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Article

Myocardial Protective Effect of Xuebijing Injection on Extracorporeal Membrane Oxygenation Perfused Isolated Langendorff Heart Based on the PI3K/AKT Pathway

Shijie Yin[1,†], Xiao Yue[2,†], Chunhua Wang[2,†], Qiangxin Huang[3], Wei Wu[1], Guanbin Qin[1], Lan Luo[1], Guixin He[4,*]

[1]Department of Anesthesiology, First Affiliated Hospital of Guangxi University of Chinese Medicine, Dongge Campus, 530028 Nanning, Guangxi, China
[2]First Clinical Medical College of Guangxi University of Chinese Medicine, 530001 Nanning, Guangxi, China
[3]Department of Thoracic and Cardiovascular Surgery, First Affiliated Hospital of Guangxi University of Chinese Medicine, Dongge Campus, 530028 Nanning, Guangxi, China
[4]Cardiology Department II, First Affiliated Hospital of Guangxi University of Chinese Medicine, Dongge Campus, 530028 Nanning, Guangxi, China
*Correspondence: he_guixin@163.com (Guixin He)
[†]These authors contributed equally.

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Abstract

Objective: This study aimed to investigate the myocardial protective effect of Xuebijing injection in a model of extracorporeal membrane oxygenation (ECMO) isolated heart perfusion and determine the role of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway. **Methods**: To establish an ECMO isolated heart perfusion model, Guangxi Bama miniature pigs were randomly divided into two groups: (1) a normal saline group (NS group), in which 50 mL of normal saline was added to the perfusion solution; and (2) a Xuebijing injection group (XBJI group), in which 10 mL of XBJI was added to the perfusion solution and then continuously pumped at 5 mL/h. Perfusion was maintained for 8 h. The hemodynamic changes, inflammatory response, and myocardial enzyme levels at T0, T2, T4, T6, and T8 were evaluated. The protein expression level of the PI3K/AKT pathway was analyzed by Western blot analysis and Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). Hematoxylin and eosin staining and transmission electron microscopy were used to observe the pathological morphology and ultrastructure of T8 cardiomyopathy. **Results**: There was no significant difference in hemodynamics between the two groups. XBJI could reduce serum inflammatory factors and myocardial enzyme levels. XBJI also upregulated the expression of PI3K and AKT mRNA and the phosphorylation levels of PI3K and AKT proteins. The results of pathological and electron microscopy showed that XBJI effectively reduced myocardial cell and mitochondrial damage. **Conclusion**: XBJI can reduce myocardial inflammation and myocardial cell and mitochondrial damage in isolated heart perfusion. XBJI may play a role in the myocardial protection of ECMO isolated heart perfusion by activating the PI3K/AKT pathway.

Keywords

Xuebijing injection; bama miniature pig; extracorporeal membrane oxygenation (ECMO); PI3K/AKT pathway; preservation of the heart; myocardial protection

Introduction

Heart transplantation is the preferred strategy for the treatment of end-stage heart failure, but supply and demand are seriously mismatched. Therefore, it is of great significance to explore the dominant donor heart protection strategy to improve the quality of donor heart preservation, the prognosis of transplantation, and the utilization rate of donor hearts. At present, static cold storage is still the standard donor organ preservation technology, but it carries the risk of donor cold ischemia injury, and the safe preservation time is only approximately 4–6 h. Mechanical cardiac perfusion preserves the donor heart in a continuous beating state, which can provide the substances required for metabolism, remove metabolic waste in time, and effectively improve the quality of donor heart preservation. The preservation time of an isolated donor heart can reach 12 h or more [1]. However, the heart is in a non-physiological state during *in vitro* perfusion, and myocardial ischemia and reperfusion injury (MIRI) is inevitable [2]. The phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/AKT) signaling pathway is an important pathway regulating the function and survival of cardiomyocytes and is involved in the regulation of cold ischemia–reperfusion injury of donor hearts. This pathway plays an important role in regulating autophagy and apoptosis, reducing inflammatory response, and mediating cell proliferation and differentiation [3]. Xuebijing injection (XBJI)

is a traditional Chinese medicine intravenous preparation primarily made of safflower, Salvia miltiorrhiza, Ligusticum chuanxiong, Radix Paeoniae Rubra, and Angelica sinensis. It contains safflower yellow A, Danshensu, ligustrazine, paeoniflorin, ferulic acid, and other active ingredients. Study has shown that XBJI regulates apoptosis and autophagy by mediating the PI3K/AKT/mTOR signaling pathway, thereby preventing myocardial injury caused by sepsis [4]. XBJI can also effectively improve the cardiac function and structure of rats with myocardial hypoxia/reoxygenation injury. A medium dose of XBJI has the best effect [5]. At present, there is no research on the myocardial protective effect of XBJI on isolated donor heart preservation. We used extracorporeal membrane oxygenation (ECMO) to establish an isolated heart perfusion model. Considering that MIRI may be caused by a variety of factors, such as temperature changes, blood and foreign body contact, aortic occlusion and reopening, oxygen free radical production, and release of inflammatory mediators, we added XBJI to the donor heart preservation solution and monitored the indicators to evaluate its effect on myocardial injury. This study aimed to provide new experimental support for clinical donor heart protection strategies.

Reagents and Instruments

Reagents

The reagents used in this study included XBJI (Tianjin Hongri Pharmaceutical Co., Ltd., batch number: 2112242, Wuqing Development Zone, Tianjin, China); interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) ELISA kits (Jiangsu Jingmei Biotechnology Co., Ltd., Yancheng, Jiangsu, China, lot numbers: 202304, 202308, 202308, 202307); rabbit anti-GAPDH polyclonal antibody (Biosharp, Hefei, Anhui, China, catalog number: BL006B); PI3K antibody (Proteintech, Wuhan, Hubei, China, catalog number: 20584-1-AP) and p-PI3K antibody (Bioss, Beijing, China, catalog number: bs-6417R); AKT and p-AKT (Proteintech, Wuhan, Hubei, China, catalog numbers: 10176-2-AP, 66444-1-Ig); and PCR Reverse Transcription Reagent and PCR Amplification Reagent (Mona Biotechnology Co., Ltd., Suzhou, Jiangsu, China, catalog numbers: MR05101, MQ10101).

Instruments

The instruments used in this study included an ECMO machine (Germany Mikovel, model: CARDIOHELP-i), high-speed frozen centrifuge (Germany Eppendorf), automatic biochemical analyzer (Shanghai Hitachi Diagnostic Products Co., Ltd., Shanghai, China, equipment number: GYZ-LAB-SH-008), microplate reader (Theromo Fisher Scientific, MA, USA), and aI600 multifunction imager (GE, USA).

Experimental Animals and Methods

Experimental Animals and Grouping

Sprague-Dawley (SD) miniature pigs were purchased from the Animal Experimental Center of Guangxi University. The experimental animal license number was SCXKGui 2018-0003. The animal experiment was approved by the Ethics Committee of Guangxi University of Chinese Medicine (No. DW20190305-047). Twelve healthy adult Guangxi Bama miniature pigs (25–30 kg) were randomly divided into the normal saline group (NS group) and Xuebijing group (XBJI group) (n = 6 each).

Anesthesia Protocol

The animals were fasted for 12 h before surgery and prevented from drinking for 4 h. Midazolam 5 mg was injected intramuscularly into the buttocks. After the animal was subdued, the ear vein was opened, and an intravenous injection of propofol 2 mg/kg, sufentanil 0.5 μg/kg, and rocuronium 0.5 mg/kg was administered. Mechanical ventilation was performed after tracheal intubation under visual laryngoscope. The respiratory rate was maintained at 20–30 times/min, an inspiratory/expiratory ratio of 1:2, and tidal volume of 10 mL/kg. Propofol 2 mg/kg, sufentanil 1 μg/kg, and rocuronium 0.5 mg/kg were injected intravenously before thoracotomy. For anesthesia maintenance, a continuous infusion of propofol 4–12 mg/kg/h, remifentanil 0.01–0.02 mg/kg/h, and dexmedetomidine 0.5–1 μg/kg/h was administered.

Donor Heart Acquisition

A chest midline incision was used to expose the heart. A purse-string suture was performed at the distal end of the ascending aorta near the innominate artery. An aortic perfusion needle was inserted, and the exhaust was fixed. The tip of the catheter did not exceed the aortic valve. A volume of 600 mL heparinized arterial blood was collected, and white blood cells and platelets were removed via a white blood cell filter. The heart was slowly perfused with 4 °C HTK cardioplegic solution at a constant rate. After the heart was arrested, the heart was dissociated and placed in an ice-water mixture (4 °C). The arterial perfusion needle was replaced as an arterial catheter. Care should be taken throughout the whole process not to allow gas to enter the heart.

Establishment of ECMO Isolated Heart Perfusion Model

As shown in Fig. 1, the isolated heart perfusion circuit was assembled. Prefilled liquid and 600 mL of leukocyte-free blood were injected into the blood storage tank, and the oxygenated blood perfusion fluid was formed after oxygenation by the membrane oxygenator. Saline was perfused

through the arterial catheter, and the heart was pressed to discharge the HTK solution. The isolated heart perfusion model was established after the heart was re-beating. In the XBJI group, 10 mL XBJI was used for pre-filling, and the remaining 40 mL was continuously pumped at 5 mL/h. In the NS group, 50 mL normal saline was added to the perfusate. Heart perfusion was maintained for 8 h, and the other treatments were the same in the two groups. The perfusion pressure was maintained at 40–50 mmHg, the perfusion flow rate was 400–500 mL/min, the oxygen flow rate was 0.1 L/min, the hematocrit was >15%, and the water tank temperature was maintained at a constant 35 °C.

Specimen Collection and Processing

Hemodynamics and blood gas indexes were monitored. Oxygenated blood (20 mL) was collected at 0 h (T0), 2 h (T2), 4 h (T4), 6 h (T6), and 8 h (T8). A volume of 8 mL of blood was left to stand at room temperature (26 °C) for 30 min, after which it was centrifuged at 3000 r/min for 15 min. The serum was sub-packed and frozen at –80 °C. The remaining 12 mL was divided into blood vessels for the determination of myocardial injury markers. Ventricular muscle tissues from T0, T4, and T8 were collected and stored at –80 °C. Western blot and RT-qPCR were used to detect the expression of pathway-related proteins and mRNA. Next, 80 mg T8 ventricular samples were collected to observe the myocardial pathological structure and ultrastructure under an electron microscope.

Detection of Indicators

ELISA Detection of Serum Inflammatory Biomarkers

In strict accordance with the ELISA kit instructions, the levels of inflammatory factors such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) in the serum of the two groups were detected at T0, T2, T4, T6, and T8, and the concentration was quantified by referring to the standard curve.

