Original Paper

ω -3 Polyunsaturated Fatty Acid Postconditioning Protects the Isolated Perfused Rat Heart from Ischemia-Reperfusion Injury

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Keywords

ω -3 polyunsaturated fatty acids · Ischemia-reperfusion injury · Myocardial postconditioning · Cardioprotection

Abstract

Aims: This study aimed to evaluate the cardioprotective effects of ω -3 polyunsaturated fatty acids (PUFAs) postconditioning against ischemia-reperfusion (I/R) injury. **Methods:** Sixty Sprague-Dawley rats were randomly divided into 4 groups ($n = 15$ for each) and used to generate the Langendorff isolated perfused rat heart model. The sham group received a continuous perfusion of 150 min. The remaining three I/R-treated groups sequentially received a 30-min perfusion, a 30-min cardioplegia, and a 90-min reperfusion. The I/R-ischemic preconditioning (IP) group additionally received three cycles of 20-s reperfusion and 20-s coronary reocclusion preceded the 90 min of reperfusion. The I/R- ω group were perfused with ω -3 PUFAs for 15 min before the 90 min of reperfusion. The myocardial infarct size, the degree of mitochondrial damage, the antioxidant capacity of the myocardium, and the cardiac functions during reperfusion were compared among groups. **Results:** Compared with the I/R group, the I/R- ω group had significantly reduced myocardial infarct size, reduced levels of lactate dehydrogenase and malondialdehyde, elevated superoxide dismutase level, and elevated rising ($+dp/dt_{max}$) and descending ($-dp/dt_{max}$) rate of left ventricular pressure. The I/R- ω group had a significantly lower rate of mitochondrial damage in myocardial tissue compared with the I/R and I/R-IP groups. **Conclusion:** ω -3 PUFA postconditioning possesses good cardioprotective effects and may be developed into a therapeutic strategy for myocardial I/R injury.

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Introduction

Ischemia-reperfusion (I/R) injury refers to the tissue damage caused by restoration of blood supply to the tissue after a period of ischemia [1]. Intraoperative myocardial I/R injury is a common and unpreventable problem occurring in several cardiac interventions, such as cardiac surgery with extracorporeal circulation, intracoronary thrombolysis, and percutaneous transluminal coronary angioplasty, and has attracted more and more attention of cardiac physicians. Significant efforts have been made to develop the methods for protecting myocardium from I/R injury. In 1986, Murry et al. [2] introduced the concept of ischemic preconditioning (IP) by demonstrating that multiple brief ischemic episodes have a protective effect on the canine heart against subsequent prolonged myocardial I/R injury. A large number of studies have confirmed that myocardial IP can effectively protect myocardium from I/R injury [3, 4]. However, myocardial IP needs to be conducted before the onset of ischemia, which greatly limits its clinical application.

In 2003, Zhao et al. [5] found that after myocardial ischemia, multiple short cycles of ischemia before the onset of reperfusion (e.g., ischemic postconditioning) can also protect the myocardium from subsequent myocardial I/R injury. In addition, the protective effect of ischemic postconditioning is comparable with that of IP. On the other hand, accumulating evidence has shown that drug treatments for ischemic myocardium at the onset of reperfusion (e.g., pharmacological postconditioning) are capable of providing a similar protective effect with ischemic postconditioning [6–9]. Pharmacological postconditioning represents an ideal alternative for ischemic postconditioning since its clinical operation is simpler than ischemic postconditioning. Therefore, it is clinically significant to identify a safe, effective, and easy-to-use postconditioning drug.

ω -3 polyunsaturated fatty acids (PUFAs) including α -linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are mainly found in marine plants and animals [10], possessing immunomodulatory, anti-inflammatory, antiarrhythmic, and antithrombotic properties [11]. In recent years, the protective capacity of ω -3 PUFAs against cardiovascular disease has been extensively reported in plenty of animal studies [12, 13] and clinical trials [14], namely that ω -3 PUFAs can effectively reduce the incidence of cardiovascular disease and mortality [15]. It has been demonstrated that ω -3 fatty acid supplementation can modulate the cardiac ion channels involved in cardiac action potentials on the cardiomyocytes, and reduce the ω -6/ ω -3 fatty acid ratio in the cardiomyocytes membrane phospholipid bilayer to exhibit antiarrhythmic properties and attenuate myocardial I/R injury [16]. In addition, ω -3 PUFAs combined with flax lignan concentrate treatments significantly reduce cardiomyocyte apoptosis and cellular oxidative stress against myocardial hypertrophy in rats [17]. Moreover, McGuinness et al. [18] have shown that ω -3 PUFA preconditioning can effectively reduce infarct size by upregulating the protein level of myocardial heat shock protein 72 (HSP72), a key preconditioning protein, and increase the myocardial membrane ω -3 fatty acid content in a New Zealand white rabbit model of regional cardiac I/R injury. Taken together, the aforementioned findings indicate that ω -3 PUFAs possess good cardioprotective effect against I/R injury.

Although the cardioprotective effects of ω -3 PUFA preconditioning have been demonstrated, the effect of ω -3 PUFA postconditioning remains to be evaluated. We hypothesized that ω -3 PUFAs may have the potential to be a safe and effective postconditioning agent for the prevention and treatment of clinical myocardial I/R injury. Therefore, the aim of this study was to evaluate the protective effect of ω -3 PUFA postconditioning on myocardium against I/R injury using an isolated perfused rat heart model of I/R.

Materials and Methods

Animals

A total of 60 SPF-class male Sprague-Dawley rats weighing 200–300 g were obtained from the Laboratory Animal Center, Guangzhou University of Traditional Chinese Medicine, Guangdong, China (license No. SCXK (Yue) 2008-0020). The rats were randomly divided into four groups: sham, I/R, I/R-IP, and I/R- ω groups ($n = 15$ for each group). The protocol of this study was approved by the Institutional Animal Care and Use Committee (IACUC). All experiments were performed in accordance with the guidelines and regulations of the IACUC.

Generating the Langendorff Isolated Perfused Rat Heart Model

To generate the Langendorff isolated perfused rat heart model, the rats were intraperitoneally injected with 1,000 U/kg heparin for anticoagulation. After 30 min, the rats were anesthetized with 50 mg/kg pento-barbital sodium, and fixed on an animal operating table. The hearts were quickly extirpated and cannulated via the aorta using a Langendorff isolated perfused heart system (LGF-HL538; Chengdu Instrument Factory, China) and perfused with standard Krebs-Henseleit buffer (KH buffer: NaCl 118 mmol/L, KCl 3.2 mmol/L, KH_2PO_4 1.2 mmol/L, NaHCO_3 25 mmol/L, CaCl_2 2.5 mmol/L, glucose 5.5 mmol/L, MgSO_4 1.6 mmol/L) under a constant flow with a rate of 5 mL/min and a perfusion pressure of 100 cm H_2O . The KH buffer was oxygenated for more than 1 h with carbogen (95% O_2 and 5% CO_2) and kept at 37.0 °C and pH 7.4 during perfusion.

The sham group received a continuous perfusion of 150 min. The three I/R-treated groups sequentially received a 30-min perfusion, a 30-min cardioplegia by perfusion with 10 mL St Thomas' II cardioplegic solution (4 °C, NaCl 147 mmol/L, NaHCO_3 10 mmol/L, KCl 20 mmol/L, MgCl_2 16 mmol/L, CaCl_2 2 mmol/L, pH 7.8) at 20 °C, and a 90-min reperfusion. The I/R-IP group additionally received three cycles of 20-s reperfusion and 20-s coronary reocclusion preceded by the 90 min of reperfusion, whereas the I/R- ω group were perfused with ω -3 PUFAs (0.025% ω -3 fish oil fat emulsion; Huarui Pharmaceutical Co., China) for 15 min before the 90 min of reperfusion. After the 90 min of reperfusion, the isolated perfused rat heart was placed in the ice-cold KH buffer for further use. The ω -3 fish oil fat emulsion consists of EPA (12.50–28.20%), DHA (14.40–30.90%), bean curd acid (1.00–6.00%), palmitic acid (2.50–10.00%), palmitoleic acid (3.00–9.00%), stearic acid (0.50–2.00%), oleic acid (6.00–13.00%), linoleic acid (1.00–7.00%), linolenic acid (<2.00%), octadecatetraenoic acid (0.50–4.00%), eicosanoic acid (0.50–3.00%), arachidonic acid (1.00–1.00%), dodecanoic acid (<1.50%), herring oleic acid (1.50–4.50%), and vitamin E (0.15–0.30%).

Determining the Levels of Markers of Oxidative Stress and Cardiomyocyte Oxidative Injury

Left ventricular free wall myocardial tissue (100 mg) was taken from the isolated rat heart ($n = 5$ for each group), and then cut and mixed with 9 times (w/v) volume of ice-cold saline for ultrasonic homogenization (Dy89 I glass homogenizer; Ningbo Xinzhi Biotechnology Co., China). The myocardial tissue homogenate sample was then centrifuged at 1,000 g at 4 °C for 15 min. The supernatant was placed in liquid nitrogen and then kept at –80 °C for further use. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), and lactate dehydrogenase (LDH) were determined using the SOD assay kit (Cat. No. A001-1-1), MDA assay kit (Cat. No. A003-1), and LDH assay kit (Cat. No. A020-1; all three kits from NanJing JianCheng Bioengineering Institute, China) according to the manufacturer's protocols, respectively.

Transmission Electron Microscopy

A small tissue block of apical myocardium was taken ($n = 5$ for each group). The myocardial tissue block was immersed in 2.5% glutaraldehyde and quickly cut into small tissue blocks with a volume of about 1.0 mm^3 , and then fixed with 2.5% glutaraldehyde. The above procedure should be operated at 4 °C and completed within 1 min to minimize the cardiomyocyte injury. The fixed tissue block was immersed in 1% osmium acid for 2 h, and gradually dehydrated by ethanol and propanol, and embedded in resin. The resin-embedded specimen was cut into ultra-thin slices, and double stained with uranyl acetate and lead citrate. The mitochondrial ultrastructure in the cardiomyocytes was observed by a Philips CM10 transmission electron microscope (Philips Electronics, USA) at 11,500 times magnification.

Determining the Myocardial Infarct Size

The myocardium was cut into 1.0-mm-thick slices from the apex to the bottom of the heart, and stained with triphenyltetrazolium chloride solution at 37 °C for 30 min. After washing with PBS buffer, the samples

Fig. 1. The myocardial infarction ratio of the 4 groups. I/R, ischemia-reperfusion; I/R-IP, I/R-ischemic preconditioning; I/R- ω , perfused with ω -3 PUFAs for 15 min before the 90 min of reperfusion.

were observed by a Nikon eclipse e400 phase contrast microscope (Nikon, Japan). The survival myocardium was stained brick red, and the infarct area was not stained (gray color). The percentage of myocardial infarct area was calculated using Image J 1.37 software (NIH, USA).

Cardiac Function Assessment

Cardiac function assessment was conducted on the isolated perfused rat heart during perfusion and reperfusion. Briefly, a small incision was made at the left atrium, and the pressure measuring sensor was inserted into the left ventricle via the left atrium and mitral valve opening. The sensor was connected to a biomedical signal acquisition and processing system (PCLAB-UE; Beijing Microsignalstar, China) to continuously monitor the hemodynamic parameters. The heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), and rising (+dp/dt_{max}) and descending (−dp/dt_{max}) rate of left ventricular pressure were measured at the end of the 30-min perfusion and at 30, 60, and 90 min after the onset of reperfusion, respectively.

Statistical Analysis

All data are expressed as mean $\pm$ standard deviation. The primary endpoints of the study were myocardial infarct size, the rate of mitochondrial damage, the antioxidant capacity of the myocardium, and the cardiac functions during reperfusion. A one-way analysis of variance was used to compare among groups. When significance was observed, the post hoc Student-Newman-Keuls) method was used for comparison between two groups. A value of $p < 0.05$ was considered statistically significant. All statistical analysis was performed with SPSS version 13.0 for Windows (Chicago, IL, USA).

Results

ω -3 PUFA Postconditioning Reduced Myocardial Infarct Size

To evaluate the protective effect of ω -3 PUFA postconditioning, the myocardial infarct size was compared among the four groups (Fig. 1). The result showed that the myocardial infarction ratio was significantly elevated in the three I/R groups compared with the sham group (all $p < 0.05$). Compared with the I/R group, both the I/R-IP and I/R- ω groups had a significantly lower myocardial infarction ratio (both $p < 0.01$). However, there was no significant difference between the I/R-IP and I/R- ω groups ($p > 0.05$).

Morphology of Myocardial Mitochondria

Since the mitochondrion is a critical organelle involved in I/R injury, the morphology of myocardial mitochondria was evaluated by electron microscopy (EM). As shown in Figure 2a, the myocardial mitochondria in the sham group had normal volume, intact membrane, regular cristae, and normal density of the matrix. Meanwhile, there was no swelling and disruption

Fig. 2. Structural damage of mitochondria of the 4 groups. **a** The electron microscopy (EM) images of myocardial mitochondria of the 4 groups. **b** The structural damages of mitochondria in the EM images were quantitated and represented as the ratio of mitochondria with structural damages (including swelling or disrupted membrane) within a single EM image ($n = 4$ for each group). * $p < 0.05$, compared with sham group; # $p < 0.05$, compared with I/R group; + $p < 0.05$, compared with I/R-IP group. I/R, ischemia-reperfusion; I/R-IP, I/R-ischemic preconditioning; I/R- ω , perfused with ω -3 PUFAs for 15 min before the 90 min of reperfusion.

in the muscle fibers. In the I/R group, severe swellings and deformations could be observed in the myocardial mitochondria. The number of cristae markedly reduced, and many of them were disrupted or vacuous. The electron density was reduced in the mitochondrial matrix, while some mitochondria had condensed matrix, reduced volume, and dense electron density. The muscle fibers were disrupted and dissolved. The injuries in the mitochondria had been reduced in the I/R-IP group, but there remained swellings, deformations, vacuoles, and disrupted cristae in some mitochondria. As for I/R- ω group, the injuries were further reduced, and the majority of the mitochondria had normal morphology. Only a few mitochondria had slight swellings and a decreased number of cristae. Nevertheless, no significant disruption and dissolved could be observed. The quantitative analysis of EM images showed that the rates of mitochondrial damage were significantly elevated in the I/R and I/R-IP groups

Table 1. Comparison of levels of LDH, MDA, and SOD among groups

	LDH, U/g protein	MDA, nmol/mg protein	SOD, U/mg protein
Sham	4,647.77 $\pm$ 1,633.84	1.64 $\pm$.14	374.98 $\pm$ 37.62
I/R	16,602.72 $\pm$ 1,852.67 ^a	3.14 $\pm$.74 ^a	267.09 $\pm$ 23.01 ^a
I/R-IP	11,997.29 $\pm$ 1,394.37 ^{a, b}	2.40 $\pm$ 1.14 ^{a, b}	302.32 $\pm$ 42.35 ^a
I/R- ω	10,089.32 $\pm$ 519.72 ^{a-c}	2.38 $\pm$.54 ^{a-c}	318.72 $\pm$ 46.13 ^{a-c}

LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase; I/R, ischemia-reperfusion; I/R-IP, I/R-ischemic preconditioning; I/R- ω , perfused with ω -3 PUFAs for 15 min before the 90 min of reperfusion. ^a $p < 0.05$ compared with the sham group; ^b $p < 0.05$ compared with the I/R group; ^c $p < 0.05$ compared with the I/R-IP group.

compared with the sham group (both $p < 0.05$; Fig. 2b). The I/R- ω group had a significantly lower rate of mitochondrial damage compared with the I/R and I/R-IP groups (both $p < 0.05$; Fig. 2b).

ω -3 PUFA Postconditioning Improved the Markers of Oxidative Stress and Cardiomyocyte Oxidative Injury

The markers of oxidative stress and cardiomyocyte oxidative injury were assessed and compared among groups. As shown in Table 1, all 3 I/R groups had significantly higher levels of LDH (a cardiomyocyte oxidative injury marker) and MDA (a lipid peroxidation marker) as well as a significantly lower level SOD (a natural antioxidant enzyme) compared with the sham group (all $p < 0.05$). Compared with the I/R group, the levels of the three markers were improved in the I/R-IP and I/R- ω groups (all $p < 0.05$ except for SOD in I/R-IP). Furthermore, the I/R- ω group had significantly lower levels of LDH and MDA and a higher SOD level compared with the I/R-IP group. These results suggested that ω -3 PUFA postconditioning treatment was capable of providing a better protective effect on cardiomyocyte against I/R-induced oxidative stress compared with the conventional ischemic postconditioning.

Cardiac Function Assessment

To determine whether ω -3 PUFA postconditioning has an effect on cardiac function, hemodynamic parameters were evaluated and compared among groups. As shown in Table 2, after the 30-min stabilization period (baseline), there was no statistical significance in all the hemodynamic parameters among the four groups, suggesting that the generated model had a good consistency. However, all the hemodynamic parameters were reduced in the three I/R groups after the onset of reperfusion at three time points compared with the sham group, indicating I/R caused different degrees of damage to cardiac function. Compared with the I/R group, the I/R- ω group had a significantly higher $+dp/dt_{\max}$ ($p < 0.01$) and $-dp/dt_{\max}$ ($p < 0.05$). The $+dp/dt_{\max}$ and $-dp/dt_{\max}$ were also higher in the I/R-IP group than in the I/R group, but the differences did not reach significance ($p > 0.05$). There was no significant difference in LVSP, LVEDP, LVDP, and heart rate between the two postconditioning groups and the I/R group.

Discussion

In this study, we evaluated the cardioprotective effects of ω -3 PUFA postconditioning against myocardial I/R injury in an isolated perfused rat heart model. The results showed that ω -3 PUFA postconditioning significantly reduced I/R-induced myocardial infarct and

Table 2. Comparison of hemodynamic parameters among groups

	Baseline	Time after the onset of reperfusion		
		30 min	60 min	90 min
<i>LVSP</i>				
Sham	107.91±19.36	106.95±18.33	99.46±17.02 ^{bb}	91.14±16.77 ^{bb}
I/R	97.79±13.75	94.6±19.36	81.76±19.42 ^a	73.43±16.01 ^a
I/R-IP	104.93±16.86	103.86±15.83	93.48±19.81	85.43±9.09 ^b
I/R-ω	105.44±15.61	97.54±14.96	91.18±112.68	83.10±11.00
<i>LVEDP</i>				
Sham	3.88±2.59	4.19±4.31 ^{bb}	5.22±5.15 ^{bb}	7.17±5.90 ^{bbc}
I/R	5.39±2.23	13.19±10.42 ^a	15.06±9.10 ^a	18.843±12.94 ^a
I/R-IP	4.48±2.87	8.67±4.50	11.76±6.34 ^a	14.35±7.80
I/R-ω	4.15±2.77	8.58±6.19	12.73±10.04 ^a	15.27±12.53 ^a
<i>LVDP</i>				
Sham	101.69±19.59	101.24±20.02 ^{bb}	92.28±19.86 ^{bbc}	75.87±26.95 ^{bb}
I/R	92.77±17.28	80.88±23.46 ^a	66.70±19.51 ^a	54.82±17.36 ^a
I/R-IP	98.62±18.53	93.83±17.89	80.43±12.50 ^b	68.75±11.92
I/R-ω	99.70±15.13	87.50±16.76	77.01±17.43 ^a	66.00±15.85
<i>HR</i>				
Sham	255.26±29.76	251.00±21.83	240.73±32.91	222.53±48.37
I/R	240.33±32.10	228.40±42.01	215.93±40.72	198.86±49.36
I/R-IP	282.46±38.95	248.61±56.94	229.76±48.55	207.30±51.79
I/R-ω	261.61±40.23	242.15±39.89	211.76±70.30	214.41±45.99
<i>+dp/dt_{max}</i>				
Sham	3,538.35±728.27	3,617.92±699.85 ^{bb}	3,268.36±693.69 ^{bb}	2,881.64±702.13 ^{bb}
I/R	3,153.07±588.84	2,626.97±678.64 ^a	2,204.96±618.53 ^{a, c}	1,901.82±652.10 ^{a, c}
I/R-IP	3,388.38±802.96	3,089.23±679.04 ^a	2,265.59±601.60 ^a	2,317.46±591.02 ^a
I/R-ω	3,710.50±825.20	3,334.76±574.85 ^{bb}	3,015.83±651.67 ^{bb}	2,472.70±712.01 ^{bb}
<i>−dp/dt_{max}</i>				
Sham	2,475.23±662.68	2,422.53±740.21 ^{bb, c}	2,010.99±680.30 ^{bb}	1,739.15±623.90 ^{bb}
I/R	2,067.01±365.91	1,546.02±403.39 ^{a, c}	1,403.29±613.59 ^{a, c}	1,050.91±388.37 ^{a, c}
I/R-IP	2,296.08±472.56	1,744.58±334.55 ^a	1,538.39±286.29 ^a	1,240.27±274.55 ^{a, c}
I/R-ω	2,396.64±58.77	1,987.68±337.37 ^{a, b}	1,860.82±409.43 ^c	1,653.86±327.31 ^{bb}

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; HR, heart rate; +dp/dt_{max}, rising rate of left ventricular pressure; -dp/dt_{max}, descending rate of left ventricular pressure; I/R, ischemia-reperfusion; I/R-IP, I/R-ischemic preconditioning; I/R- ω , perfused with ω -3 PUFAs for 15 min before the 90 min of reperfusion. ^a $p < 0.05$ compared with the sham group; ^b $p < 0.05$, ^{bb} $p < 0.01$ compared with the I/R group; ^c $p < 0.05$ compared with the I/R-IP group.

protected myocardial mitochondria from I/R-induced swellings, ruptures, vacuoles, and cristae disruption. In addition, ω -3 PUFA postconditioning significantly reduced the levels of LDH and MDA and elevated the SOD level. Hemodynamic data showed that ω -3 PUFA postconditioning significantly elevated +dp/dt_{max} and -dp/dt_{max}. These results suggested that ω -3 PUFA postconditioning treatment was capable of providing good cardioprotective effects against I/R injury. To the best of our knowledge, this is the first study reporting the cardioprotective effects of ω -3 PUFA postconditioning.

Free radical damage plays an important role in the pathogenesis mechanism of I/R injury. During ischemia, anaerobic metabolism in cardiomyocytes results in rapid consumption of creatine phosphate and ATP, and accumulation of lactic acid, hypoxanthine, and xanthine

oxidase [19]. During reperfusion, the reactivation of aerobic metabolism induces an accumulation of oxygen-derived free radicals (OFR), which markedly elevate oxidative stress in cardiomyocytes [20, 21]. In this study, ω -3 PUFA postconditioning significantly reduced the levels of MDA (a lipid peroxidation marker) and LDH (a cardiomyocyte oxidative injury marker), as well as increased the level of SOD (a natural antioxidant enzyme) in the myocardium compared with those in untreated I/R animals. Since ω -3 PUFAs are naturally occurring antioxidants, these results suggested that the cardioprotective effects of ω -3 PUFA postconditioning may be associated with the elimination of OFR and relief of oxidative stress, which subsequently attenuate the consumption of SOD, lipid peroxidation, and cardiomyocyte oxidative injury. The detailed molecular mechanism remains to be further investigated. We found that ω -3 PUFA preconditioning treatment can simultaneously reduce infarct size and mitochondrial damage, associated with a significant elevation of SOD level. Likewise, McGuinness et al. [18] have demonstrated that ω -3 PUFA preconditioning significantly elevated the expression of HSP72 in cardiomyocytes and reduced the myocardial infarction size in a New Zealand white rabbit model. Suzuki et al. [22] have reported that overexpression of HSP72 in cardiomyocytes enhances both the level and activity of manganese SOD during myocardial I/R injury, associated with mitochondrial protection in rat. Thereby it is worthy to investigate whether HSP72 participates in the cardioprotective effects of ω -3 PUFA preconditioning.

In addition to the production of a large number of OFR, I/R injury also induces calcium overload in cardiomyocytes. Both OFR and calcium overload contribute to mitochondrial permeability transition pore (MPTP) opening [23]. MPTP opening in early myocardial reperfusion is a critical determinant of I/R injury [24], and is closely associated with I/R-induced mitochondrial damage [23]. I/R-induced MPTP opening can lead to mitochondrial matrix swelling due to an elevation of osmotic pressure, energy depletion due to loss of the mitochondrial transmembrane potential, damage of mitochondrial outer membrane, and cytochrome C release, eventually leading to apoptotic cell death [23]. In this study, ω -3 PUFA postconditioning can markedly attenuate the conditions of swellings, vacuoles, and disrupted cristae in myocardial mitochondria, suggesting that maintenance of the mitochondrial function in cardiomyocytes is implicated in the cardioprotective effects of ω -3 PUFA postconditioning. Recently, Mączewski et al. [25] reported that DHA supplementation prevented calcium overload in rat cardiomyocytes. Meanwhile, Madingou et al. [26] demonstrated that DHA supplementation conferred resistance to MPTP opening in a murine model of myocardial infarction. Therefore, the mechanism by which ω -3 PUFA postconditioning protects mitochondrial function may be due to the reduction of OFR and calcium overload to inhibit MPTP opening. Further study is necessary to elucidate its mechanism.

It has been demonstrated that administration of triglycerides during reperfusion significantly improves myocardial contractility in a canine I/R injury model [27], suggesting that lipid treatment can improve cardiac function. Our hemodynamic data showed that compared with the sham group, the three I/R-treated groups had reductions of all hemodynamic parameters, demonstrating that I/R injury induced damage to cardiac function. Compared with the I/R group, ω -3 PUFA postconditioning significantly elevated $+dp/dt_{\max}$ and $-dp/dt_{\max}$ during reperfusion, suggesting that ω -3 PUFA postconditioning can improve cardiac function. Meanwhile, LVEDP, LVDP, and LVSP were also elevated, but the difference did not reach significance. Our observation that ω -3 PUFA postconditioning can effectively maintain the morphology of myocardial mitochondria may indicate that ω -3 PUFA postconditioning ensured myocardial oxygen supply by maintaining mitochondrial function to improve myocardial contractility.

The cardioprotective effects of ischemic postconditioning have been validated in many studies [28–30]. However, our observations of mitochondrial morphology, oxidative stress markers, and hemodynamic parameters consistently suggested that the cardioprotective effects seem better in ω -3 PUFA postconditioning than in ischemic postconditioning. One

possible explanation for this phenomenon may be that the ischemic time of 30 min in this study may not be long enough to induce a large myocardial ischemic infarction size, and therefore the therapeutic efficacy of ischemic postconditioning was not as good as that of ω -3 PUFA postconditioning.

There are still some limitations in the current study. First, the cardioprotective effects of ω -3 PUFA postconditioning were observed in an isolated perfused rat heart model, which need to be further validated by intravenous administration of PUFAs to a live animal. Because this is a pilot exploratory study, the dose of ω -3 PUFAs used in the isolated heart model was originally designed based on the intravenous dose of ω -3 PUFAs for a human adult (about 0.1–0.2 g/kg body weight). Therefore, this intravenous dose of ω -3 PUFAs could be considered in other in vivo animal models or clinical trials in humans. In addition, we only demonstrated the cardioprotective effects of ω -3 PUFA postconditioning but the detailed molecular mechanisms were not further investigated. For example, the molecular mechanisms by which PUFAs protect against mitochondrial damage and oxidative injury should be further elucidated. Furthermore, the conditions for ω -3 PUFA postconditioning, such as the amount of ω -3 PUFAs and the time and number of cycles of I/R, may still have room for improvement since several hemodynamic parameters did not significantly elevate upon ω -3 PUFA postconditioning. All these limitations should be addressed in a future study.

Conclusion

In summary, our results suggest that ω -3 PUFA postconditioning can significantly reduce infarct size, attenuate myocardial mitochondrial damage, and improve antioxidant capacity and cardiac function during reperfusion, eventually protecting the myocardium against myocardial I/R injury, and may be developed into a therapeutic strategy for myocardial I/R injury.

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Disclosure Statement

The authors declare no conflicts of interest.

References

- 1 Mendes-Braz M, Elias-Miró M, Jiménez-Castro MB, Casillas-Ramírez A, Ramalho FS, Peralta C: The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. *J Biomed Biotechnol* 2012;2012:298657.
- 2 Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124–1136.
- 3 Iliodromitis EK, Lazou A, Kremastinos DT: Ischemic preconditioning: protection against myocardial necrosis and apoptosis. *Vasc Health Risk Manag* 2007;3:629–637.
- 4 Tomai F, Crea F, Chiariello L, Gioffre PA: Ischemic preconditioning in humans: models, mediators, and clinical relevance. *Circulation* 1999;100:559–563.
- 5 Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J: Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol* 2003;285:H579–H588.

- 6 Andreadou I, Iliodromitis EK, Lazou A, Görbe A, Giricz Z, Schulz R, Ferdinandy P: Effect of hypercholesterol-aemia on myocardial function, ischaemia-reperfusion injury and cardioprotection by preconditioning, post-conditioning and remote conditioning. *Br J Pharmacol* 2017;174:1555–1569.
- 7 Chen Z, Spahn DR, Zhang X, Liu Y, Chu H, Liu Z: Morphine postconditioning protects against reperfusion injury: the role of protein kinase c-epsilon, extracellular signal-regulated kinase 1/2 and mitochondrial permeability transition pores. *Cell Physiol Biochem* 2016;39:1930–1940.
- 8 Liu CW, Yang F, Cheng SZ, Liu Y, Wan LH, Cong HL: Rosuvastatin postconditioning protects isolated hearts against ischemia-reperfusion injury: the role of radical oxygen species, PI3K-Akt-GSK-3 β pathway, and mitochondrial permeability transition pore. *Cardiovasc Ther* 2017;35:3–9.
- 9 Zhang WP, Zong QF, Gao Q, Yu Y, Gu XY, Wang Y, Li ZH, