Clinics of Surgery

Research Article ISSN: 2638-1451 | Volume 8

Vitamin C Post Conditioning Alleviates Myocardial is-Chemia-Reperfusion Injury by Inhibiting Oxidative Stress in A Rat Model

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Received: 26 Oct 2022
Accepted: 05 Nov 2022
Published: 11 Nov 2022
J Short Name: COS

Citation:

Cui W. Vitamin C Post Conditioning Alleviates Myocardial is-Chemia-Reperfusion Injury by Inhibiting Oxidative Stress in A Rat Model.. Clin Surg. 2022; 8(5): 1-9

Keywords:

Vitamin C; Reperfusion; Cardioprotective agents; Antioxidants

1. Abstract

1.1. Background: The objective of this study was to investigate whether vitamin C can alleviate myocardial ischemia-reperfusion injury in vivo, and to explore its possible mechanisms.

1.2. Material and Methods: Sprague Dawley rats were randomly divided into sham, vitamin C-treated, ischemia-reperfusion, and hydrochloric acid groups. Myocardial H2O2 and NO were measured in real time using a sensor placed in the heart and a free radical analyzer. Cardiac function parameters were monitored using a PowerLab data acquisition system (AD Instruments Pty Ltd.). Myocardial infarct size was measured by triphenyl tetrazolium chloride staining, and hematoxylin and eosin staining were used to determine tissue morphology. Blood samples were collected for detection of lactate dehydrogenase and superoxide dismutase release, to evaluate the degree of cardiac injury. One-way analysis of variance and post-hoc testing was performed.

1.3. Results: Bcl-2 and p-ERK expression was statistically significantly increased and decreased (P<0.001), respectively, in the vitamin C group compared to the ischemia-reperfusion group. Infarcts were smaller in the vitamin C than in the ischemia-reperfusion group (21.38 ±1.58% vs. 41.65±1.51%, P=0.005). Compared with the ischemia-reperfusion group, vitamin C postconditioning reduced lactate dehydrogenase (374.66±7.58 vs. 305.54±16.46 U/L, P=0.011) and increased superoxide dismutase (433.00±25.04 vs. 566.67±25.50 U/L) concentrations. Vitamin C effectively restrained the production of H2O2 and NO in cardiomyocytes.

1.4. Conclusion: The present study demonstrated that vitamin C postconditioning in a myocardial ischemia-reperfusion rat model can protect cardiomyocytes from a burst of reactive oxygen species, and can inhibit the ERK signaling pathway and apoptotic proteins. Thus, vitamin C may protect the myocardium from ischemia-reperfusion injury.

2. Background

Cardiovascular disease is a major cause of death worldwide. Immediate restoration of coronary blood flow is the basic strategy for treatment of acute myocardial ischemia. On the other hand, after prolonged myocardial ischemia, reperfusion may lead to myocardial injury [1]. Common complications include reperfusion arrhythmia, myocardial infarction, and cardiac dysfunction [2]. Sudden reperfusion may result in a burst of reactive oxygen species (ROS), Ca2+ overload, NO disorder, rapid pH recovery, as well as the activation of neutrophils and inflammation factors [3]. These changes, alone and in combination, may lead to damage, necrosis, and apoptosis of cardiomyocytes. Thus, it is necessary to develop a method that can reduce myocardial reperfusion injury. In myocardial ischemia-reperfusion (I/R) injury, the myocardial cell membranes are exposed to the rapidly changing environment described above, which facilitates the sudden opening of the mitochondrial permeability transition pore (MPTP), and the release of cytochrome c (Cyto-c) and the activation of Caspase-9 [4]. B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) are involved in the process of I/R in the heart, and the ratio of Bcl-2/Bax appears to determine cell survival or death following an apoptotic stimulus [5]. Extracellular signal-regulated kinase (ERK)

also plays an important role in myocardial apoptosis [6]. Adluri et al. [7] discovered that vitamin E can effectively inhibit myocardial I/R injury in rats by reducing the ROS burst and apoptosis. Others have also demonstrated that low-pH reperfusion can reduce myocardial injury [8]. Vitamin C (VC), also known as L-ascorbic acid, is widely used as a clinical antioxidant that can eliminate excess oxygen free radicals in the human body [9]. The objective of this study was to investigate whether VC can alleviate myocardial I/R injury in vivo, and to explore its possible mechanisms. Because VC has two biological properties of interest, oxidation resistance and acidity, we prepared a hydrochloric acid (HCL) control solution with the same pH value as that of the VC solution, to determine which underlying mechanism is involved. Our results revealed that postconditioning with VC played an important cardioprotective role via oxidation resistance, by inhibiting the ERK pathway in vivo.

