Received: 27 November 2020 | Accepted: 31 August 2021

DOI: 10.1113/EP089289

Experimental Physiology WILEY

RESEARCH PAPER

Remote limb ischaemic conditioning produces cardioprotection in rats with testicular ischaemia–reperfusion injury

Zhibing Yan[1] | Lei Du[2] | Quanhua Liu[1] | Leng Zhou[2] | Zhaoyang Hu[1]

[1] Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China

[2] Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China

Correspondence
Zhaoyang Hu, Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, Sichuan, China.
Email: zyhu@hotmail.com

Funding information
Sichuan University West China Hospital setup funding to ZH

Edited by: Jason Peart

Abstract

Remote ischaemic conditioning can protect hearts against arrhythmia. Testicular ischaemia–reperfusion (TI/R) injury is associated with electrocardiographic abnormalities. We investigated the effect of remote limb ischaemia preconditioning (RIPre) and postconditioning (RIPost) on arrhythmogenesis in TI/R rats, and determined the potential role of c-Jun N-terminal kinase (JNK)/connexin 43 (Cx43) signalling. Rats were randomized to sham-operated, control, TI/R, RIPre and RIPost groups. TI/R rats were more predisposed to myocardial reperfusion-induced atrioventricular block (AVB). RIPre and RIPost reduced the incidence of sudden cardiac death (SCD) or AVB, and duration of ventricular tachyarrhythmias during myocardial reperfusion. RIPre and RIPost decreased myocardial I/R-induced phosphorylation level of JNK, while preserving myocardial Cx43 expression in TI/R rats. Taken together, TI/R rats were predisposed to myocardial reperfusion-induced AVB. RIPre and RIPost protected TI/R hearts against ischaemia-provoked ventricular arrhythmia and ultimately reduced the incidence of SCD by suppressing JNK activation and restoring Cx43 expression.

KEYWORDS
arrhythmia, JNK/Cx43 signalling pathway, remote limb ischaemic postconditioning, remote limb ischaemic preconditioning, testicular ischaemia–reperfusion injury

1 | INTRODUCTION

Testicular torsion is a medical emergency (Pentyala et al., 2001), but the re-establishment of blood flow is an ischaemia–reperfusion (I/R) process of the testis which may cause seminiferous tubular injury (Arena et al., 2017). Besides this reperfusion-induced testicular damage, including disturbance in hormone release, decreased spermatogenesis and induction of infertility, testicular reperfusion has recently been found to cause electrocardiographic abnormalities (Hamed et al., 2011). It is well recognized that a low testosterone level is associated with higher risk of various heart diseases, including arrhythmia (Goodale et al., 2017). A study has also shown that changes with electrical consequences for cardiac myocytes may partly be due to the reperfusion-induced reduction of testosterone secretion (Turner et al., 2005). Although clinical intervention recovers the testicular vascular blood flow after reperfusion, it is unknown whether the body is more susceptible to lethal arrhythmias after torsion repair. Therefore, understanding the pathological role of testicular I/R (TI/R) injury-induced cardiac abnormality is of great importance for patients with testicular torsion in the perioperative period. In the current study, we artificially provoked a lethal arrhythmia event in a model of TI/R, and examined the pathogenesis of TI/R-related arrhythmogenesis and susceptibility to sudden cardiac death (SCD). In addition, further identification of therapeutic approaches to improve myocardial

tolerance would facilitate the optimization of a clinical therapeutic strategy at the point of care.

Murry et al. (1986) demonstrated that ischaemia of shorter duration to the heart afforded protection against sustained I/R-induced injury. This concept of ischaemic preconditioning has been expanded to include remote ischaemic preconditioning (RIPre) (Przyklenk et al., 1993) or postconditioning (RIPost) (Aimo et al., 2015), both of which are treatment strategies in which brief episodes of I/R stimuli applied in remote tissues or organs can exert cardioprotection against the harmful effects of acute I/R injury. It has been shown that RIPre and RIPost limited infarction (White et al., 2015; Yang *et al*., 2017) or exerted antiarrhythmic activity (Hu et al., 2016); however, TI/R injury may generate electrical substrates that predispose the body to lethal cardiac rhythm disturbances, and whether remote conditioning may enhance myocardial tolerance to artificially induced lethal arrhythmia post-TI/R is incompletely understood.

Remote conditioning, especially limb conditioning, is a non-invasive, clinically feasible strategy, but its underlying endogenous cardioprotective mechanisms remain to be fully elucidated. Remote ischaemic preconditioning and postconditioning may share similar signal transduction pathways. Studies have demonstrated the roles of several intrinsic signalling molecules as potential signalling components in cardioprotective signalling pathways, including the stress-activated protein kinase/c-Jun N-terminal kinase (JNK), which belongs to the mitogen-activated protein kinase (MAPK) superfamily and can be activated in response to stress stimuli such as I/R by phosphorylation of amino-terminal residues (Rose et al., 2010). Recent data also support that pharmacological activation of JNK leads to the development of atrial arrhythmia (Yan et al., 2013). Moreover, it has been shown that the inhibition of JNK decreased cardiomyocyte apoptosis and limited infarct size after myocardial I/R injury (Ferrandi et al., 2004). Nevertheless, it is unknown if the beneficial effect of remote conditioning on myocardial I/R-induced ventricular arrhythmia post-TI/R results from alternation of the JNK signalling molecule. Connexin 43 (Cx43) is the predominant ventricular gap junction protein and plays an important role in maintaining cardiac rhythm. It has been well recognized that suppression of the expression of Cx43 or disruption the physical structure of Cx43 is associated with the occurrence and development of cardiac arrhythmia (Michela et al., 2015). Furthermore, there is a growing body of evidence indicating that the activation of JNK causes Cx43 remodelling and therefore leads to cardiac conduction disturbances (Petrich et al., 2004). However, the exact roles of JNK and Cx43 in remote ischaemic conditioning-induced cardioprotection in TI/R rats are unclear.

Here our aims were, first, to determine whether TI/R predisposes to arrhythmia induced by myocardial I/R injury; second, to determine whether remote preconditioning or postconditioning of the limb exerts an anti-arrhythmic effect and reduces predisposition to SCD post-TI/R; and third, to identify whether this effect is associated with JNK/Cx43-dependent signalling pathways.

New Findings

- **What is the central question of this study?**
  Can remote limb ischaemic conditioning produce cardioprotection in rats with testicular ischaemia–reperfusion injury?
- **What is the main finding and its importance?**
  Testicular ischaemia–reperfusion (TI/R)-injured rats were predisposed to myocardial reperfusion-induced atrioventricular block. Remote limb ischaemia preconditioning and postconditioning protected TI/R hearts against ischaemia-provoked ventricular arrhythmia and ultimately reduced the incidence of sudden cardiac death, with a possible role of c-Jun N-terminal kinase inhibition and connexin 43 activation.

2 | METHODS

2.1 | Ethical approval

The experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University (Approval No. 2015035A) and conformed to the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (8th edition, 2011). All experiments comply with the ethical principles and regulations as described in the Editorial by Grundy (2015).

2.1.1 | Animals

Sprague–Dawley male rats weighing 220–250 g were purchased from Chengdu Dashuo Experimental Animal Research Center (Chengdu, China). All rats were housed with a 12 h light–dark cycle in a pathogen-free animal facility. The controlled temperature was 20–25°C and the relative humidity was 60 ± 5%. Rats were free to eat and drink before the start of the experiments.