Detection of Cardiac Injury Biomarkers

The levels of the myocardial injury markers troponin T (cTnT), creatine kinase (CK), creatine kinase isoenzymes (CK-MB), and lactic dehydrogenase (LDH) were detected by automatic biochemical analyzer at T0, T2, T4, T6, and T8.

Western Blot Analysis of PI3K and AKT Protein Expression in Myocardial Tissue

To 40 mg of cryopreserved myocardial tissue, 400 µL of RIPA lysate containing phosphatase inhibitor and 4 µL of PMSF were added and mixed well. After the mixture was fully ground up, it was lysed on ice for 30 min and then centrifuged (12,000 r/min, 4 °C, 10 min). The supernatant was collected, and the protein content was determined using the BCA method. SDS-PAGE separation was performed, followed by wet transfer and blocking. The samples were incubated overnight at 4 °C with primary antibody PI3K (1:600), p-PI3K (1:1000), AKT (1:4000), p-AKT (1:1000), and GAPDH (1:1000). The samples were then incubated with secondary antibody at room temperature for 1 h. The signal was visualized using enhanced chemiluminescence. The optical density was analyzed by ImageJ software (Wayne Rasband and contributors National Institutes of Health, USA, https://imagej.net/, Java 1.8.0_322 (64-bit)), and the ratios of p-PI3K/PI3K and p-AKT/AKT were calculated as the activity index of the PI3K/AKT pathway.

Quantification of Myocardial PI3K and AKT mRNA Relative Expression Levels Using Reverse Transcription Quantitative Polymerase Chain Reaction(RT-qPCR)

First, 40 mg of myocardial tissue was homogenized thoroughly, and total RNA was extracted using the Trizol method. The RNA concentration was measured, followed by reverse transcription of RNA into cDNA using a reverse transcription kit. RT-qPCR amplification was performed. GAPDH was used as the internal reference gene, and the $2^{-\Delta\Delta CT}$ method was employed to calculate the relative expression levels of mRNA. The gene sequence is shown in Table 1.

Observation of Myocardial Tissue Ultrastructure by Transmission Electron Microscopy

Left ventricular muscle tissue (1 mm^3) was cut and fixed in electron microscope fixative at 4 °C for 3 h. After rinsing, fixing, gradient dehydration, and embedding, ultrathin sections (60–80 nm) were stained with uranium-lead double staining (2% uranium acetate saturated alcohol solution, lead citrate; each stained for 15 min). The samples were then observed under a transmission electron microscope and subjected to image analysis.

Observation of Myocardial Histopathological Changes by Hematoxylin and Eosin (HE) Staining

Left ventricular myocardial tissue collected at T8 was fixed in 4% paraformaldehyde for 48 h, followed by dehydration, embedding in paraffin, sectioning, drying, dewaxing twice, staining with hematoxylin for 10 min, rinsing with running water to remove excess stain, staining with eosin for 3 min, mounting with neutral resin, and observation and image acquisition under a microscope.