3. Material and Methods

3.1. Materials

VC was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA) with a purity of more than 99%. Male Sprague Dawley (SD) rats (280-320 g, 10-12 weeks old) were provided by the Experimental Animal Center in Hebei Medical University. Antibodies against Cyto-c, Caspase-9, Bcl-2, and Bax were provided by Abcam plc. (Cambridge, UK). ERK1/2 and p-ERK1/2 were provided by Epitomics, Inc. (Burlingame, CA, USA).

3.2. Myocardial I/R Rat Model and Grouping

The coronary artery was occluded and perfused as previously described [17]. Rats were anesthetized with chloral hydrate (300 mg/kg, intraperitoneally). During the surgical procedure, rats were artificially ventilated (75 cycles/min, tidal volume 20 mL, inhalation/exhalation 1:1) with a rodent ventilator (Taimeng Technology Institute; Chengdu, China). Briefly, the chest was opened via a left thoracotomy, and the heart was exposed via an incision in the pericardium. A suture was passed around the left anterior descending artery (LAD), approximately 4-5 mm away from its origin, and tightened. Ischemia was induced by ligation of the LAD for 30 min, followed by reperfusion for 2 h.

In total, 48 rats were randomly divided into four groups of equal size: the sham, I/R, VC, and HCL groups. For the sham group, left thoracotomy was performed without LAD ligation; at the time when other groups underwent reperfusion, this group was treated with a identical relative volume of physiological saline via the carotid artery. The I/R, VC, and HCL groups were subjected to LAD ligation, and were respectively treated with a relative volume of physiological saline, VC solution (25, 50, and 100 mg/kg), or HCL solution at the onset of reperfusion. The optimal dose of VC was considered to be 50 mg/kg, which had a pH of 2.5; therefore, the HCL solution was set to pH 2.5.

3.3. Hemodynamic Measurements

To evaluate left ventricular systolic and diastolic function, a PE-50 catheter (AD Instruments) connected to a pressure sensor was advanced through the left carotid artery and aorta into the left ventricle (LV), under continuous monitoring of the pressure curve. When the value of the curve ranged from 0 to 120, it indicated that the catheter was inside the LV. Cardiac function parameters were continuously monitored using a PowerLab data acquisition system (AD Instruments PL3508 Pty Ltd.; Bella Vista, Australia; (Figure 1)). During the procedure, heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and the maximal rate of change of left ventricular pressure (±dP/dtmax) were recorded on a computer. Stable hemodynamic parameters before ischemia were selected for the baseline. After the ischemic procedure was completed, the 1st, 30th, 60th, 90th, and 120th minutes during reperfusion were selected as time points around which to record six to eight consecutive cardiac cycles

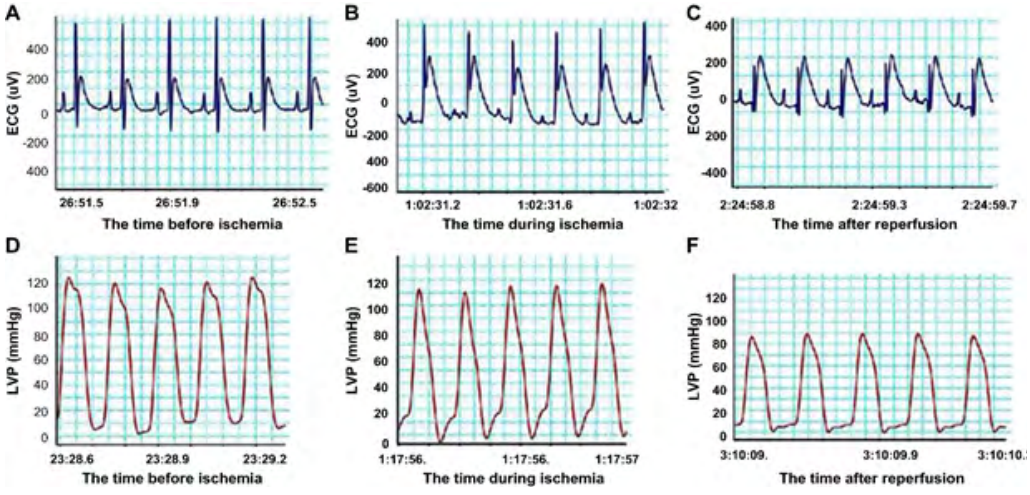

Figure 1: Electrocardiograms and left ventricular pressure changes of the ischemia-reperfusion rat model in the VC group.
A. The electrocardiogram (ECG) before ischemia. B. The ECG during ischemia.
C. The ECG after VC reperfusion. D. The left ventricular pressure (LVP) before ischemia.
E. The LVP during ischemia. F. The LVP after VC reperfusion.