2.1.2 | Experimental protocol

A total of 46 rats were used in the study. Rats were randomly divided into five groups, as shown in Figure 1. In the sham-operated group (sham), the femoral arteries, right testis and left main coronary artery were exposed, but there was no additional intervention (n = 5). In the control group (CON), the femoral arteries and right testis were exposed, and the coronary artery was occluded for 5 min followed

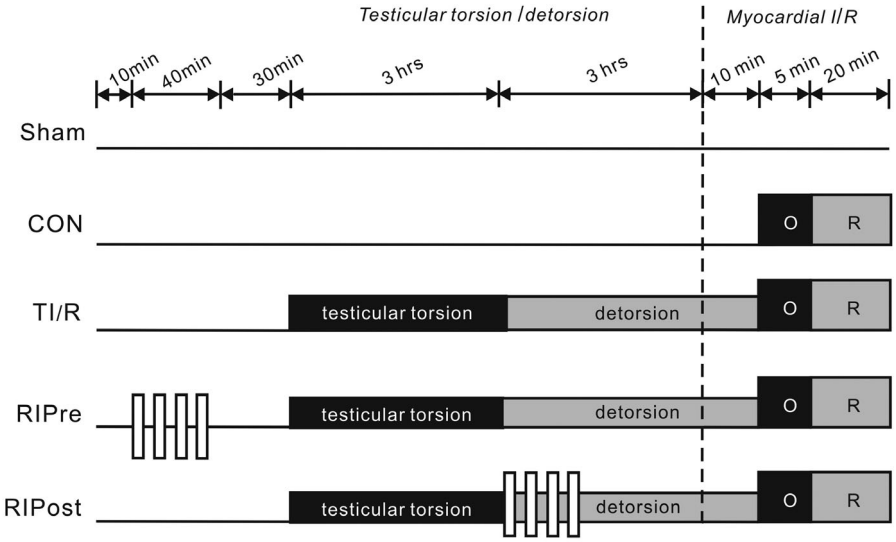

FIGURE 1 Experimental protocols. All rats were randomly divided into five groups: (1) sham group (sham, no intervention); (2) control group (CON, no testis intervention), (3) testicular torsion (ischaemia)/detorsion (reperfusion) group (TI/R), (4) testicular torsion/detorsion+remote ischaemia preconditioning group (RIPre), (5) testicular torsion/detorsion+remote ischaemia postconditioning group (RIPost). All groups were subjected to 5-min left main coronary artery occlusion followed by 20-min reperfusion except for the sham-operated group. TI/R group and RIPre/RIPost-treated TI/R groups had 3 h of testicular torsion (testicular ischaemia), followed by 3 h of testicular detorsion (testicular reperfusion) before myocardial ischaemia. Four cycles of 5-min bilateral hindlimb ischaemia followed by 5-min reperfusion were used for remote limb ischaemic preconditioning (30 min before testicular torsion, RIPre) or postconditioning (at the onset of testicular reperfusion, RIPost). Hearts were taken at 20 min for protein phosphorylation analysis post-myocardial reperfusion. Before myocardial ischaemia, rats were acclimatized to the invasive procedures and instrumentation for 10 min

by 20 min of reperfusion ($n = 14$). All other rats in the TI/R injury group (TI/R, $n = 10$), remote ischaemic preconditioning group (RIPre, $n = 9$), or remote ischaemic postconditioning group (RIPost, $n = 8$) had 3 h of testicular ischaemia and 3 h of testicular reperfusion, followed by another 5 min of coronary artery occlusion with 20 min of reperfusion. Death was characterized by discontinuation of respiration and cardiac activity. The coronary artery was then retightened. One per cent Evans Blue (Sigma-Aldrich, St Louis, MO, USA) was injected into the left ventricular cavity to identify the non-ischaemic portion of the heart. The areas of myocardium at risk (AAR) within the left ventricle were harvested and then stored at −80°C in a freezer until further use.

2.1.3 | Surgical procedures

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, I.P. injection). The depth of anaesthesia was controlled by monitoring the pedal reflex and pinna reflex. Rats were given additional sodium pentobarbital (20 mg/kg, I.P.) every 30 min throughout the experiment. Nalbuphine (2 mg/kg, S.C.) was used for analgesia.

2.2 | Testicular ischaemia–reperfusion injury model

After anaesthesia, rats were placed in a fixed position and were subjected to 10 min stabilization. Needle electrodes were attached subcutaneously to each rat, and ECG was recorded in limb lead II throughout the experiment. A 1 cm mid-scrotal vertical incisions was performed along the linea alba to expose the right tunica. Another midline incision in the tunica was made afterwards. The right testis and spermatic cord were then exposed, isolated and taken out through the incision. The testis was rotated 720 degrees in a clockwise direction around the longitudinal axis of the spermatic cord for 3 h to induce testicular ischaemia. The right testis was maintained in the torted position and was fixed to the scrotal wall with a 5-0 silk suture passing through the tunica dartos muscle. The testis was placed on top of the incision and was covered with a sterile gauze pad with sterile isotonic saline. After 3 h of testicular ischaemia, the fixation sutures were cut off, and the right testis was detorted and replaced in its natural position for 3 h of reperfusion. Body temperature was maintained using a heating blanket.

2.3 | Induction of remote ischaemic conditioning

A longitudinal skin incision was performed in the femoral region. The femoral arteries were isolated at the proximal position from the femoral neurovascular bundle near the groin. Ischemic conditioning treatment was initiated either 30 min ahead of TI/R (RIPre) or at the onset of testicular reperfusion (RIPost) by four cycles of 5-min occlusion and subsequent 5-min reperfusion of the bilateral femoral arteries. Successful limb ischaemia was confirmed by changes in limb skin colour and temperature. After limb reperfusion, skin

temperature of the lower limbs returned to baseline and the underskin area regained normal skin coloration.

2.4 | Myocardial reperfusion-induced ventricular arrhythmia

Details of the surgical procedures have been described previously (Hu et al., 2014). Briefly, at the end of 3 h of testicular reperfusion, a tracheostomy was performed and the trachea was exposed. Lungs were mechanically ventilated by connecting the tracheal tubing to a rodent ventilator (Chengdu Taimeng Technology Co., Ltd, Chengdu, China). The tidal volume was set at 8 ml, frequency was 80 times per minute, and expiration:inspiration was 5:4. The chest of each rat was opened via a lateral left-sided thoracotomy. The heart was exposed and a segment of the proximal main left coronary artery (MLCA) was identified. After pericardectomy, a 6-0 silk ligature (Ethicon, Somerville, NJ, USA) was placed around the MLCA for the production of coronary artery occlusion and reperfusion. Proper ligation of the MLCA was verified by observing blanching and dyskinesia of the ischaemic myocardium distal to the occlusion. The suture was loosened after 5 min of ischaemia to allow myocardial reperfusion for 20 min. Reperfusion was confirmed by visual observation of epicardial hyperaemia. At the end of myocardial reperfusion, surviving animals were killed with an overdose of sodium pentobarbital (200 mg/kg, I.P.).

2.4.1 | Electrocardiography and arrhythmia analysis

A standard limb Lead II was used to record and analyse electrocardiograms in rats during the experiment using a PowerLab/8sp system (ADInstruments, Colorado Springs, CO, USA). Data were acquired and analysed offline by LabChart 7.2.1 software (ADInstruments). Incidence of arrhythmias during the myocardial reperfusion period was recorded and analysed (CON: $n = 14$; TI/R: $n = 10$; RIPre: $n = 9$; RIPost: $n = 8$), such as ventricular tachycardia (VT), sustained VT (>1 min) (SVT), polymorphic VT (PVT) and other types of arrhythmias, including atrioventricular block (AVB), SCD and ventricular fibrillation (VF). We also recorded durations of VT and longest episode of VT (LVT), as well as the start time of the first run of VT.

2.4.2 | Serum tests

At the end of the experiment, blood samples were taken from the heart followed by centrifugation (1000 *g*, 10 min, 4°C). Levels of lactate dehydrogenase (LDH, $n = 5–6$ per group), creatine kinase MB (CK-MB, $n = 5–7$ per group) and α-hydroxybutyrate dehydrogenase (α-HBDH, $n = 5–7$ per group) were measured with a BS-120 biochemical analyser (Mindray, Shenzhen, China). An enzyme-linked immunosorbent assay (ELISA) kit was used to detect the serum cardiac troponin I (cTnI) level (Quanzhou Ruixin Biological Technology Co., Ltd, China).

2.4.3 | Testosterone measurements

Serum quantification of total testosterone ($n = 5–7$ per group) was carried out in duplicate using ELISA according to manufacturer's instruction (Quanzhou Ruixin Biological Technology Co.).

