoptotic cells/total number of cells × 100%.

*Quantitative reverse transcription polymerase chain reaction (qRT-PCR) method*

The myocardial tissue was collected, and total RNA was extracted from cells with Trizol method in accordance with the kit instructions. The concentration and purity of RNA were detected by UV spectrophotometer, and cDNA was synthesized by reverse transcription. Reverse transcription to cDNA was conducted using RNA PCR Kit (AMV) Ver.3.0 kit, and the mRNA expression levels of Bcl-2, Bax, Caspase3, and cleaved-caspase3 were measured according to qRT-PCR kit. The internal reference gene was GAPDH. Reaction procedure: 94°C, 15 s (denaturation); 58°C, 15 s (annealing), 72°C, 15 s (extension), 40 cycles. The relative quantitative $2^{-\Delta\Delta CT}$ method was applied to calculate the mRNA expression level of the target gene in each group. Primer sequences are listed in **Table 1**.

*Western blot test*

After the myocardial tissue was taken to make tissue a homogenate, RIPA cell lysate was used to lyse the protein, and the concentration was detected with the BCA method (Beyotime Institute of Biotechnology) and a standard curve was made.

The total protein (20 μg) was taken, denatured at 94°C for 3 min, subjected to 12% SDS-PAGE electrophoresis, transferred to PVDF membrane, and blocked with 5% nonfat dry milk in TBS buffer for 1 h. The primary antibodies such as Bcl-2 (1:1000) (bs-0032P Bioss, USA), Bax (1:1000) (PA570418, Thermo Scientific, USA), caspase3 (1:1000) (abx125617 Abbexa, UK), cleaved-caspase3 (1:1000) (abs132005, absin, China), GADPH (1:500) (HA101-02, Tiangen, China) were added and incubated at 4°C for 12 h. The membrane was rinsed three times with PBS, incubated with the secondary antibody (1:2000 dilution) abx103653 (Abbexa, UK) for 1 h, rinsed with TBST, and exposed to light in the dark room for development. The relative gray value of each sample protein and GADPH was calculated and recorded.

*Statistical method*

GraphPad Prism 6 was used for data analysis. The Shapiro-Wilk test was conducted to test the normality of the data, and the normally distributed measured data were described by mean ± standard deviation (mean ± SD). For data analysis of multiple groups or time points, the repeated measures ANOVA was used, and one-way ANOVA was used for multiple comparisons, followed by Bonferroni's post hoc test. Counted data were described by frequency (n) or percentage (%) and were analyzed by chi-

**Table 2.** General condition of rats

| Index | Sham group | HF group | MV group | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| Total (n) | 12 | 12 | 12 | | |
| Weight (g) | 463.3±11.2 | 467.1±10.6 | 460.3±12.7 | 3.76 | 1.38 |
| Death (n) | 0 | 8 | 2* | 17.87 | 0.001 |
| Mortality rate (%) | 0 | 66.7 | 16.7* | 19.4 | 0.001 |

*$P$<0.05 compared to the HF group.

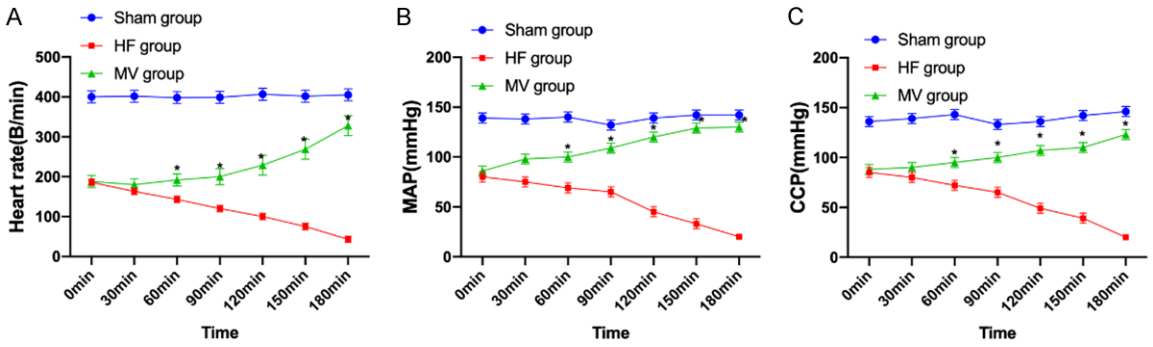


**Figure 1.** Effect of mechanical ventilation on hemodynamics in rats with acute heart failure (n = 12). (A) Heart rate (HR), (B) Mean arterial pressure (MAP), and (C) Coronary perfusion pressure (CPP). Data are shown as mean ± SD. *$P$<0.05 compared to HF group.