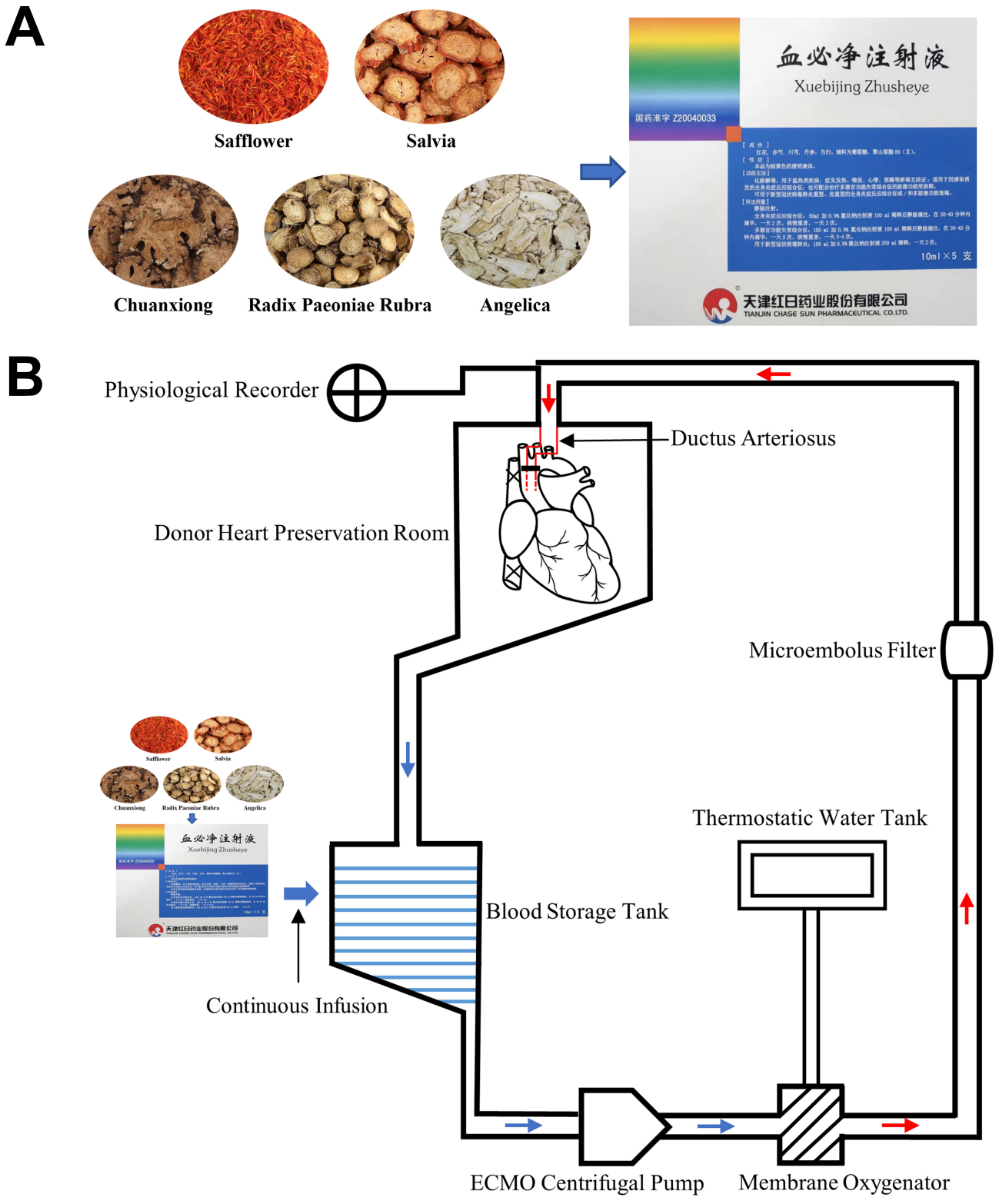

Fig. 1. Diagram of cardiac preservation. (A) Preparation of Xuebijing injection of Chinese herbal medicine raw materials. (B) Schematic diagram of extracorporeal membrane oxygenation (ECMO) isolated heart perfusion process. × denotes ligation.

Statistical Analysis

Data processing was conducted using SPSS 25.0 statistical software (IBM, Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA, USA). Normality tests were performed for continuous variables and expressed as. For comparisons among different time points, repeated measures analysis of variance (ANOVA)

Table 1. Primer Sequences.

Gene name		Sequence
PI3K	Forward primer	5′-TCTGCTTTTCTTTAACTTTGCAGTC-3′
	Reverse primer	5′-TCGTACACTAAGGATTGTTTGGAC-3′
AKT	Forward primer	5′-TCTGCAAGGAGGGCATCAAG-3′
	Reverse primer	5′-CCGTAGTCGTTGTCCTCCAG-3′
GAPDH	Forward primer	5′-CCTCCCCGTTCGACAGAC-3′
	Reverse primer	5′-GCGGCCAAATCCGTTCA-3′

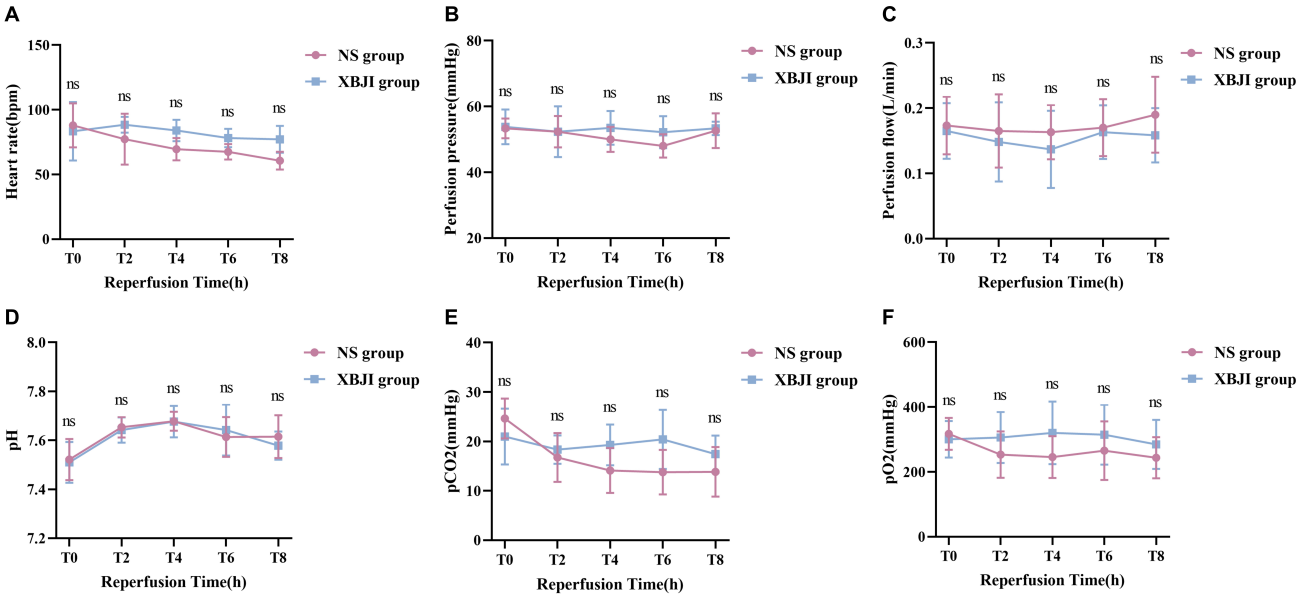

Fig. 2. The effect of Xuebijing injection group (XBJI) on heart rate (A), perfusion pressure (B), perfusion flow rate (C), pH (D), pCO_2 (E), and pO_2 (F) during *ex vivo* heart perfusion at T0, T2, T4, T6, and T8 (n = 6). Data are presented as the $\bar{x} \pm s$. $^{ns}p > 0.05$ *vs.* the normal saline (NS) group.

was employed. A p value < 0.05 was considered statistically significant.

Results

Comparison of Hemodynamics and Blood Gas Indexes between the Two Groups of the ECMO Isolated Heart Perfusion Model

The differences in heart rate, perfusion pressure, perfusion flow rate, pH, pCO_2, and pO_2 at each time point between the two groups were not statistically significant ($p >$ 0.05), as shown in Fig. 2.

XBJI Improves Myocardial Inflammation in Isolated Heart Perfusion

At T4, T6, and T8, the levels of IL-1β, IL-6, and TNF-α in the XBJI group were significantly lower than those in the NS group ($p < 0.05$). Additionally, at T6 and T8, the levels of IL-8 in the XBJI group were significantly lower than those in the NS group ($p < 0.05$), as shown in Fig. 3.

XBJI Improves the Level of Myocardial Injury Markers in Isolated Heart Perfusion

The levels of cTnT, CK, CK-MB, and LDH in the XBJI group were significantly lower than those in the NS group at T4, T6, and T8, and the difference was statistically significant ($p < 0.05$), as shown in Fig. 4.

XBJI Activates the PI3K/AKT Signaling Pathway in Myocardial Tissue of the Isolated Heart Perfusion Model

As shown in Fig. 5A–C, compared with those in the NS group, the p-PI3K/PI3K and p-AKT/AKT ratios at T4 and T8 in the XBJI group were significantly increased, and the difference was statistically significant ($p < 0.05$). XBJI upregulated the expression levels of p-PI3K and p-AKT proteins in myocardial tissue. As shown in Fig. 5D,E, compared with that in the NS group, the relative expression of PI3K and AKT mRNA in the XBJI group at T8 was significantly increased, and the difference was statistically significant ($p < 0.001$) (Fig. 5).

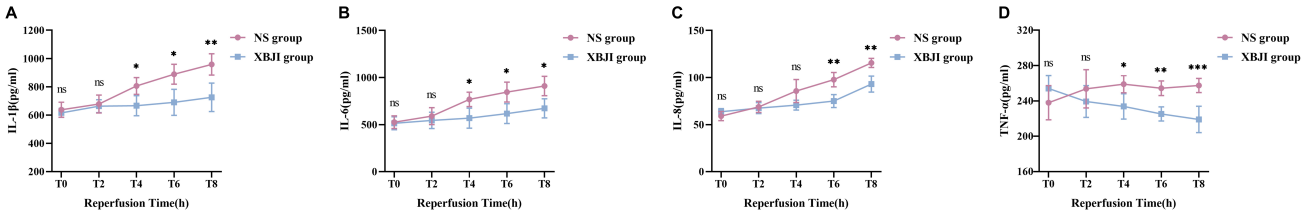

Fig. 3. The impact of XBJI on serum inflammatory factors interleukin (IL)-1β (A), IL-6 (B), IL-8 (C), and tumor necrosis factor-α (TNF-α) (D) during *ex vivo* heart perfusion at T0, T2, T4, T6, and T8 (n = 6). Data are presented as $\bar{x} \pm s$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* the NS group. ns indicates $p \geq 0.05$ *vs.* the NS group. The levels of serum inflammatory factors were detected by ELISA.

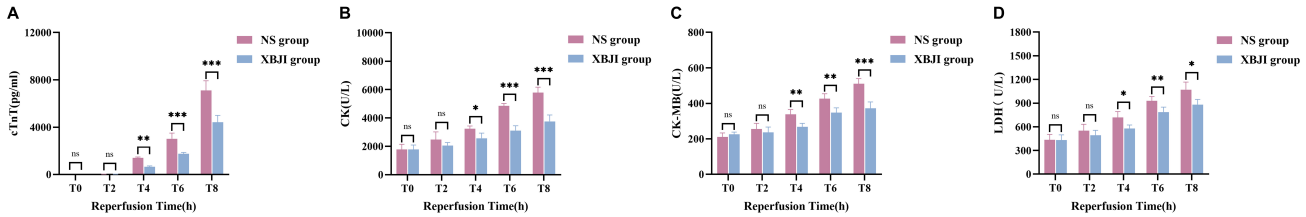

Fig. 4. The effect of XBJI on serum inflammatory factors myocardial injury markers troponin T (cTnT) (A), creatine kinase (CK) (B), creatine kinase isoenzymes (CK-MB) (C), and lactic dehydrogenase (LDH) (D) at T0, T2, T4, T6, and T8 after isolated heart perfusion (n = 6). Data are presented as $\bar{x} \pm s$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* the NS group. ns indicates $p \geq 0.05$ *vs.* the NS group. The levels of serum myocardial injury markers were detected by automatic biochemical analyzer.

XBJI Alleviates Myocardial Tissue Structural Damage

As shown in Fig. 6A, the HE staining results showed that T8 myocardial fibers in the NS group were irregularly arranged; some myocardial fibers were broken, and myocardial interstitial edema was observed, along with a small amount of inflammatory cell infiltration. In the XBJI group, the T8 myocardial fibers were arranged neatly, the structure was clear, and the myocardial tissue morphology was basically normal. As shown in Fig. 6B, transmission electron microscopy scans revealed significant degenerative changes in the myocardial tissue of the NS group, characterized by moderate to severe degeneration, including intracellular matrix dissolution; disorganized arrangement, extensive fragmentation, and fusion of muscle fibers; blurred sarcomeric structures; severe mitochondrial swelling with uneven sizes; membrane dissolution and damage; extensive fragmentation and disordered arrangement of cristae; dissolution of matrix presenting vacuolation; and dissolution of Z lines and H bands. In contrast, myocardial cells in the XBJI group exhibited mild hypertrophic changes with relatively minor cellular structural damage, uniform intracellular matrix, orderly arrangement of muscle fibers, clear sarcomeric structures, mild mitochondrial swelling with uniform sizes, and well-arranged Z lines and H bands.

Discussion

Cardiovascular diseases (CVDs) are the most common cause of death in member countries of the European Society of Cardiology (ESC), characterized by high morbidity and mortality. Ischemic heart disease (IHD), which accounts for 45% of female deaths and 39% of male deaths, is one of the major threats to global health [6]. With myocardial ischemia at 1–5 min, 5–20 min, and more than 20 min after reperfusion, there will be arrhythmia, myocardial stunning, myocardial infarction, and three different degrees of MIRI. Arrhythmia manifests as ventricular tachycardia or ventricular fibrillation. The primary feature of myocardial stunning is reversible ventricular systolic dysfunction. Myocardial infarction can cause myocardial cell apoptosis and necrosis and other irreversible damage [7]. Although we strictly maintained the time of myocardial ischemia within 20 min, MIRI is inevitable.

Hydroxysafflor yellow A (HSYA), the main active ingredient of safflower, has the effects of dredging collaterals and activating blood, removing blood stasis, relieving pain, anti-inflammation, and anti-oxidation [8]. Studies have shown that HSYA can reduce serum levels of inflammatory factors and myocardial enzyme levels [9]. By activating the PI3K/AKT/hexokinase II pathway, HSYA restores mitochondrial energy metabolism, reduces the production of reactive oxygen species (ROS), increases Ca^{2+} uptake by calcium stores, alleviates oxidative stress, and inhibits apoptosis [10]. Salvia miltiorrhiza has the effects of dilating blood vessels, improving microcirculation, and anti-inflammation [11]. It can reduce the expression of pro-inflammatory factors such as TNF-α, IL-6, and IL-β [12]. Danshen-honghua herb pair (DHHP) improves MIRI by inhibiting the opening of the mitochondrial permeability tran-

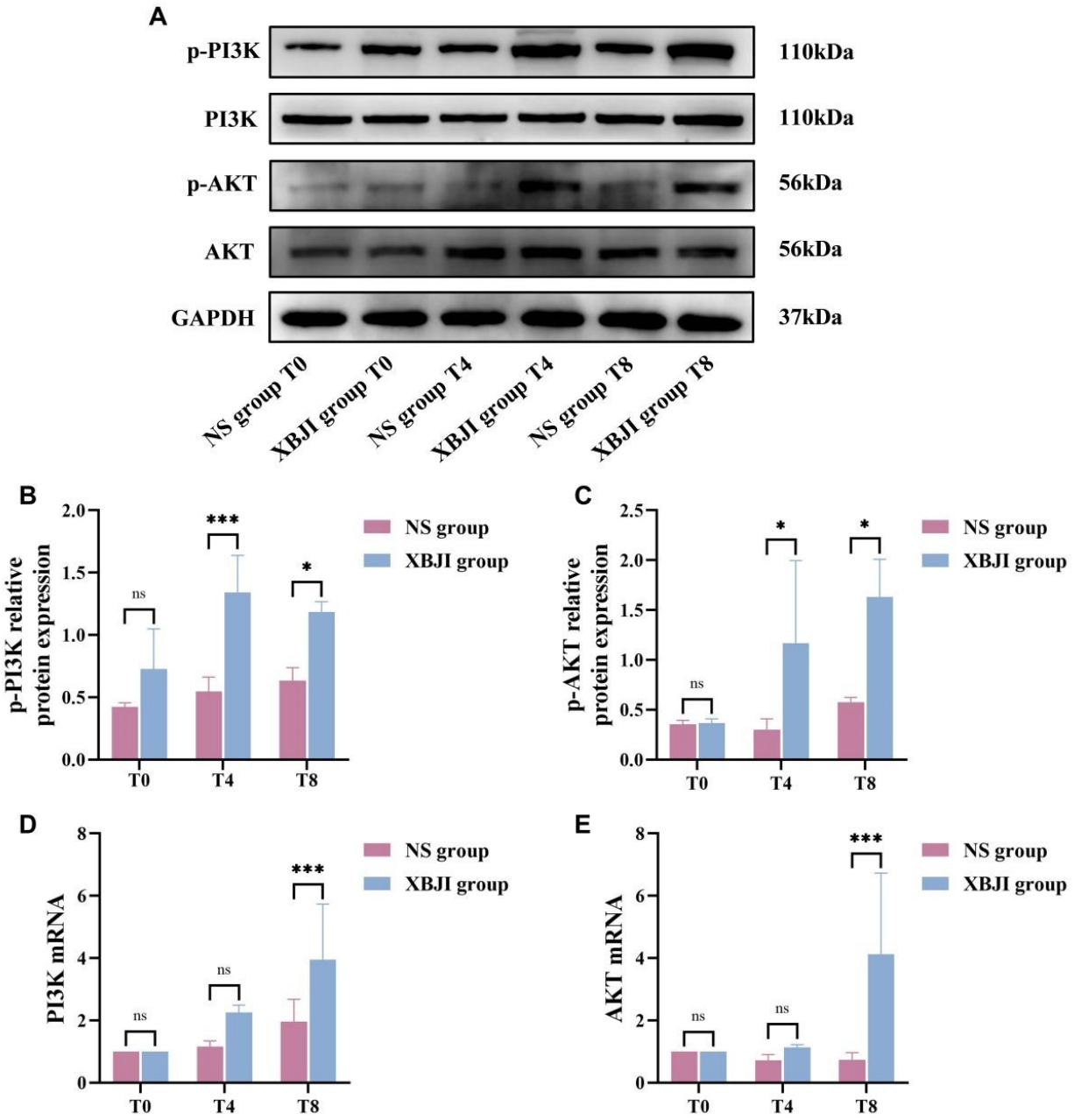

Fig. 5. The effect of XBJI on the phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/AKT) signaling pathway in myocardial tissue at T0, T2, T4, T6, and T8 after isolated heart perfusion. (A) Western blot representative images of p-PI3K, PI3K, p-AKT, and AKT. (B) Relative expression of p-PI3K. (C) Relative expression of p-AKT. (D) PI3K mRNA relative expression. (E) AKT mRNA relative expression (n = 3). Data are presented as $\bar{x} \pm s$. * $p < 0.05$, *** $p < 0.001$ *vs.* the NS group. ns indicates $p \geq 0.05$ *vs.* the NS group.

sition pore (mPTP), reducing ischemic injury, and inhibiting cardiomyocyte apoptosis [13]. Other active ingredients in XBJI, such as paeoniflorin, ferulic acid, and ligustrazine, also have the effects of anti–oxidative stress and myocardial inflammation inhibition [14,15]. The results of this study showed that the expression levels of TNF-α, IL-6, IL-8, IL-β, CK, CK-MB, LDH, and cTnT in the NS group were significantly increased in a time-dependent manner, and there was a significant difference between the NS group and the XBJI group. In contrast, XBJI significantly improved myocardial inflammation and alleviated myocardial injury in a time-dependent manner.

The PI3K/AKT signaling pathway is activated in the early stage of myocardial ischemia and hypoxia, which is an important cascade signaling pathway for the prevention and treatment of MIRI. Numerous studies have shown that