2.4.4 | Western blotting

Protein was extracted from the heart tissue by homogenizing samples in ice-cold RIPA buffer containing 50 mM Tris–HCl (pH7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.25% sodium deoxycholate, phosphatase inhibitor cocktail (Sigma-Aldrich), and a protease inhibitor cocktail (Sigma-Aldrich), followed by two cycles of centrifugation at 10,000 *g* for 10 min at 4°C ($n = 4–5$ per group). Protein concentration was determined with a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein from each sample were separated by 12% SDS-PAGE and subsequently transferred to a nitrocellulose membrane (BioTrace NT, Pall Corp., Port Washington, NY, USA). The membrane was blocked with 5% non-fat dry milk in phosphate buffered saline and 0.1% Tween-20 (PBST) for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies raised against the following: phosphorylated stress-activated protein kinase/c-Jun N-terminal kinase (Thr183/Tyr185) (p-JNK), total JNK, Cx43 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (all 1:1000 dilution, from Cell Signaling Technology, Danvers, MA, USA). The membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (all 1:5000 dilution, from Bio-Rad, Hercules, CA, USA) for 2 h at room temperature and finally visualized using a chemiluminescence ECL detection system (Millipore, Billerica, MA, USA). Images were captured using an Amersham Imager 600 system (GE Healthcare, Little Chalfont, UK). The processing and quantification of protein bands densities were performed using ImageJ data acquisition software (National Institutes of Health, Bethesda, MD, USA)

2.4.5 | Statistics

All data were analysed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as the mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to test for normality. For two independent groups, an unpaired Student's *t*-test (two-tailed) was used to determine significance. Fisher's exact test was used to calculate categorical variables, that is, the numbers of rats falling into one of two groups. Homogeneity of variance was tested by Levene's test. One-way analysis of variance followed by the Newman–Keuls test was used to compare the data of three or more groups if variances were equal. Dunnett's T3 *post hoc* test was used if variances were not homogeneous. $P < 0.05$ was considered statistically significant.

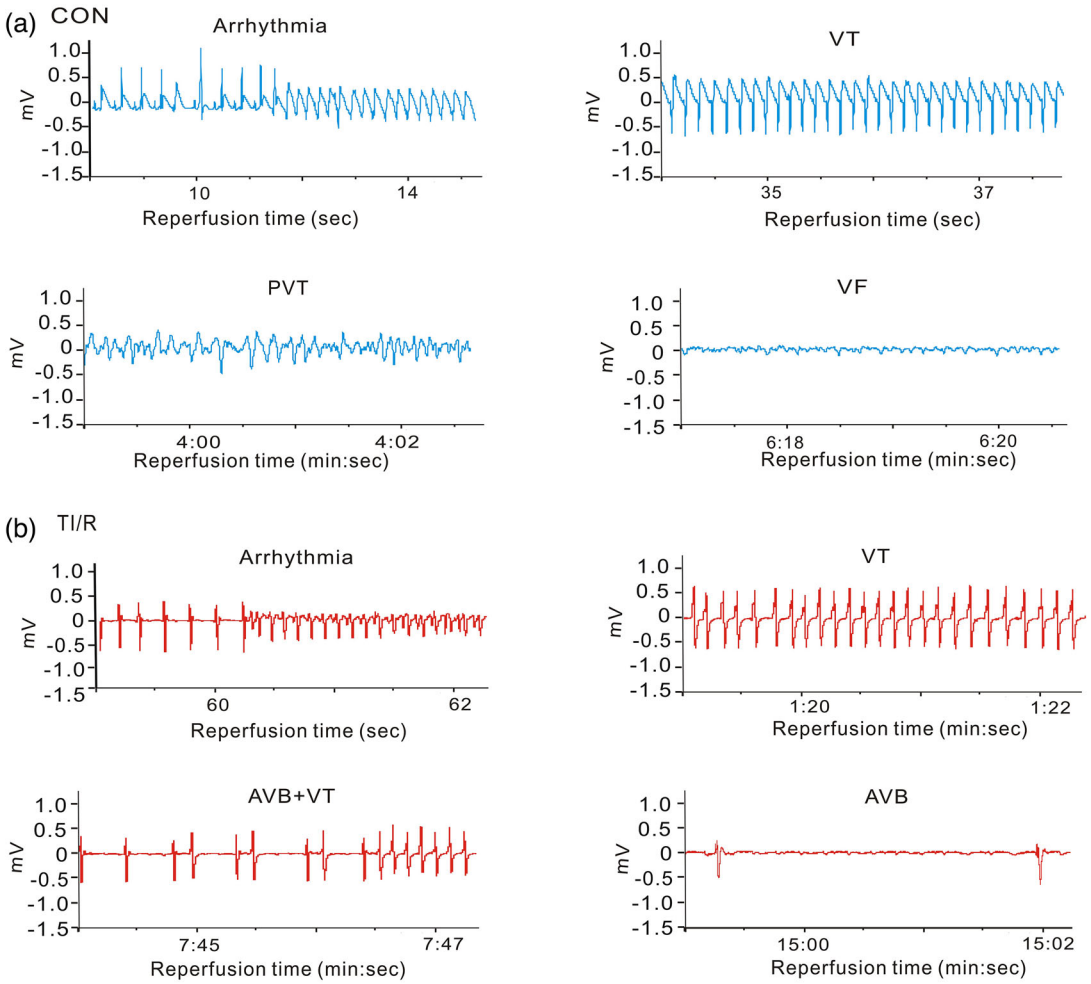

FIGURE 2 The influence of testicular I/R injury on myocardial I/R injury-induced ventricular arrhythmias. (a) Representative ECG traces from CON rats during myocardial reperfusion period. Arrhythmia, showing the first run of VT. (b) Representative ECG traces from TI/R rats during myocardial reperfusion period. AVB, AV block; CON, control group; PVT, polymorphic VT; TI/R: testicular I/R group; VF, ventricular fibrillation; VT, ventricular tachycardia

3 | RESULTS

3.1 | Testicular I/R injury predisposes rats to cardiac reperfusion-induced AV block

We found that TI/R injury led to a reduction of testicular testosterone secretion in our study (7.1 ± 3.1 ng/ml in TI/R, $n = 7$, vs. 27.7 ± 5.4 ng/ml in sham, $n = 5$, and 27.0 ± 4.4 ng/ml in CON, $n = 6$, $P < 0.0001$), indicating impaired testicular function in TI/R rats. However, a lower testosterone level was shown to be associated with arrhythmogenesis (Magnani et al., 2014). To study the effect of TI/R injury on cardiac susceptibility to arrhythmias, myocardial reperfusion was performed in each heart to induce ventricular arrhythmia. Rats with (TI/R group) or without (CON group) TI/R injury were exposed to a 5-min main left coronary ligation followed by 20-min reperfusion. Electrocardiogram tracings are shown in Figure 2, and their analysis in Table 1. Representative classes of reperfusion-induced arrhythmia included VT, AVB, VF or SCD. During the entire 20 min reperfusion

TABLE 1 Quantification of mortality and different cardiac arrhythmia incidence after coronary artery ischaemia in CON and TI/R rats

Parameters	CON ($n = 14$)	TI/R ($n = 10$)	P
Arrhythmia	13 (92.9)	10 (100.0)	1
VT	13 (92.9)	9 (90.0)	1
AVB	7 (50.0)	10 (100.0)	0.0188
SCD	10 (71.4)	7 (70.0)	1
VF	8 (57.1)	1 (10.0)	0.0333
VT duration (s)	205.5 ± 118.5	222.8 ± 158.2	0.762
LVT duration (s)	136.9 ± 92.2	48.3 ± 43.3	0.0102
VT starting time (s)	22.31 ± 16.4	62.33 ± 41.2	0.0047

Categorical variables ae reported as n (%); continuous variables are expressed as means ± SD. P-values: CON versus TI/R rats. Abbreviations: AVB, atrioventricular block; LVT, longest VT; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

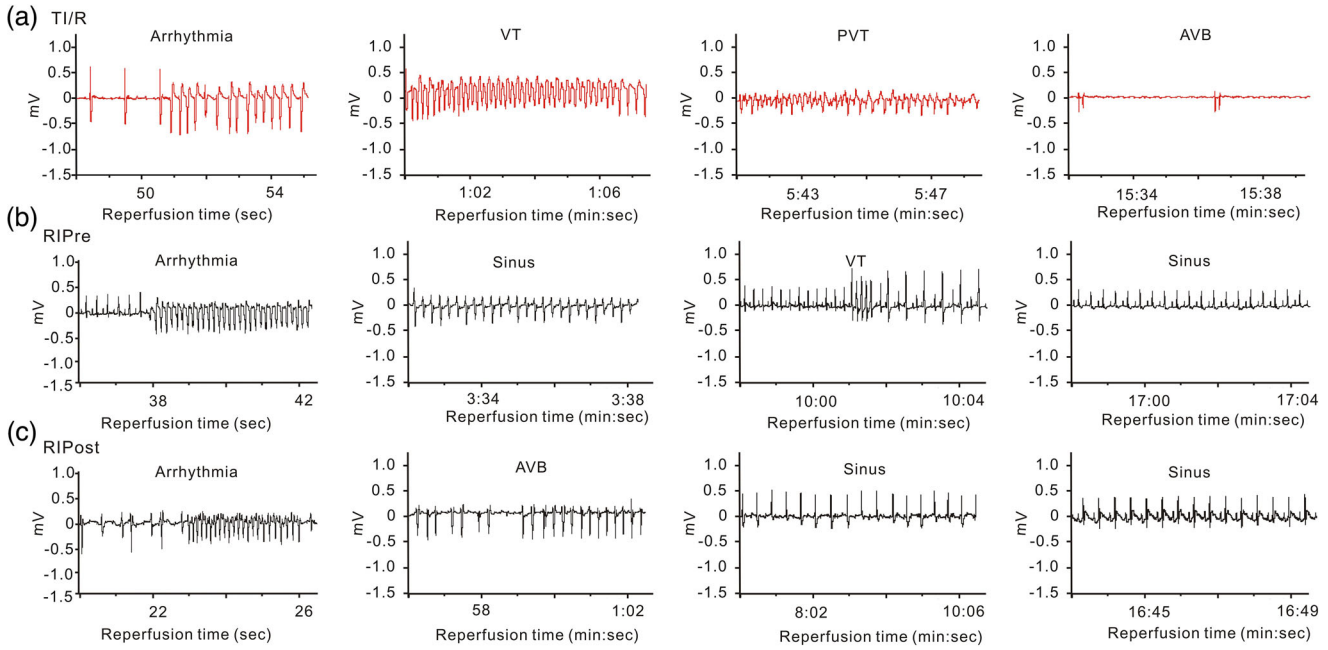

FIGURE 3 Representative ECG traces. (a) Representative ECG traces from TI/R rats during 20-min of myocardial reperfusion period. Arrhythmia, showing the first run of VT. (b) Representative ECG traces from RIPre rats during 20 min myocardial reperfusion period. (c) Representative ECG traces from RIPost rats during 20-min of myocardial reperfusion period. AVB, AV block; PVT, polymorphic VT; RIPost: testicular I/R rats with remote ischaemic postconditioning; RIPre: testicular I/R rats with remote ischaemic preconditioning; Sinus, remained in sinus rhythm; TI/R: testicular I/R group without conditioning treatment; VT, ventricular tachycardia

period, there was no significant difference in SCD incidence between CON and TI/R rats ($P = 1.000$). All control rats (14 out of 14) and TI/R rats (10 out of 10) exhibited different types of arrhythmias, switching between one other. VT was seen in most of the groups (CON: 13/14, TI/R: 9/10), but VF was more prevalent in control rats; the proportion of induced VF was almost 6-fold greater in the control group (8/14, 57.1%) than in the TI/R group (1/10, 10%, $P = 0.0333$). In contrast, TI/R rats had severe AVB after cardiac manipulation and the ratio of rats exhibiting AVB was almost doubled in the TI/R group post-myocardial I/R (10/10, 100%) when compared to the control group (7/14, 50%, $P = 0.0188$). This severity of AVB would predispose to cardiogenic shock or SCD in patients. Further comparison between these two groups revealed that the major reason for mortality was either VF or severe VT in control rats or severe AVB in TI/R rats. During the 20-min post-ischaemic reperfusion period, although mean VT durations were not different in control and TI/R groups ($P = 0.762$, Table 1), the average longest episode of VT duration was >2.8-fold longer in control rats (136.9 ± 92.2 s) compared to TI/R rats (48.3 ± 43.3s, $P = 0.0102$, Table 1). Notably, the TI/R injury prolonged the latency to first run of the VT episode after the onset of myocardial reperfusion from 22.3 ± 4.6 s in the control group to 62.3 ± 13.7 s ($P = 0.0047$, Table 1). Taken together, despite the similar SCD incidence for control and TI/R rats, lethal AVB was more common in TI/R rats during the cardiac reperfusion period, while control rats were more predisposed to longer and more severe ventricular tachycardia.

3.2 | Remote ischaemic conditioning reduces susceptibility to post-ischaemic ventricular arrhythmias in testicular-I/R rats

To characterize the influence of remote ischaemic conditioning (including RIPre and RIPost) on reperfusion-induced ventricular arrhythmia in TI/R rats, we quantified electrocardiographic phenotypes and variability in TI/R rats with or without conditioning stimuli. Rats in all three groups were exposed to a 5-min left main coronary ligation followed by 20-min reperfusion, and their representative ECG tracings are presented in Figure 3. Here, we found that although VT was still prevalent in all groups, there was a trend towards a decreased value for sustained VT (1/9, RIPre; 0/8, RIPost) or PVT (3/9, RIPre; 1/8, RIPost) incidence after conditioning treatment, compared to SVT (3/10) or PVT (5/10) in non-treated TI/R rats. TI/R rats rarely had VF after myocardial reperfusion, but the cause of death was dominated by severe AVB, as we have previously shown in Figure 2 and Table 1. Here, importantly, we found that RIPre and RIPost were highly effective in reducing SCD incidence (0 out of 9, 0% in RIPre or 0 out of 8, 0% in RIPost rats), compared to TI/R rats (7 out of 10, 70%, $P = 0.0031$ vs. RIPre, or $P = 0.004$ vs. RIPost), largely due to the reduction of AVB incidence from 100% (10/10) in TI/R rats to 44% (4/9, $P = 0.0108$ vs. TI/R rats) in RIPre, or 50% (4/8, $P = 0.0229$ vs. TI/R rats) in RIPost-treated TI/R rats (Table 2) during the entire 20 min cardiac reperfusion period. Furthermore, ischaemic conditioning had a prompt and substantial cardioprotective effect at a very early stage of

TABLE 2 Quantification of mortality and different cardiac arrhythmia incidence after coronary artery ischaemia in TI/R, RIPre and RIPost groups

Parameters	TI/R ($n = 10$)	RIPre ($n = 9$)	*P* (RIPre vs. TI/R)	RIPost ($n = 8$)	*P* (RIPost vs. TI/R)
Arrhythmia	10 (100.0)	8 (88.9)	0.474	6 (75.0)	0.1830
VT	9 (90.0)	8 (88.9)	1	6 (75.0)	0.559
SVT	3 (30.0)	1 (11.1)	0.582	0 (0)	0.216
PVT	5 (50.0)	3 (33.3)	0.650	1 (12.5)	0.152
SCD	7 (70.0)	0 (0)	0.0031	0 (0)	0.004
VF	1 (10.0)	0 (0)	1	0 (0)	1
AVB (20 min post-cardiac I/R)	10 (100.0)	4 (44.4)	0.0108	4 (50)	0.0229
AVB (10 min post-cardiac I/R)	10 (100.0)	4 (44.4)	0.0108	4 (50)	0.0229
AVB (5 min post-cardiac I/R)	10 (100.0)	2 (22.2)	0.0007	1 (12.5)	0.0003
VT starting time (s)	62.33 ± 41.2	284.3 ± 263.7	0.0245	249.8 ± 88.4	<0.001

Categorical variables are reported as *n* (%); continuous variables are expressed as mean ± SD.
Values for TI/R rats are repeated from Table 1 for comparison. Abbreviations: AVB, atrioventricular block; PVT, polymorphic VT; SCD, sudden cardiac death; SVT, sustained VT (>1 min VT); VF, ventricular fibrillation; VT, ventricular tachycardia.

cardiac reperfusion; for example, when compared with TI/R rats, both RIPre and RIPost induced highly variable decrease in AVB incidence during the first 5 min (RIPre: $P = 0.0007$ vs. TI/R; RIPost: $P = 0.0003$ vs. TI/R) or 10 min (RIPre: $P = 0.0108$ vs. TI/R; RIPost: $P = 0.0229$ vs. TI/R) reperfusion period (Table 2, $P < 0.05$ or $P < 0.01$), which was associated ultimately with lower SCD rate. At the same time, we also observed that RIPre or RIPost successfully postponed the latency to the first run of the VT episode from 62.3 ± 41.2 s in the TI/R group to 284.3 ± 263.7 s in the RIPre group ($P = 0.0345$, vs. TI/R rats) or 249.8 ± 88.4 s in the RIPost group ($P < 0.001$ vs. TI/R rats, Table 2).

We also evaluated the effects of ischaemic conditioning on VT duration and longest VT duration and found that, as expected, VT duration was about 4-fold longer in non-treated TI/R rats, compared to RIPre-treated ($P = 0.0099$) or RIPost-treated TI/R rats($P = 0.0116$). Meanwhile, TI/R rats (48.3 ± 43.3 s) had markedly longer LVT duration than that of RIPre-treated (15.2 ± 9.1 s, $P = 0.0387$ vs. TI/R rats) or RIPost-treated (7.8 ± 6.6 s, $P = 0.0194$ vs. TI/R rats) TI/R rats throughout the whole 20 min reperfusion period (Figure 4a, b). Notably, the average mean durations of VT and LVT for the rats, calculated throughout the first 5 or 10 min post-cardiac reperfusion recording period, were significantly shorter in the conditioned rats than in the non-treated TI/R rats (first 5 min VT: RIPre: $P = 0.0632$ vs. TI/R; RIPost: $P = 0.0123$ vs. TI/R; first 5 min LVT: RIPre: $P = 0.08$ vs. TI/R; RIPost: $P = 0.046$ vs. TI/R; first 10 min VT: RIPre: $P = 0.0065$ vs. TI/R; RIPost: $P = 0.012$ vs. TI/R; first 10 min LVT: RIPre: $P = 0.107$ vs. TI/R; RIPost: $P = 0.026$ vs. TI/R, Figure 4c–f).

Taken together, these results suggested that both remote ischaemic preconditioning and postconditioning have strong anti-arrhythmic effects against cardiac reperfusion-induced ventricular arrhythmias in TI/R rats. In addition, we also tested if these conditioning-induced cardioprotection effects were testosterone-dependent. However, serum testosterone levels in RIPre (8.7 ± 2.6 ng/ml, $n = 6$) and RIPost (7.4 ± 1.9 ng/ml, $n = 7$) groups were similar to that in the TI/R group (7.1 ± 3.1 ng/ml, $n = 7$, $P = 0.529$), suggesting that the conventional testosterone level was not a crucial factor in the RIPre- and RIPost-specific aspects of post-I/R cardioprotection in TI/R rats.

3.3 | Serum enzymes

The levels of LDH, CK-MB, α-HBDH and cTnI are indicators of myocardial damage. We found in our study that the serum levels of these four enzymes were elevated after myocardial reperfusion in CON, TI/R and conditioned TI/R hearts as compared to sham hearts (LDH: $P = 0.0161$; CK-MB: $P = 0.0001$; α-HBDH: $P = 0.0012$; cTnI: $P = 0.0067$), suggesting that myocardial reperfusion led to myocardial injury. However, RIPre- and RIPost-treated TI/R hearts had similar levels of LDH, CK-MB, cTnI and α-HBDH as compared to CON or TI/R groups after 20 min of myocardial reperfusion (Figure 5a, d), indicating that remote conditioning may increase the cardiac electrical stability and exert its anti-arrhythmic effect independently of myocyte salvage in TI/R rats.

3.4 | Remote ischaemic conditioning alters protein expression

Remote organ ischaemic preconditioning and postconditioning may share mutual signalling pathways during cardioprotection, and may exert a potent cardioprotective effect via activation of the JNK signalling molecule, a key component in the MAPK signalling pathway. In our study, the JNK level was normalized by its total protein level. There were significant differences among experimental groups in the level of phosphorylated JNK ($P < 0.0001$, Figure 6a). Our western blot data demonstrated that JNK phosphorylation was significantly increased in control ($P < 0.001$) and TI/R ($P < 0.001$) hearts versus

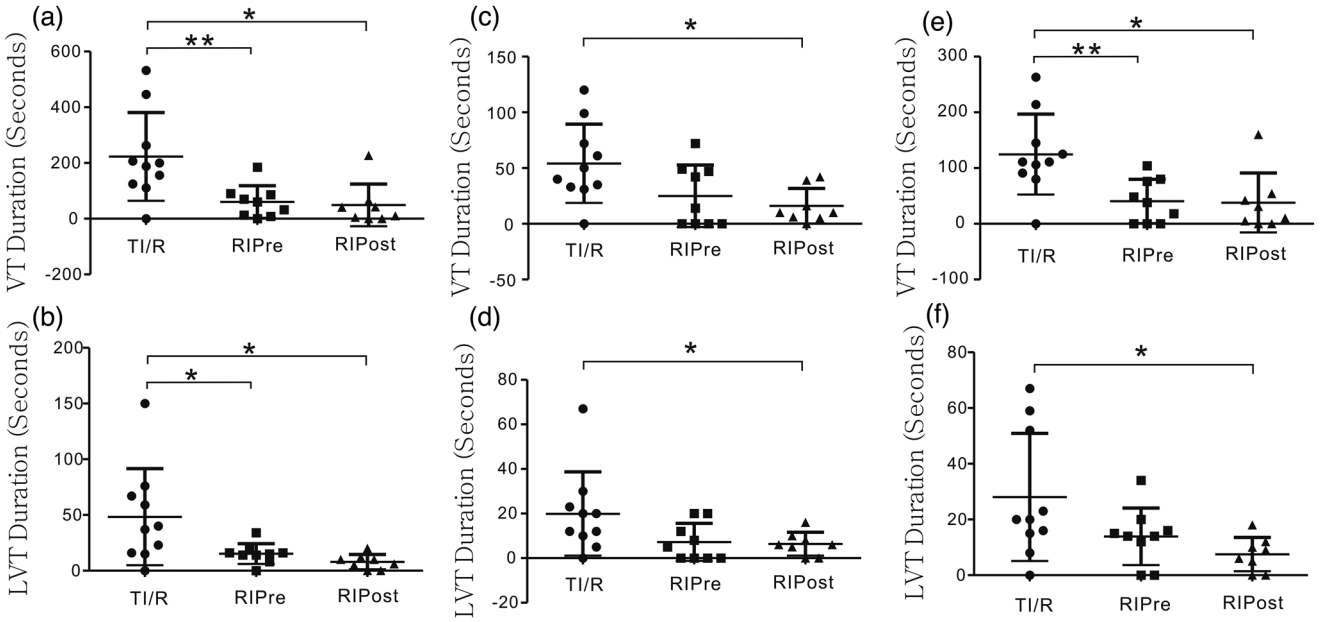

FIGURE 4 Remote ischaemic conditioning confers cardioprotection against myocardial I/R-induced ventricular tachycardia in testicular-I/R rats. (a) Mean durations of VT during the entire 20 min myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$, $^{**}P < 0.01$ versus TI/R rats. Values for TI/R rats are repeated from Table 1 for comparison. (b) Mean durations of longest VT (LVT) during the entire 20 min of myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$ versus TI/R rats. Values for TI/R rats are repeated from Table 1 for comparison. (c) Mean durations of VT during the first 5 min of myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$ versus TI/R rats. (d) Mean durations of LVT during the first 5 min of myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$ versus TI/R rats. (e) Mean durations of VT during the first 10 min of myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$, $^{**}P < 0.01$ versus TI/R rats. (f) Mean durations of LVT during the first 10 min of myocardial reperfusion injury period for TI/R, RIPre and RIPost rats (each group, $n = 8–10$). Rats without VT are indicated as 0 duration. $^{*}P < 0.05$ versus TI/R rats

sham-operated rats (Figure 6a). Strikingly, this elevated p-JNK level was completely blocked by applying RIPre ($P < 0.001$ vs. CON or TI/R hearts) or RIPost ($P < 0.001$ vs. CON or TI/R hearts, Figure 6a). Cx43 is the main gap junction component of the heart. It has been shown that ischaemic arrhythmias may be linked with the alternation of cardiac Cx43 level. We further examined the expression of Cx43 by western blotting. Differences in Cx43 expression among groups were detected ($P = 0.0002$, Figure 6b). We found that myocardial reperfusion injury significantly reduced the expression of Cx43 in control ($P < 0.01$) and TI/R ($P < 0.01$) rats as compared to the sham group. Interestingly, RIPre ($P < 0.01$ vs. CON, $P < 0.01$ vs. TI/R) and RIPost ($P < 0.01$ vs. CON, $P < 0.01$ vs. TI/R) could increase Cx43 expression as compared to CON and TI/R rats, indicating activation of Cx43.

4 | DISCUSSION

The major findings of the present study are as follows. First, TI/R rats were predisposed to myocardial reperfusion-induced AVB. Second, RIPre and PIPost protected cardiac function by reducing susceptibility to myocardial ischaemia-provoked ventricular arrhythmias in TI/R rats. Third, this beneficial effect is mediated by the inhibition of JNK and the activation of Cx43.

Remote ischaemic conditioning, intermittent episodes of ischaemia–reperfusion stimuli exerted in remote organs or limbs, has been shown to confer potent cardioprotection by stimulating various signalling pathways. Previous data from our laboratory and others have shown that remote conditioning protected hearts against arrhythmia and infarction (Hu et al., 2016, 2017; Johnsen et al., 2016), but its effects on cardiac arrhythmia in subjects with TI/R injury are completely unclear. It has been reported that TI/R injury could cause a loss of testicular testosterone secretion (Ahmed et al., 2016). Consistently, we also found that serum testosterone levels dropped significantly in rats after TI/R injury. However, low testosterone level is linked to arrhythmogenesis in humans (Magnani et al., 2014). To investigate if rats are predispose to arrhythmia after testicular torsion (during the testicular reperfusion period), an experimentally induced lethal arrhythmia model was applied. Here, a 3 h period of testicular ischaemia followed by 3 h of reperfusion was imposed in rats by a 720° rotation–derotation of the spermatic cord, ensuring that TI/R injury would be present; then we conducted the model of myocardial I/R injury-induced arrhythmias by an imposed ischaemic event (main coronary artery ligation), and tested the anti-arrhythmic effect of RIPre and RIPost in TI/R rats. We found that SCD incidence was equivalent between rats with and without TI/R stimuli, as was the incidence of VT. The major cause of death was either VF or severe AVB

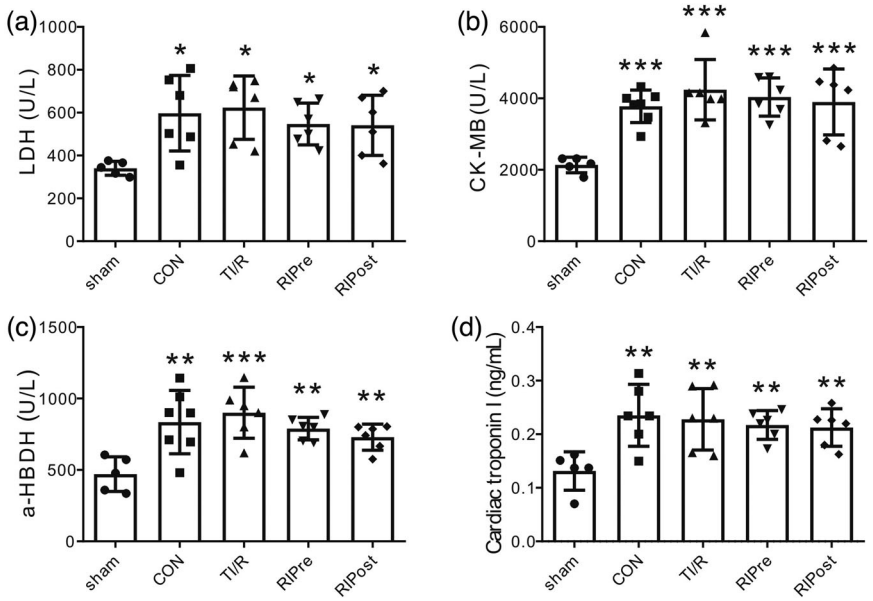

FIGURE 5 Levels of cardiac enzymes in serum. Post-reperfusion injury mean serum levels of lactate dehydrogenase (LDH, $n = 5–6$ per group, a), creatine kinase MB (CK-MB, $n = 5–7$ per group, b), α-hydroxybutyrate dehydrogenase (α-HBDH, $n = 5–7$ per group, c) and cardiac troponin I (cTnI, $n = 5–7$ per group, d) for sham, CON, TI/R and conditioned TI/R rats. Data are means ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus sham (by one-way ANOVA)

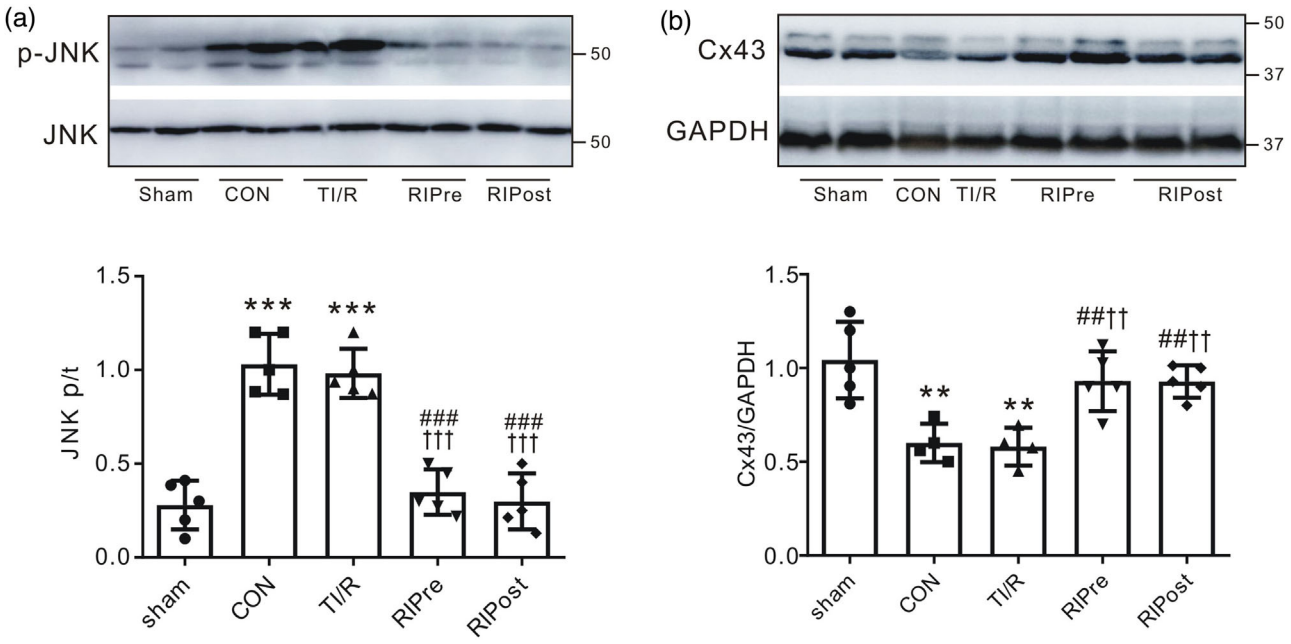

FIGURE 6 Remote ischaemic preconditioning alters ventricular protein expression. (a) Representative western blots of phospho (p)-JNK and total (t)JNK (upper) in sham, CON, non-treated TI/R (TI/R), RIPre- and RIPost-treated TI/R hearts post-myocardial I/R injury. The intensities of phospho-JNK band were normalized to its corresponding total protein band densities (lower). Each group, $n = 4–5$ per group. $^{***}P < 0.001$ versus sham, $^{###}P < 0.001$ versus CON, $^{†††}P < 0.001$ versus TI/R. (b) Representative western blots of Cx43 and GAPDH in sham, CON, non-treated TI/R (TI/R), RIPre- and RIPost-treated TI/R hearts post-myocardial I/R injury (upper). Cx43 expression levels were normalized by GAPDH (lower). Each group, $n = 4–5$ per group. $^{**}P < 0.01$ versus sham, $^{##}P < 0.01$ versus CON, $^{††}P < 0.01$ versus TI/R

in our study. It is likely that control rats had increased VF incidence contributing to the cases of SCD. Meanwhile, AVB, the major cardiac conduction disorder, has been shown in multiple clinical studies to contribute to increased mortality of patients with cardiovascular diseases (Simon et al., 1972). Unexpectedly, however, AVB was the leading cause of death for TI/R rats. After myocardial I/R, TI/R rats exhibited different types of severe atrioventricular blocks, including intermittent third-degree AVB. It has been demonstrated that the occurrence of AVB is associated with hormone secretion in elderly patients (Schoenmakers *et al*., 2008), and therefore the potential underlying reason for the increased prevalence of high-degree AVB in TI/R rats may be related to an imbalance in male sex hormone secretion and utilization after testicular damage. Most importantly, the present study provided evidence for the therapeutic efficacy of

RIPre and RIPost by demonstrating their ability to increase survival (no rats died after RIPre or RIPost) in TI/R rats with AVB. We also found that treatment with RIPre or RIPost was associated with a better cardiac outcome after induced arrhythmia, as evidenced by reduced severity of ventricular tachycardia (shorter durations of VT and LVT).

To further elucidate the mechanism of remote conditioning-induced cardioprotection in TI/R rats, we examined signalling pathway induction after myocardial I/R. I/R stimuli are a potent mediator of signalling networks involving a number of intracellular kinases, such as JNK (Luo et al., 2018; Rose et al., 2010). JNK, a stress-activated mitogen-activated protein kinase, was found to be activated (via phosphorylation) by cellular stress such as ischaemia. JNK plays a dual role in the setting of I/R injury, mediating both protective and detrimental aspects (Rose et al., 2010). Previous studies have shown that the phosphorylation levels of JNK were significantly increased after I/R (Xu et al., 2019), and thus inhibition of JNK phosphorylation may be a potential therapeutic approach for treating myocardial I/R injury (Ferrandi et al., 2004). Ischemic conditioning was found to inhibit I/R-provoked JNK activation, suggesting a potential role for JNK as a detrimental mediator in the setting of conditioning (Gu et al., 2000). Our study extends these findings by showing that myocardial I/R-induced activation of JNK (phosphorylation) was decreased in TI/R rats after remote conditioning treatment. Contrary to this, studies have shown that inhibition of JNK phosphorylation negated the cardioprotection afforded by ischaemic conditioning, demonstrating the opposite protective role of JNK during I/R (Strohm et al., 2002).

Cx43 is mainly expressed in ventricular cardiomyocytes. It is critical for maintaining normal cardiac electrical conduction (Michela et al., 2015). Abundant experimental evidence has shown its role during arrhythmogenesis. Under normal physiological conditions, Cx43 is highly phosphorylated. In respond to triggers such as ischaemia or hypoxia, Cx43 is dephosphorylated and downregulation of Cx43 occurs, accompanied by the opening of Cx43-formed hemichannels (Schulz et al., 2015), which may cause excessive Ca^{2+} influx and cardiac electrical disturbance. The reduction of Cx43 expression is associated with increased ventricular arrhythmogenicity, and therefore the absence of Cx43 may result in SCD (Danik et al., 2004). It is recognized that ischaemic preconditioning or postconditioning could effectively limit infarct size via preserving myocardial Cx43 expression during ischaemia in rat hearts (Michela et al., 2015). Furthermore, Chen et al. (2011) showed that ischaemic preconditioning protected hearts against reperfusion-induced arrhythmias in hypertrophic rabbit hearts by increasing Cx43 protein expression. The current results also confirm these previous findings demonstrating that remote ischaemic conditioning could effectively prevent decreases in Cx43 expression post-cardiac reperfusion in TI/R rats.

Notably, JNK and Cx43 have been identified as major mediators of arrhythmogenesis. Yan et al. (2013) found that the activation of JNK (phosphorylation) induced Cx43 suppression, which is critical in atrial fibrillation development. Petrich et al. (2002) further showed that the loss of Cx43 occurred in conjunction with the activation of JNK in cardiac myocytes under pathological stresses. Therefore, JNK may serve as a critical regulator of Cx43 expression. Interestingly, the role of the JNK/Cx43 signalling pathway under ischaemic stimuli was supported by our observations that JNK was phosphorylated, while Cx43 was down-regulated after myocardial reperfusion in TI/R hearts, but remote conditioning treatment could exert cardioprotective effects by suppressing JNK activation and restoring Cx43 expression. Our current results may implicate a pathway involving these two proteins in anti-arrhythmic effects of ischaemic conditioning in rats with TI/R injury.

There are several limitations to this study. First, because of the unpredictability of the occurrence of testicular torsion, postconditioning offers a more attractive and promising approach in clinical settings, but whether the combined treatment of preconditioning and postconditioning would exert a stronger cardioprotective effect in our study remains unknown. Second, we observed that remote conditioning effectively reduced susceptibility to post-ischaemic ventricular arrhythmias in TI/R rats. However, based on the current study design protocol, the independent testicular and cardiac mechanisms cannot be delineated, and thus it remains unclear whether the beneficial electrical effects we observed in RIPre and RIPost groups were testicular-dependent and whether it may involve an amalgam of both testicular and cardiac protection. Third, we found that the cardioprotection offered by ischaemic conditioning was less likely due to the preservation of circulating testosterone level in the current study. We used a protocol of 3 h of testicular ischaemia, followed by another 3 h of reperfusion; one may be unable to detect the alternation of hormone level in such a short time frame, and therefore the long-term effect of RIPre and RIPost on testosterone levels in TI/R rats may deserve future study. In the meantime, it is likely that other hormones, cytokines or active factors, such as catecholamine, microRNA, etc., may also be released into the blood during TI/R that may possibly influence cardiac kinase activity and promote arrhythmias. Fourth, we found that TI/R rats were predisposed to myocardial reperfusion-induced AVB, rather than ventricular tachycardia. The detailed underlying electrophysiological mechanism needs further attention. Furthermore, JNK and Cx43 were found to be involved in RIPre- and RIPost-mediated anti-arrhythmia activity in rats with TI/R injury, and other signalling molecules could also participate in this cardioprotective cascade. Thus, future studies may be necessary to address the detailed involvement of signalling pathways in this protective phenomenon, and meanwhile, understanding the detailed mechanisms of crosstalk between JNK and Cx43 signalling pathways may facilitate the translation of experimental results into new clinical therapies in the future.

In conclusion, rats with TI/R injury were predisposed to myocardial reperfusion-induced AVB. Remote preconditioning and postconditioning protected TI/R hearts against post-ischaemic ventricular tachyarrhythmias and effectively lowered the incidence of AVB and SCD. These remote conditioning-induced anti-arrhythmic effects may be exerted via suppressing JNK activation and restoring Cx43 expression.

ACKNOWLEDGEMENTS

This work was supported by Sichuan University West China Hospital setup funding to Z.H.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Z.H. designed the research; Z.Y. and Z.H. conducted the experiments; Z.Y., L.D., Q.L., L.Z. and Z.H. analysed the data; Z.H. drafted the article. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Zhaoyang Hu https://orcid.org/0000-0003-3642-9610

REFERENCES

Ahmed, A. I., Lasheen, N. N., & El-Zawahry, K. M. (2016). Ginkgo biloba ameliorates subfertility induced by testicular ischemia/reperfusion injury in adult Wistar rats: A possible new mitochondrial mechanism. *Oxidative Medicine and Cellular Longevity*, *2016*, 6959274.

Aimo, A., Borrelli, C., Giannoni, A., Pastormerlo, L. E., Barison, A., Mirizzi, G., Emdin, M., & Passino, C. (2015). Cardioprotection by remote ischemic conditioning: Mechanisms and clinical evidences. *World Journal of Cardiology*, *7*, 621–632.