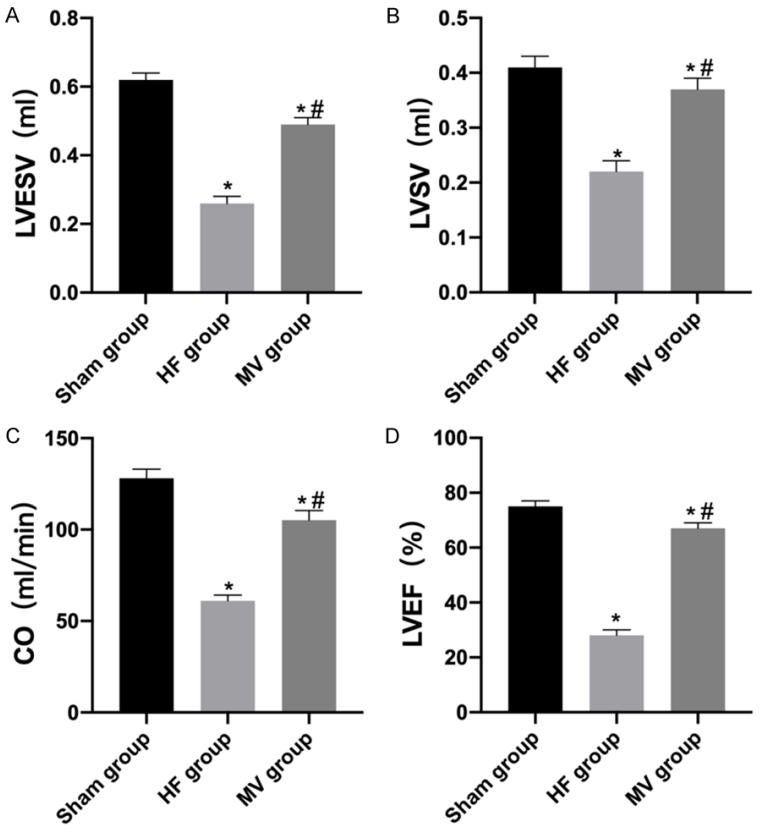


**Figure 2.** Effect of mechanical ventilation on cardiac function in rats with acute heart failure (n = 12). (A) Left ventricular end-systolic volume (LVESV), (B) Left ventricular stroke volume (LVSV), (C) Cardiac output (CO), (D) Left ventricular ejection fraction (LVEF). Data are shown as mean ± SD. *$P$<0.05 compared to sham group; #$P$<0.05 compared to HF group.

square test. $P$<0.05 was a significant difference.

## Results

### *General conditions of experimental rats*

The three groups exhibited no significant difference in body weight, which was comparable. No rats died in the sham group within 180 min. Eight rats died in HF group, with a mortality rate of 66.67%. Two rats died in the MV group within 180 min, with a mortality rate of 16.67%. There was a marked difference in mortality rate between HF and MV groups ($P$<0.05) (**Table 2**).

Symptoms and signs of heart failure during operation were observed in both MV and HF groups, such as a depressed mental state, decreased activity, decreased eating, accelerated breathing, dull hair, increased hair removal, and cyanosis of lips and toes.

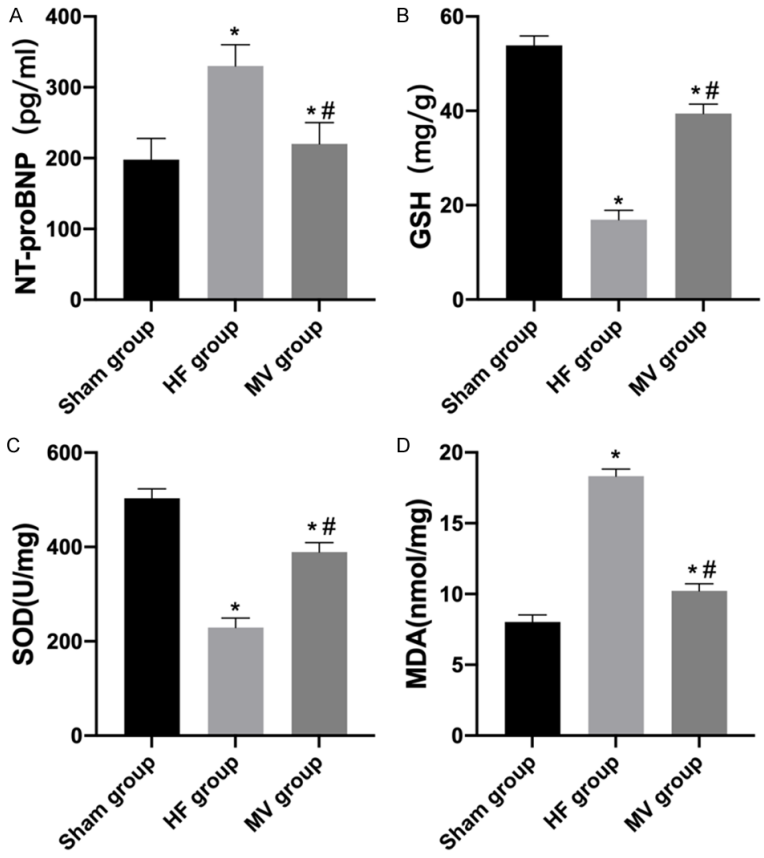


**Figure 3.** Effect of mechanical ventilation on changes of serum related indices in rats with acute heart failure (n = 12). (A) N-terminal pro brain natriuretic peptide (NT-proBNP), (B) Glutathione (GSH), (C) Superoxide dismutase (SOD), (D) Malondialdehyde (MDA). Data are mean ± SD. **P*<0.05 compared to sham group; #*P*<0.05 compared to HF group.

Compared to the MV group, the rats in the HF group had more severe symptoms and the highest mortality rate. Rats in the sham group showed no symptoms as mentioned above.

*Effects of mechanical ventilation on hemodynamics and myocardial function in AHF rats*

The heart failure model rats were observed for 180 min, and the myocardial dysfunction caused by AHF was obvious in each rat. Compared to the sham group, the heart rate was markedly reduced in MV and HF groups, while MAP and CPP decreased (*P*<0.05). In contrast to the HF group, the heart rate was increased in the MV group, and MAP and CPP also increased continuously during the observation period (*P*<0.05) (**Figure 1**).

In contrast to the sham group, the LV end-systolic volume (LVESV), LV SV, CO, and EF of rats in MV and HF groups were reduced (*P*<0.05). In contrast to the HF group, LVESV, LV SV, CO, and EF of rats in the MV group was elevated (*P*<0.05) (**Figure 2**).

*Effect of mechanical ventilation on changes of NT-proBNP in AHF rats*

Compared to the sham group, NT-proBNP was increased in rats of the MV and HF groups (*P*<0.05). Compared to the HF group, NT-proBNP was decreased in rats of the MV group (*P*<0.05) (**Figure 3A**).

*Effect of mechanical ventilation on changes of oxidative stress-related indices in AHF rats*

Compared to the sham group, rats in MV and HF groups showed decreased GSH and SOD, but had increased MDA (all *P*<0.05). Compared to the HF group, rats in MV groups showed increased serum GSH and SOD, but had decreased MDA (all *P*<0.05) (**Figure 3B-D**).

*Myocardial histopathology in each group*

In the sham group, the myocardial tissue was intact, and the myocardial fibers were arranged regularly; HF group rats had serious pathologic damage such as myocardial fiber morphologic disorder and inflammatory cell infiltration. Compared to the HF group, the injury of the myocardial tissue was reduced in rats in the MV group (**Figure 4**).

*Myocardial cell apoptosis in each group*

Compared to the sham group, myocardial apoptosis index was increased in rats of MV and HF groups (*P*<0.05). Compared to the HF group, the myocardial apoptosis index was decreased in rats of the MV group (*P*<0.05) (**Figure 5**).

*Effect of mechanical ventilation on apoptosis-related proteins in AHF rats*

Compared to the sham group, rats in MV and HF groups showed decreased apoptosis-related protein and mRNA Bcl-2, increased Bax,

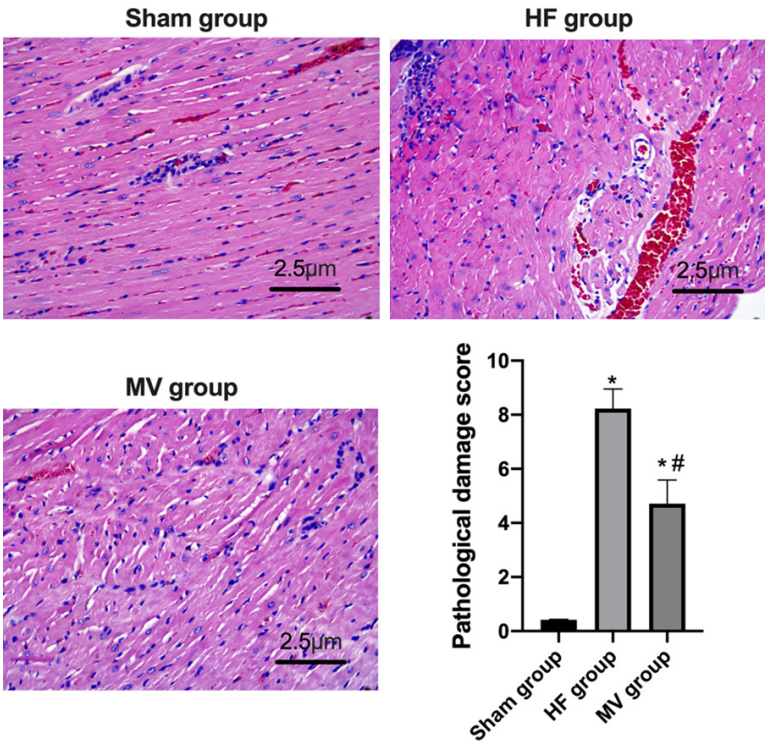


**Figure 4.** H&E staining showed the influence of myocardial histopathology in each group (n = 12). Data are shown as mean ± SD. *$P$<0.05 compared to sham group; #$P$<0.05 compared to the HF group.

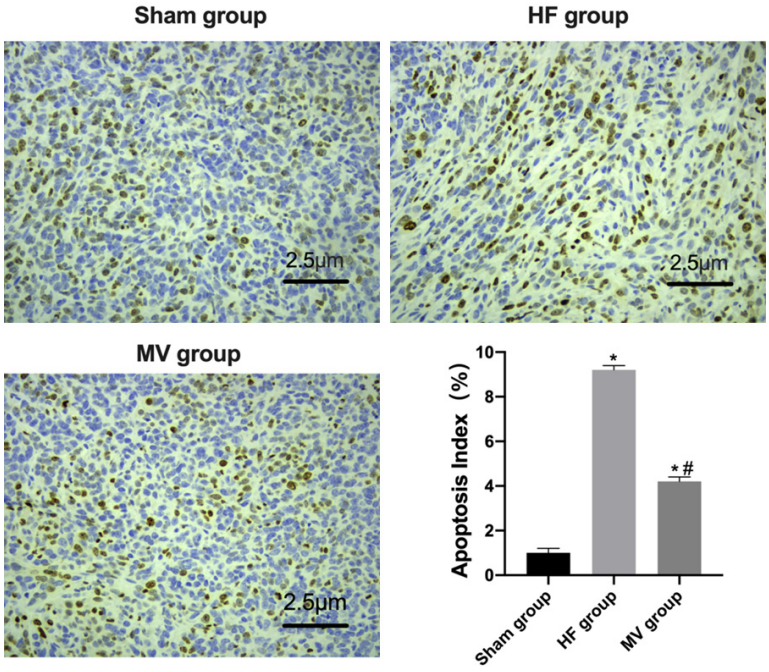


**Figure 5.** TUNEL test shows the myocardial cell apoptosis of rats in each group (n = 12). Data are mean ± SD. *$P$<0.05 compared to sham group; #$P$<0.05 compared to the HF group.

decreased Caspase3, and increased cleaved Caspase3 (all $P$<0.05). After mechanical ventilation, apoptosis was significantly reduced, apoptosis-related protein and mRNA Bcl-2 were increased, Bax was decreased, Caspase3 was increased, and cleaved Caspase3 was decreased (all $P$<0.05) (**Figures 6** and **7**).

## Discussion

Acute heart failure (AHF) is common [23]. The causes are mainly acute myocardial infarction or more serious myocardial injury and acute hemodynamic changes [24]. In this study, male SD rats were used to build a rat model of AHF by pentobarbital perfusion under right internal jugular vein monitoring. NT-proBNP, oxidative stress-related indicators, cardiomyocyte apoptosis index and expression of apoptosis-related proteins were compared, and the results showed that mechanical ventilation in the early stage of heart failure could significantly reduce the excessive occurrence of oxidative stress in rats and the occurrence of cardiomyocyte apoptosis, thereby effectively improving the symptoms of AHF and reducing mortality in AHF rats.

When AHF occurs, myocardial oxygen supply is insufficient, which increases cardiac load, aggravates heart failure, and causes a vicious cycle [3]. The main hemodynamic features include impaired LV systolic function, CO, and arterial pressure [25]. Our results show that compared to the sham group, the heart rate of rats in MV and HF groups decreased

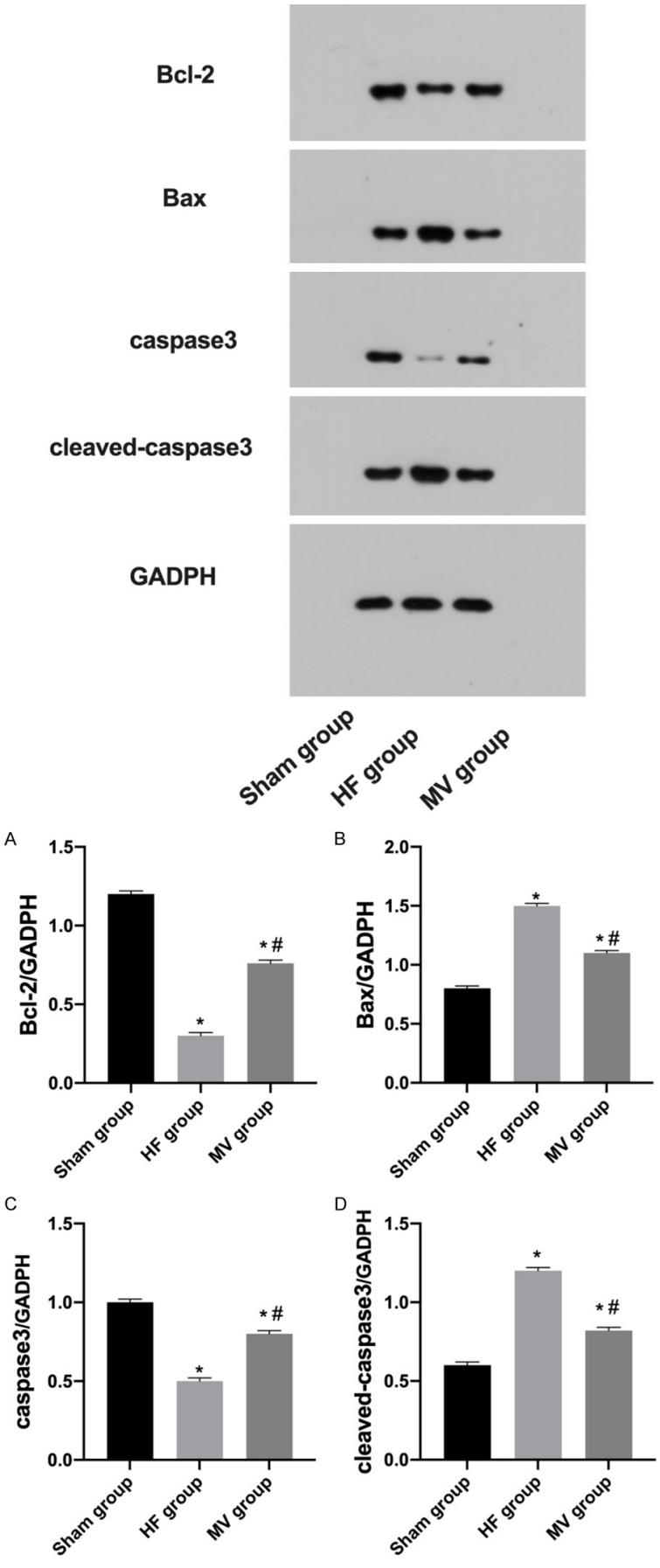


**Figure 6.** Effect of mechanical ventilation on apoptosis-related proteins in rats with acute heart failure (n = 12). (A) Protein expression of Bcl-2, (B) Protein expression of Bax, (C) Protein expression of caspase3, (D) Protein expression of cleaved-caspase3. Data are mean ± SD. **P*<0.05 compared to sham group; #*P*<0.05 compared to the HF group.

significantly, while the MAP and CPP continued to decrease during the observation period, consistent with the above findings. At present, relevant studies have found that the cardiac function-related indicators of patients are decreased when AHF occurs [3], and cardiac function is improved after mechanical ventilation. Cardiac function-related indicators such as SV, CO, and EF were elevated. We showed that active mechanical ventilation in the early stage of AHF could markedly improve the hemodynamics and myocardial function in AHF.

NT-proBNP is also known as amino-terminal pro-brain natriuretic peptide [26]. When NT-proBNP is increased, the heart is in a state of failure. In order to detect the effect of mechanical ventilation on the state of heart failure in rats, the serum NT-proBNP content of rats was detected after mechanical ventilation, and it was found that the serum NT-proBNP was increased. This suggests that mechanical ventilation can raise serum NT-proBNP in AHF rats to control the progression of heart failure.

After the occurrence of AHF, imbalance of the oxidative stress response further aggravates the injury of cardiomyocytes [4]. GSH, SOD, and MDA

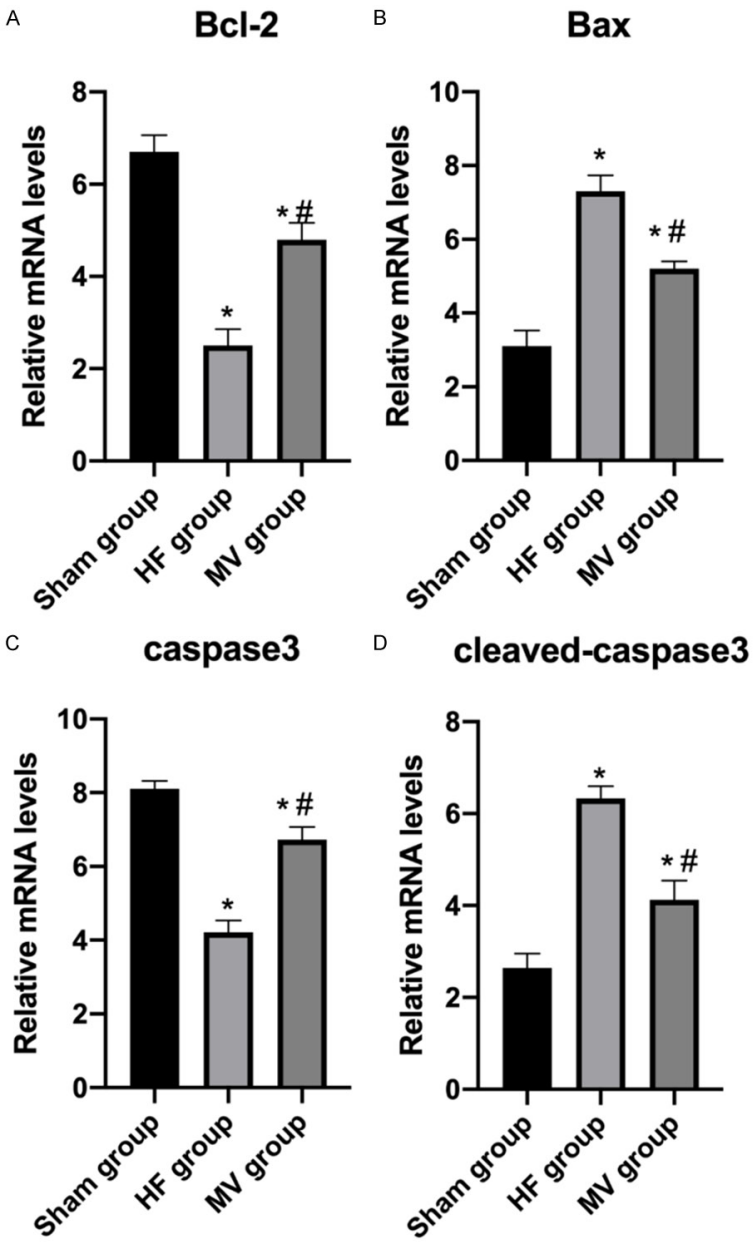


**Figure 7.** Effect of mechanical ventilation on apoptosis-related mRNA in rats with acute heart failure (n = 12). (A) mRNA expression of Bcl-2, (B) mRNA expression of Bax, (C) mRNA expression of caspase3, (D) mRNA expression of cleaved-caspase3. Data are shown as mean ± SD. **P*<0.05 compared to sham group; #*P*<0.05 compared to the HF group.

levels were measured to evaluate the oxidative stress in AHF rats. Relevant studies have found that oxidative stress occurs in AHF patients and related animal models [27, 28]. Mechanical ventilation can improve the level of oxidative stress in patients with severe pneumonia and acute respiratory distress syndrome, but there are few related studies on patients with AHF. In our experiment, it was indicated that mechanical ventilation could significantly decrease the oxidative stress response in patients, indicating that under mechanical ventilation treatment, with gradual improvement in the hypoxic state of AHF patients, the imbalance in the proportion of pulmonary ventilation and blood flow was corrected, and the level of oxidative stress was improved. The lipid peroxidation caused by ROS was alleviated, and the increase in SOD content was synchronized with a decrease of MDA content, suggesting that the antioxidant function was gradually repaired and enhanced with the correction of hypoxia. At the same time, the activity of SOD was increased, indicating that the oxidative stress level of patients was markedly lower than that before treatment during this period, antioxidant capacity was enhanced, and the oxygen-like antioxidant imbalance was significantly corrected.