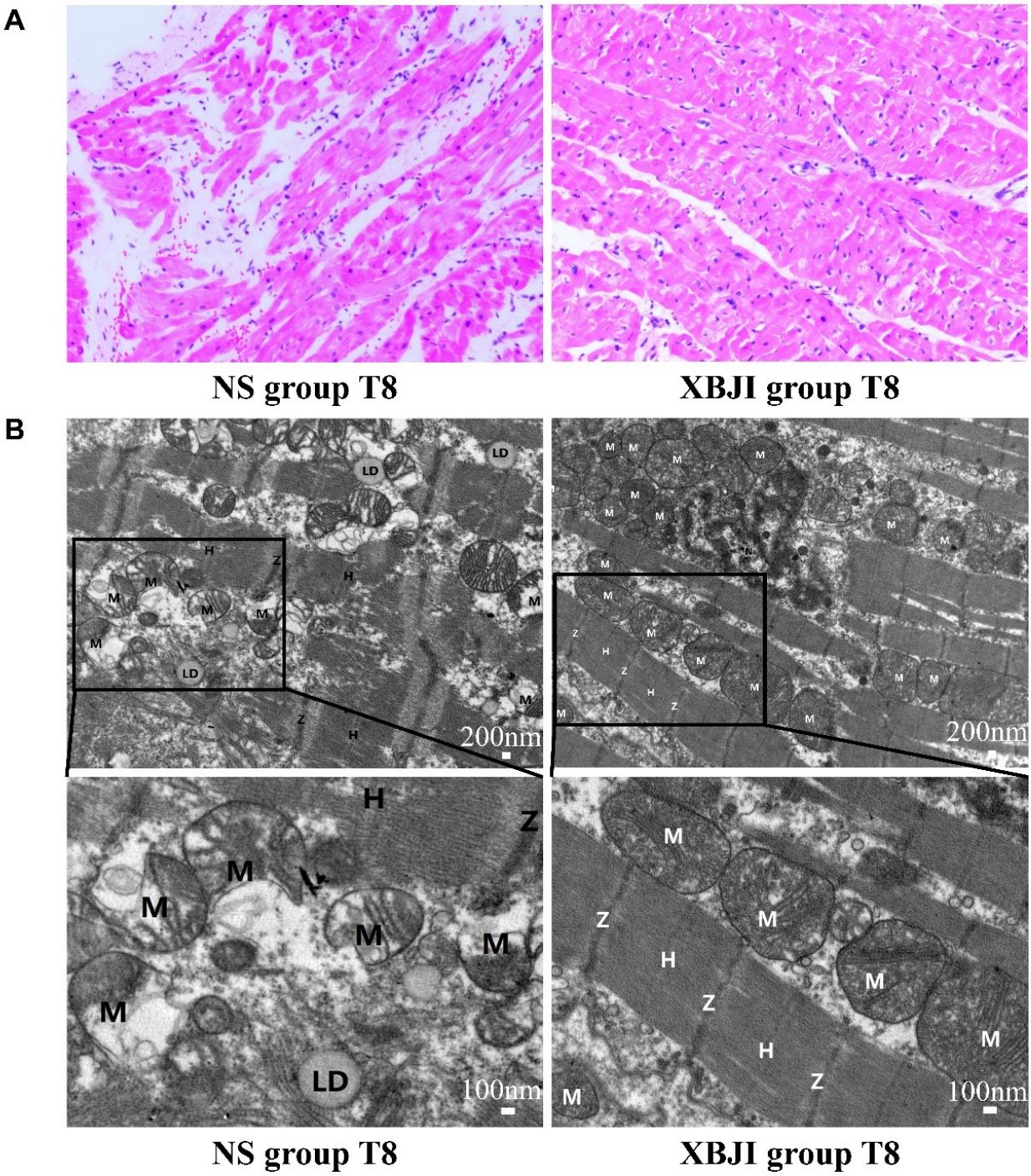

Fig. 6. The effects of XBJI on the ultrastructure and pathological morphology of T8 myocardial tissue in the two groups. (A) Hematoxylin and eosin (×200) staining for myocardial histopathological analysis (n = 3). (B) Transmission electron microscopy (×25.0 k) was used to observe the effect of XBJI on myocardial ultrastructure and mitochondrial damage in the isolated heart perfusion model (n = 3).

[16–18] the PI3K/AKT signaling pathway is involved in the regulation of myocardial autophagy and apoptosis, reducing ischemia–reperfusion injury. Xuebijing has also been shown to have a good protective effect against myocardial injury in sepsis after continuous treatment. It is speculated that in the early stage of sepsis, it may activate the PI3K/AKT/mTOR pathway, inhibiting apoptosis and promoting autophagy; in the late stage of sepsis, it may inhibit the PI3K/AKT/mTOR pathway, promoting apoptosis and inhibiting autophagy [4]. In this study, compared with those in the NS group, the phosphorylation levels of PI3K and AKT proteins in the myocardial tissue of the XBJI group

were significantly increased at T4 and T8, suggesting that Xuebijing may activate the PI3K/AKT signaling pathway and thus exert a myocardial protective effect on ECMO preservation of isolated empty beating hearts and reduce myocardial injury.

The heart is a high energy–demanding organ, and mitochondria account for about 35% of the volume of adult cardiomyocytes. Mitochondria are cardiac protective organelles and key effectors involved in MIRI. After MIRI, mitochondria produce a large amount of ROS, which hinders the production of ATP, and together with a Ca^{2+} imbalance, induces the continuous opening of mPTP, leading to mitochondrial swelling, structural changes and dysfunction, and autophagy disorders. This eventually leads to cell necrosis and apoptosis, which in turn induces myocardial injury [19,20]. The results of pathology and transmission electron microscopy in this study showed that the degree of mitochondrial and myocardial injury in the ventricular myocardium of the NS group was more serious than that of the XBJI group. It can be seen that XBJI can effectively reduce mitochondrial and myocardial cell injury.

The miniature pig heart has similar advantages to the human heart structure. As a traditional Chinese medicine intravenous preparation, XBJI has a certain clinical effect on cardiovascular and cerebrovascular diseases and lung diseases. However, this *in vitro* perfusion model has some limitations. First, this study only maintained the beating heart for 8 h. In the future, the perfusion conditions should be optimized, a feasible scheme with a longer duration should be implemented, and the deeper myocardial protection mechanism should be verified by the effectiveness of clinical practice. Second, myocardial systolic and diastolic functions, such as left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP), were not fully evaluated. Third, the dose-effect relationship of XBJI in ECMO donor heart preservation was not explored, and the optimal dose was not determined. Fourth, the specific mechanism by which XBJI reduces mitochondrial damage remains unclear.

This study primarily focused on assessing the myocardial protective effects of XBJI in an ECMO-exposed *ex vivo* heart model. Due to the limited timeframe of the experiment and the scope of the original design, we did not evaluate the total protein levels of PI3K and AKT at multiple time points, nor did we directly assess the oxidative stress response. Additionally, the potential side effects of XBJI on the heart at baseline and its role in oxidative stress during ECMO perfusion were not explored in this study. Future research should include these assessments to provide a more comprehensive understanding of XBJI's mechanisms and safety profile. These additional experiments would help elucidate the full range of effects and validate the findings reported here.

Traditional Chinese medicine has multi-target and multi-channel characteristics, as well as a variety of pharmacological activities. In the future, we should further study the pharmacodynamic mechanism of traditional Chinese medicine and optimize the perfusion formula, promote the development of safer and more effective combination strategies of traditional Chinese medicine and modern treatment, and optimize the perioperative management of patients undergoing heart transplantation. In conclusion, our study has opened up a new path for the development of auxiliary means for donor heart preservation. We expect that improving donor heart preservation will improve the clinical prognosis of patients undergoing heart transplantation, and further study is warranted.

Conclusion

Our study found that there was no statistically significant difference in heart rate, perfusion pressure, perfusion flow rate, pH, pCO_2, and pO_2 at each time point between the two groups. XBJI can reduce myocardial inflammation and myocardial cell and mitochondrial damage in isolated heart perfusion. XBJI may play a role in the myocardial protection of ECMO isolated heart perfusion by activating the PI3K/AKT pathway.

Availability of Data and Materials

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

Author Contributions

SY: Concept/Design, Drafting article. XY: Data Analysis/interpretation, Drafting article, Statistics, Data collection. CW: Data analysis/interpretation, Drafting article. QH: Data collection. WW: The acquisition, analysis, or interpretation of data for the work. GQ: Data collection. LL: Data collection. GH: Substantial contributions to the conception and design of the work, Critical revision of article, Approval of articles. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The animal experiment was approved by the Ethics Committee of Guangxi University of Chinese Medicine (No. DW20190305-047).

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Conflict of Interest

The authors declare no conflict of interest.

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