Arena, S., Iacona, R., Antonuccio, P., Russo, T., Salvo, V., Gitto, E., Impellizzeri, P., & Romeo, C. (2017). Medical perspective in testicular ischemia-reperfusion injury. *Experimental and Therapeutic Medicine*, *13*, 2115–2122.

Chen, Z., Luo, H., Zhuang, M., Cai, L., Su, C., Lei, Y., & Zou, J. (2011). Effects of ischemic preconditioning on ischemia/reperfusion-induced arrhythmias by upregulatation of connexin 43 expression. *Journal of Cardiothoracic Surgery*, *6*, 80.

Danik, S. B., Liu, F., Zhang, J., Suk, H. J., Morley, G. E., Fishman, G. I., & Gutstein, D. E. (2004). Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circulation Research*, *95*, 1035–1041.

Ferrandi, C., Ballerio, R., Gaillard, P., Giachetti, C., Carboni, S., Vitte, P. A., Gotteland, J. P., & Cirillo, R. (2004). Inhibition of c-Jun N-terminal kinase decreases cardiomyocyte apoptosis and infarct size after myocardial ischemia and reperfusion in anaesthetized rats. *British Journal of Pharmacology*, *142*, 953–960.

Goodale, T., Sadhu, A., Petak, S., & Robbins, R. (2017). Testosterone and the heart. *Methodist DeBakey Cardiovascular Journal*, *13*, 68–72.

Grundy, D. (2015). Principles and standards for reporting animal experiments in *The Journal of Physiology* and *Experimental Physiology*. *Journal of Physiology*, *593*, 2547–2549.

Gu, Z., Jiang, Q., Zhang, G., Cui, Z., & Zhu, Z. (2000). Diphosphorylation of extracellular signal-regulated kinases and c-Jun N-terminal protein kinases in brain ischemic tolerance in rat. *Brain Research*, *860*, 157–160.

Hamed, G., Ahmed, R., Emara, M., & Mahmoud, M. (2011). Effect of erythropoietin on experimental unilateral testicular torsion detorsion in rat model. *Life Science Journal*, *8*, 405–412.

Hu, Z., Chen, M., Zhang, P., Liu, J., & Abbott, G. W. (2017). Remote ischemic preconditioning differentially attenuates post-ischemic cardiac arrhythmia in streptozotocin-induced diabetic versus nondiabetic rats. *Cardiovascular Diabetology*, *16*, 57.

Hu, Z., Hu, S., Yang, S., Chen, M., Zhang, P., Liu, J., & Abbott, G. W. (2016). Remote liver ischemic preconditioning protects against sudden cardiac death via an ERK/GSK-3β-dependent mechanism. *PLoS One*, *11*, e0165123.

Hu, Z., Kant, R., Anand, M., King, E. C., Krogh-Madsen, T., Christini, D. J., & Abbott, G. W. (2014). *Kcne2* deletion creates a multisystem syndrome predisposing to sudden cardiac death. *Circulation: Cardiovascular Genetics*, *7*, 33–42.

Johnsen, J., Pryds, K., Salman, R., Lofgren, B., Kristiansen, S. B., & Botker, H. E. (2016). The remote ischemic preconditioning algorithm: Effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. *Basic Research in Cardiology*, *111*, 10.

Luo, N., Liu, J., Chen, Y., Li, H., Hu, Z., & Abbott, G. W. (2018). Remote ischemic preconditioning STAT3-dependently ameliorates pulmonary ischemia/reperfusion injury. *PLoS One*, *13*, e0196186.

Magnani, J. W., Moser, C. B., Murabito, J. M., Sullivan, L. M., Wang, N., Ellinor, P. T., Vasan, R. S., Benjamin, E. J., & Coviello, A. D. (2014). Association of sex hormones, aging, and atrial fibrillation in men: The Framingham Heart Study. *Circulation: Arrhythmia and Electrophysiology*, *7*, 307–312.

Michela, P., Velia, V., Aldo, P., & Ada, P. (2015). Role of connexin 43 in cardiovascular diseases. *European Journal of Pharmacology*, *768*, 71–76.

Murry, C. E., Jennings, R. B., & Reimer, K. A. (1986). Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation*, *74*, 1124–1136.

Pentyala, S., Lee, J., Yalamanchili, P., Vitkun, S., & Khan, S. A. (2001). Testicular torsion: A review. *Journal of Lower Genital Tract Disease*, *5*, 38–47.

Petrich, B. G., Eloff, B. C., Lerner, D. L., Kovacs, A., Saffitz, J. E., Rosenbaum, D. S., & Wang, Y. (2004). Targeted activation of c-Jun N-terminal kinase in vivo induces restrictive cardiomyopathy and conduction defects. *Journal of Biological Chemistry*, *279*, 15330–15338.

Petrich, B. G., Gong, X., Lerner, D. L., Wang, X., Brown, J. H., Saffitz, J. E., & Wang, Y. (2002). c-Jun N-terminal kinase activation mediates down-regulation of connexin43 in cardiomyocytes. *Circulation Research*, *91*, 640–647.

Przyklenk, K., Bauer, B., Ovize, M., Kloner, R. A., & Whittaker, P. (1993). Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation*, *87*, 893–899.

Rose, B. A., Force, T., & Wang, Y. (2010). Mitogen-activated protein kinase signaling in the heart: Angels versus demons in a heart-breaking tale. *Physiological Reviews*, *90*, 1507–1546.

Schoenmakers, N., de Graaff, W. E., & Peters, R. H. (2008). Hypothyroidism as the cause of atrioventricular block in an elderly patient. *Netherlands Heart Journal*, *16*, 57–59.

Schulz, R., Gorge, P. M., Gorbe, A., Ferdinandy, P., Lampe, P. D., & Leybaert, L. (2015). Connexin 43 is an emerging therapeutic target in ischemia/reperfusion injury, cardioprotection and neuroprotection. *Pharmacology & Therapeutics*, *153*, 90–106.

Simon, A. B., Steinke, W. E., & Curry, J. J. (1972). Atrioventricular block in acute myocardial infarction. *Chest*, *62*, 156–161.

Strohm, C., Barancik, M., von Bruehl, M., Strniskova, M., Ullmann, C., Zimmermann, R., & Schaper, W. (2002). Transcription inhibitor actinomycin-D abolishes the cardioprotective effect of ischemic reconditioning. *Cardiovascular Research*, *55*, 602–618.

Turner, T. T., Bang, H. J., & Lysiak, J. J. (2005). Experimental testicular torsion: Reperfusion blood flow and subsequent testicular venous plasma testosterone concentrations. *Urology*, *65*, 390–394.

White, S. K., Frohlich, G. M., Sado, D. M., Maestrini, V., Fontana, M., Treibel, T. A., Tehrani, S., Flett, A. S., Meier, P., Ariti, C., Davies, J. R., Moon, J. C., Yellon, D. M., & Hausenloy, D. J. (2015). Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment elevation myocardial infarction. *JACC: Cardiovascular Interventions*, *8*, 178–188.

Xu, W., Zhang, L., Zhang, Y., Zhang, K., Wu, Y., & Jin, D. (2019). TRAF1 exacerbates myocardial ischemia reperfusion injury via ASK1-JNK/p38 signaling. *Journal of the American Heart Association*, *8*, e012575.

Yan, J., Kong, W., Zhang, Q., Beyer, E. C., Walcott, G., Fast, V. G., & Ai, X. (2013). c-Jun N-terminal kinase activation contributes to reduced connexin43 and development of atrial arrhythmias. *Cardiovascular Research*, *97*, 589–597.

Yang, S., Abbott, G. W., Gao, W. D., Liu, J., Luo, C., & Hu, Z. (2017). Involvement of glycogen synthase kinase-3β in liver ischemic conditioning induced cardioprotection against myocardial ischemia and reperfusion injury in rats. *Journal of Applied Physiology*, *122*, 1095–1105.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Yan, Z., Du, L., Liu, Q., Zhou, L., & Hu, Z. (2021). Remote limb ischaemic conditioning produces cardioprotection in rats with testicular ischaemia–reperfusion injury. *Experimental Physiology*, *106*, 2223–2234. https://doi.org/10.1113/EP089289