In order to further analyze the mechanism of mechanical ven-tilation for myocardial protection in AHF rats, the tissue changes and apoptosis of rat cardiomyocytes were analyzed. Relevant studies have shown that the occurrence of heart failure promotes cardiomyocyte apoptosis, and apoptosis also accelerates the progression of heart failure [11]. The results of this study indicated that the apoptosis rate of cardiomyocytes was higher in HF and MV groups than in sham group, and the apoptosis rate of cardiomyocytes was lower in the MV group than in the HF group. This is similar to most results reported in the literature, indicating that cardiomyocyte apoptosis is involved in the development of AHF. This leads to the deterioration of cardi-

ac function. The regulation of the cardiomyocyte apoptosis pathway may become a new target for the treatment of heart failure. In addition, our results showed that mechanical ventilation can significantly improve cardiomyocyte apoptosis in AHF rats by increasing apoptosis suppressor genes, decreasing apoptosis promoting genes, and inhibiting the activation of caspase3, the final executor of apoptosis.

There are some deficiencies in our study. First, the direct mechanism between oxidative stress and myocardial apoptosis was not explored in the present study. Second, the effect of inhibition of the oxidative stress pathway on cardiomyocyte apoptosis and myocardial dysfunction after heart failure was not discussed.

In conclusion, we showed that early mechanical ventilation can inhibit the occurrence of oxidative stress, increase apoptosis suppressor genes, reduce apoptosis-promoting genes, and inhibit the activation of Caspase3, the final executor of apoptosis. This reduced cardiomyocyte apoptosis in AHF rats, thus playing a myocardial protective role.

## Disclosure of conflict of interest

None.

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