3.4. Lactate Dehydrogenase and Superoxide Dismutase Activity In Blood

Blood samples were collected after reperfusion. Lactate dehydrogenase (LDH) and superoxide dismutase (SOD) activity were measured using LDH and SOD assay kits (Jiancheng Bioengineering Institute; Nanjing, China).

3.5. Infarct Size and Histopathology

Myocardial infarct size was measured by triphenyl tetrazolium chloride (TTC) staining. After reperfusion, the hearts were removed from the models and frozen at -20 °C for 20 min. Thereafter, the left ventricles were sliced from apex to base into five to six 2 × 2-mm sections. The sections were incubated in a 1% TTC phosphate buffer (pH 7.4) at 37 °C for 20 min, and fixed in 10% formalin for 24 h. Normal zones were stained red with the TTC, and infarct regions, which did not incorporate the TTC, remained gray. Photos of the sections were taken for calculation of the percentage of myocardial infarction using ZEISS ZEN image analysis software (Carl Zeiss Microscopy GmbH; Jena, Germany).

For light microscopy, hearts were removed quickly after reperfusion and were snap frozen in liquid nitrogen. The frozen hearts were sliced into 4-μm sections using a freezing microtome (Leica Biosystems GmbH, Nußloch, Germany), and stained with hematoxylin and eosin (H&E). Slides were examined using a microscope (Carl Zeiss Microscopy GmbH) at 200× magnification.

3.6. Measurement of H2O2 and NO in Cardiomyocytes

The real-time release of myocardial H2O2 and NO was recorded using sensors placed in the hearts of the SD rats, and measured using a free radical analyzer (Apollo 4000, World Precision Instruments, Inc.; Sarasota, FL; (Figure 2). The changes in H2O2 and NO release during I/R were expressed relative to baseline readings. The values were converted to concentrations (nM) after correction using calibration curves. We analyzed the data at baseline, at the start of reperfusion, and at the 5th, 10th, 20th, 30th, 40th, 50th and 60th min of reperfusion.

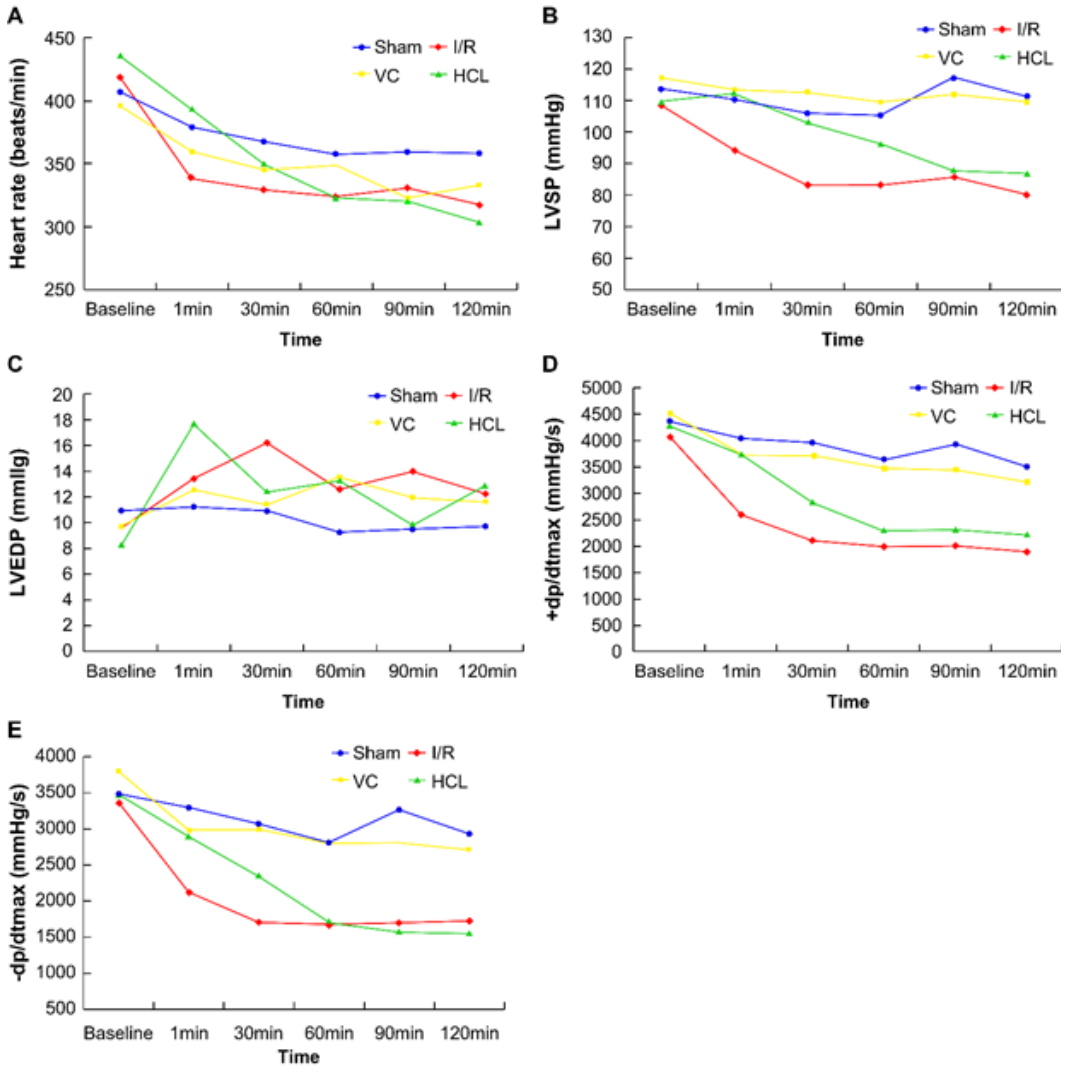

Figure 2: Cardiac function parameters among different experimental groups.

Note: Values are indicated as means of the 10 values per group

LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; $+dP/dt_{max}$: maximal rate of increase of left ventricular pressure; $-dP/dt_{max}$: maximal rate of decrease of left ventricular pressure.

3.7. Western Blotting

Hearts were collected at the second hour (i.e., at the end) of reperfusion, and the LV tissues were snap frozen in liquid nitrogen before being stored at -80 °C. Approximately 100 mg of ventricular tissue was used for protein extraction. After centrifugation at 20,000 × g for 20 min at 4 °C, the supernatant was collected and proteins were quantified using a modified Bradford assay (Bio-Rad Laboratories; Hercules, CA, USA). Aliquots of the supernatants containing equal amounts of protein (40 μg) were electrophoresed on SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. Primary antibodies were used to attach Cyto-c, Caspase-9, Bcl-2, Bax, P-ERK1/2, and ERK1/2 to the membranes. Thereafter, the membranes were incubated with the corresponding secondary antibodies. Detection was performed using the Chemi-Doc MP imaging system (Bio-Rad Laboratories).

3.8. Statistical Analysis

All values are expressed as means ± standard errors of the mean. Whenever a statistically significant result was obtained with one-way analysis of variance, we performed multiple comparison tests between pairs of groups using the Student-Newman-Keuls procedure. All procedures were performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp.; Armonk, NY, USA) after Shapiro-Wilk and Levene testing for normality and equality of variances, respectively. Differences between groups were considered statistically significant at $P<0.05$.

4. Results

4.1. VC Postconditioning Alleviates Cardiac I/R Dysfunction

As ECGs are important indicators of regional myocardial ischemia, we investigated the effects of VC on changes in ECGs. (Figure 1) demonstrates that ST-segment elevation was observed in the ischemia process (figure 1B), but was significantly decreased in the VC reperfusion process (figure 1C). There was no significant difference in any of the hemodynamic parameters at baseline among groups ($P>0.05$) (Figure 2, Table 1), and LVEDP did not differ among the four groups at any of the time points ($P>0.05$). HR differed among groups only at the 1st min time point. LVSP and ±dP/dtmax values were lower in the I/R than in the sham group at all reperfusion time points ($P=0.002$). Postconditioning with VC significantly increased the ±dP/dtmax and LVSP at all reperfusion time points ($P=0.004$). After treatment with HCL solution, the LVSP values at the 1st and 30th min time points were increased significantly compared to those in the I/R group ($P<0.001$), and the dP/dtmax value was increased significantly at the 1st min ($P<0.001$).

Table 1: Hemodynamic parameters of each group

Item	Baseline	Reperfusion				
		1 min	30 min	60 min	90 min	120 min
HR (beats/min)						
Sham	406.84±20.19	378.54±10.10	367.26±9.75	357.40±11.12	359.12±9.26	358.21±9.62
I/R	418.07±25.98	337.65±14.06▼	329.03±19.13	323.88±19.70	330.75±28.11	319.78±22.09
VC	395.63±27.32	359.53±14.03	344.83±16.46	348.70±12.30	322.45±26.50	333.17±24.07
HCL	435.63±13.24	393.03±11.39▲	349.74±16.04	322.69±28.78	320.13±19.53	303.67±24.25
LVEDP (mmHg)						
Sham	10.91±2.01	11.20±1.82	10.88±3.15	9.19±2.54	9.50±2.32	9.70±2.00
I/R	9.62±2.11	13.37±3.96	16.19±3.06	12.56±3.03	13.91±2.66	14.10±2.85
VC	9.69±0.68	12.52±1.75	11.43±1.30	13.47±2.73	11.91±1.45	11.58±1.33
HCL	8.25±1.78	17.64±2.89	12.38±1.34	13.27±2.92	9.83±1.90	12.88±3.83
LVSP (mmHg)						
Sham	113.56±6.33	110.35±1.98	105.82±5.52	105.20±5.56	117.15±5.11	111.25±4.16
I/R	108.53±3.50	94.14±4.87▼	83.04±6.91▼	83.18±6.52▼	85.70±8.07▼	80.11±7.65▼
VC	117.23±3.78	113.51±4.53▲	112.64±3.04▲	109.35±6.38▲	112.15±6.74▲	109.55±5.70▲
HCL	109.77±2.79	112.15±2.90▲	102.99±5.22▲	96.31±8.40	87.78±12.4▼	86.85±11.19▼★
dP/dtmax (mmHg/s)						
Sham	4354.89±507.92	4035.70±473.76	3950.52±658.12	3630.17±522.70	3925.78±556.26	3494.14± 543.71
I/R	4056.58±621.76	2578.25±284.95▼	2087.56±351.38▼	1966.36±219.17▼	1987.10±190.90▼	1873.47±260.00▼
VC	4504.80±278.43	3720.37±334.80▲	3700.20±302.93▲	3461.67±310.51▲	3428.85±430.78▲	3204.15±366.37▲
HCL	4264.10±398.19	3732.84±304.57▲	2818.67±489.83	2275.17±280.42▼★	2307.00±505.47▼	2194.85±407.22▼
-dP/dtmax (mmHg/s)						
Sham	3470.89±480.05	3288.67±415.56	3056.23±344.36	2797.60±271.05	3264.93±336.50	2935.49±323.33
I/R	3357.43±434.25	2112.7±235.00▼	1702.94±407.72▼	1672.59±271.84▼	1696.15±202.62▼	1534.04±229.65▼
VC	3798.63±231.62	2973.16±201.72▲	3001.05±216.43▲	2808.34±382.58▲	2796.76±543.80▲	2713.40±413.43▲
HCL	3480.73±396.94	3106.77±190.74	2349.85±323.50	1700.12±260.47▼★	1570.58±386.36▼★	1545.80±302.89▼★

Note: All values are expressed as mean ± SEM. ▼Compared with the sham group, $P<0.05$; ▲Compared with the I/R group, $P<0.05$; ★Compared with the VC group, $P<0.05$. HR: heart rate; I/R: ischemia-reperfusion; VC: vitamin C; HCL: hydrochloric acid; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; dP/dtmax: maximal rate of increase in left ventricular pressure; -dP/dtmax: maximal rate of decrease in left ventricular pressure.

4.2. VC Postconditioning Can Reduce LDH Activity and Increase SOD Activity in the Blood

The release of LDH from the heart during reperfusion is graphed in (Figure 3A). Compared with the sham group (254.55±21.71 U/L), the activity of LDH in the I/R (374.66±7.58 U/L), VC (305.54±16.46 U/L), and HCL (366.05±13.31 U/L) groups were increased significantly (P =0.007). Compared with the I/R group, LDH activity in the VC group was significantly reduced (P =0.011), while this reduction was not statistically significant (P>0.05) in the HCL group.

As (Figure 3B) illustrates, compared with the sham group (519.33±20.30 U/L), the activity of SOD in the I/R (433.00±25.04 U/L) and HCL (382.67±14.67 U/L) groups was significantly reduced (P <0.05). The activity of SOD in the VC group (566.67±25.50 U/L) was not statistically different (P>0.05) from that in the sham group. Compared with the I/R group, treatment with VC significantly increased the activity of SOD (P<0.05), but the activity of SOD was not statistically different after treatment with HCL.

4.3. VC Postconditioning has Beneficial Effects on Infarct Size and Histopathology

(Figure 3C) contains representative images of infarct size, as revealed by TTC staining. Infarcts were smaller in the VC than in the I/R group (21.38 ±1.58% vs. 41.65±1.51%, P <0.001). In contrast, infarct sizes in the HCL group (36.80±1.39%) were not significantly different from those in the I/R group (P>0.05).

The results of H&E staining of myocardial tissue are presented in (Figure 3D). In the sham group, the myocardial fibers were neat and had clear boundaries, with no swelling, disorder, or inflammatory cell infiltration in the interstitial tissues. However, in the I/R group, the myocardial fibers were very disorderly, with different degrees of swelling and widening of gaps between cardiomyocytes. Moreover, there were great quantities of inflammatory cell infiltration, and visible focal necrosis in the myocardial tissue. Compared with the I/R group, the changes were visibly reduced in the VC group (P=0.006); there was no statistical difference between the I/R and HCL groups (P>0.05).

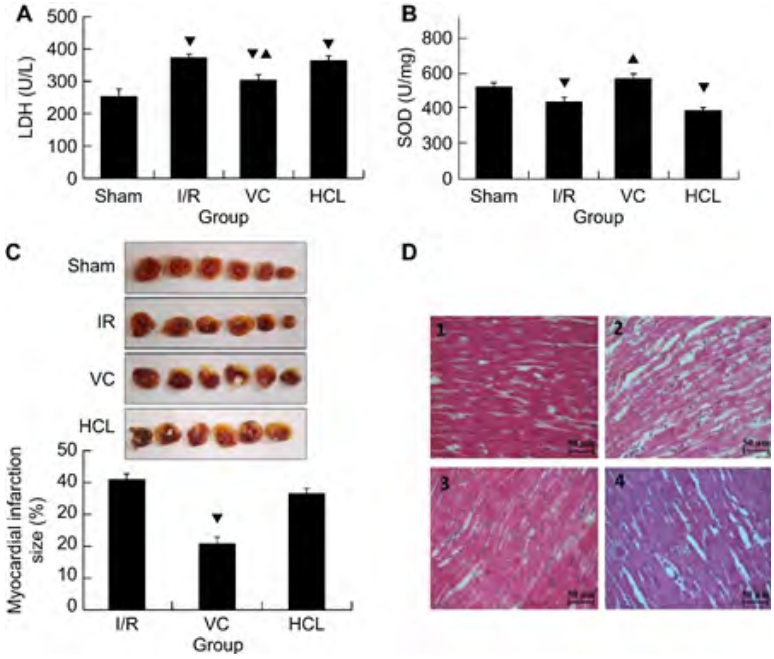

Figure 3: The comparison of myocardial injury and serum LDH and SOD in each group.

Note: Values are indicated as means (error bars are standard errors of the mean) of the 10 values per group

A. Serum LDH (U/L) in each group.

B. Serum SOD (U/mg) in each group.

C. Representative heart sections of groups after triphenyl tetrazolium chloride staining.

D. Histopathological changes of myocardial tissue. (hematoxylin and eosin staining, 200× magnification). 1: Sham group; 2: I/R group; 3: VC group; 4: HCL group.

▼Compared with sham group, *P*<0.05; ▲Compared with I/R group , P<0.05.

LDH: lactate dehydrogenase; SOD: superoxide dismutase; I/R: ischemia-reperfusion; VC: vitamin C; HCL: hydrochloric acid.

4.4. VC Postconditioning Inhibits Oxidative Stress and Reduces NO in the Myocardium

As summarized in (Figure 4A and Table 2), in the I/R group, the concentration of H2O2 peaked at approximately 164 nM from the 30th to the 40th min of reperfusion. From the 40th to the 60th min, the H2O2 concentration decreased to 133.90 nM. Compared with the I/R group, the changes in H2O2 concentration at each time point were significantly decreased in the VC group (P=0.012. The changes in H2O2 concentrations in the HCL group, from the start of reperfusion to the 30th min time point, did not significantly differ from those in the I/R group (P>0.05). However, from the 40th to the 60th min time points, the changes were significantly decreased in the HCL compared to the I/R group (P<0.05). As (Figure 4B) and (Table 2) indicate, the concentration of NO decreased over time (P=0.002) during reperfusion in the I/R group. However, the reduction in NO concentration at each time point was significantly lower in the VC than in the I/R group (P=0.015). In contrast, the reduction in NO concentration was not significantly different in the HCL than in the I/R group at any of the time points (P>0.05).

4.5. VC Postconditioning Protected Myocardial Cells from I/R Injury Via Inhibition of Apoptosis-Related Proteins and the ERK Pathway

As illustrated in (Figure 4D), Cyto-C, Caspase-9, and Bax concentrations in cardiac tissues were significantly increased by I/R treatment. VC therapy reduced the above protein levels and HCL slightly decreased the related levels. Compared with the I/R group, the expression of p-ERK in the VC group was significantly reduced (P=0.005) and the expression of Bcl-2 and the Bcl-2/Bax ratio were significantly increased in the VC group (P=0.014). Meanwhile, HCL also inhibited the expression of p-ERK (P<0.05), but less so than in the VC group (P=0.025).

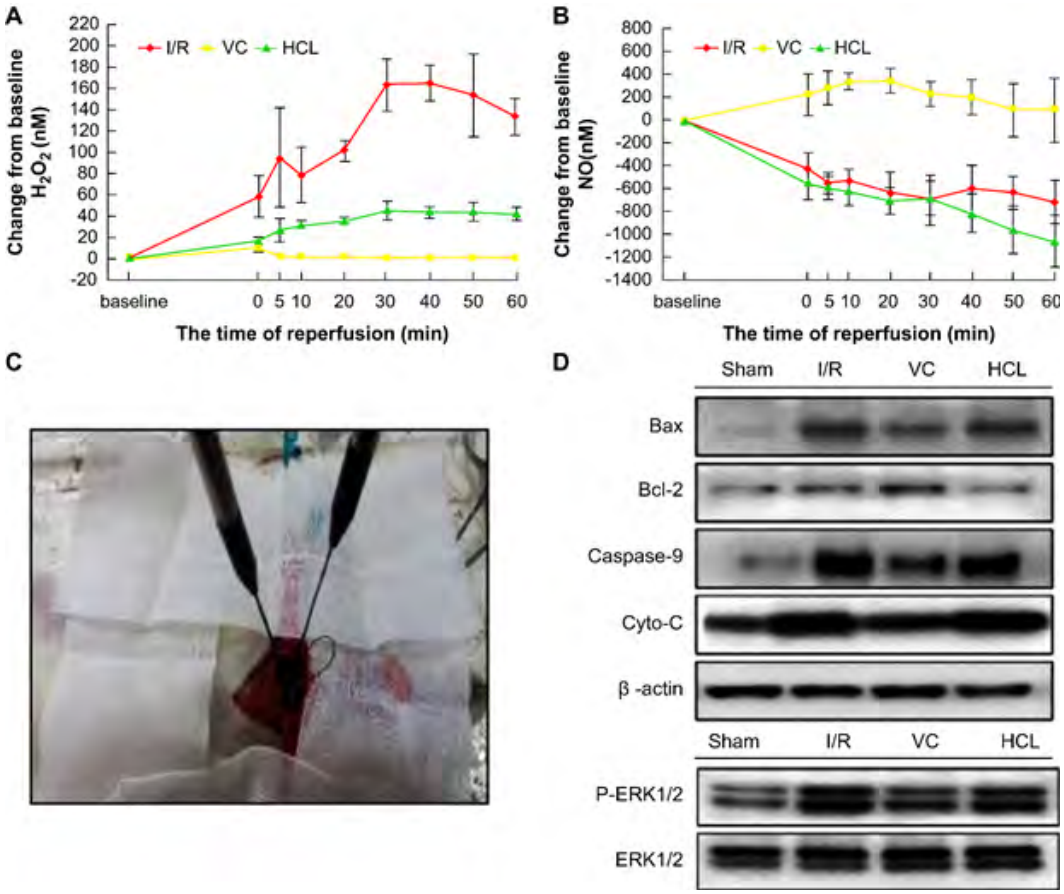

Figure 4: The relative difference in H_2O_2 and NO release in the myocardium and the expression level of apoptotic proteins in each group.

Note: Values are indicated as means (error bars are standard errors of the mean). n=10 per group

A. The relative difference in H_2O_2 release in in the myocardium of each group.

B. The relative difference in NO release in the myocardium of each group.

C. The process of measuring myocardial H_2O_2 and NO concentration.

D. Expression levels and relative intensity of Bax, Bcl-2, Caspase-9, Cyto-C, p-ERK1/2, and ERK1/2.

I/R: ischemia-reperfusion; VC: vitamin C; HCL: hydrochloric acid; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma-2; Cyto-c: cytochrome c; ERK: extracellular signal-regulated kinase.

Table 2: H_2O_2 and NO change in the myocardium of each group

Items	**Change From Baseline H2O2 (nM)**							
	0 min	5 min	10 min	20 min	30 min	40 min	50 min	60 min
I/R	57.4±	94.01±	78.38±	101.01±	164.37±	164.89±	153.39±	133.90±
	19.29	46.49	26.83	9.06	24.4	16.13	39.31	17.4
VC	8.93±	0.92±	0.54±	0.38±	-0.47±	-0.03±	0.12±	-0.28±
	5.44▲	0.90▲	0.89▲	0.33▲	0.74▲	1.21▲	1.29▲	1.43▲
HCL	15.63±	26.04±	31.35±	34.94±	44.72±	42.99±	43.07±	41.63±
	4.05	10.8	3.8	2.74	8.88	5.22▲	9.20▲	4.63▲
Items	**Changes From Baseline NO (nM)**							
	0 min	5 min	10 min	20 min	30 min	40 min	50 min	60 min
I/R	-416.10±	-547.29±	-525.10±	-627.30±	-695.24±	-594.24±	-632.2±	-713.18±
	143.24	99.1	107.65	180.27	218.84	199.38	143.22	186.88
VC	230.40±	295.13±	346.10±	354.03±	242.59±	216.37±	105.01±	99.82±
	181.71▲	152.65▲	65.26▲	113.81▲	105.31▲	150.58▲	232.06▲	282.63▲
HCL	-546.94±	-585.81±	-615.81±	-701.68±	-675.78±	-812.97±	-955.50±	-1058.79±
	142.06	96.6	123.88	119.33	146.4	161.64	211.04	224.95

Note: All data are presented as mean ± SEM, and n=10 for each. ▲Compared with the I/R group, P<0.05. I/R: ischemia-reperfusion; VC: vitamin C; HCL: hydrochloric acid.

5. Discussion

In the present study, the positive effects of VC postconditioning on myocardial I/R injury and its underlying mechanisms were investigated in vivo. The results indicate that VC ameliorated myocardial I/R injury by reducing infarct size, decreasing ROS, H2O2, and LDH concentrations, increasing SOD and NO concentrations, and inhibiting apoptosis of cardiomyocytes. ROS cause oxidative stress and act as a major mediator of I/R injury, increasing lipid oxidation of the cell membrane and mitochondria, which results in increased membrane permeability, ion transport dysfunction, MPTP opening, and Ca2+ overload [10]. Experimental studies have also demonstrated that, with reperfusion, the low extracellular pH quickly recovers, but that, because of the cell membrane and ion exchange proteins, the intracellular pH value cannot recover as quickly [11,12]. This results in a pH gradient that activates the Na+-H+ and Na+-Ca2+ exchangers, ultimately aggravating the Ca2+ overload. In addition, Ca2+ overload can activate phospholipase- and calcium-dependent proteases [13]. The former results in arachidonic acid formation, which produces a large amount of H2O2 and OH by oxidation; the latter can convert xanthine oxidase into xanthine dehydrogenase, which produces large amounts of oxygen free radicals. Maczewski et al. [14] also discovered that inhibitors of Na+-H+ and Na+-Ca2+ exchangers significantly reduced intracellular accumulation of Ca2+ and reduced the formation of oxygen free radicals. This provided evidence that I/R-related Ca2+ overload is positively correlated with the formation of oxygen free radicals. Most importantly, during I/R, the burst of oxygen free radicals, Ca2+ overload, and rapid change in pH all have their respective mechanisms resulting in injury, but these also interact with each other. It is common knowledge that NO plays an important role in the cardiovascular system, such as in regulating vascular tone, cell growth, leukocyte adhesion, and platelet aggregation [15]. NO can also reduce the intracellular concentration of Ca2+ in vascular smooth muscle cells via the cGMP signaling pathway. Li et al. [16] demonstrated that NO was reduced during early reperfusion. Thus, it is necessary to artificially supplement myocardial NO before the reperfusion procedure is initiated, to avoid necrosis of myocardial cells.

The mechanism of cardiomyocyte apoptosis is comprised mainly of two signaling pathways: the mitochondrial apoptotic pathway and the receptor-independent apoptotic pathway [17,18]. During I/R, ROS stress and other undesirable stimulation activate the mitochondrial apoptosis pathway, promoting MPTP opening and releasing Cyto-c and apoptosis inducing factor (AIF) [19,20]. Upon release from the mitochondria, Cyto-c promptly forms a trimeric complex with apoptotic protease activating factor 1 in an ATP-dependent manner. This complex activates procaspase-9, which is cleaved to form caspase-9, the most upstream caspase in the mitochondrial apoptotic pathway [19,20]. Among intracellular apoptosis-regulating proteins, the Bcl-2 family plays a pivotal role in regulating cellular responses to a wide variety of apoptotic signals. For example, Bax is crucial for inducing permeabilization of the outer mitochondrial membrane, resulting in the opening of the MPTP and the subsequent release of apoptogenic molecules (such as Cyto-c and AIF). On the other hand, Bcl-2 can inhibit apoptosis by blocking the opening of MPTP. The relative ratio between anti- and pro-apoptotic proteins determines the cell's sensitivity or resistance to a variety of stimulating signals [18,21–23].

ERKs are key regulatory proteins that mediate cell survival, proliferation, and differentiation [24,25]. ROS are involved in the activation of the ERK pathway, but the apoptotic effects of ERK in I/R injury remain controversial. Several studies have revealed that ERK1/2 activation can reduce apoptosis [26]. However, others have indicated that it can increase apoptosis [27]. Overall, its effect remains to be further validated. In the current study, VC postconditioning statistically significantly reduced myocardial I/R injury in rats. The reasons are that its antioxidant property can reduce the burst of ROS during I/R and that its acidity may inhibit the rapid recovery of pH. Therefore, HCL post-treatment with the same pH value also played a certain protective effect.

6. Conclusion

We revealed that VC postconditioning can protect myocardial tissue from I/R injury mediated by the ERK pathway in a rat model. These findings warrant further research and may ultimately lead to the clinical use of VC during reperfusion in patients with ischemic heart diseases.

7. Acknowledgement

We would like to thank Editage (www.editage.cn) for English language editing.

8. Conflicts of Interest

None

9. Funding

This work was supported by the Hebei Province Natural Science Foundation (No. H2018206066) and Xinxin-Merck Cardiovascular Research Fund.

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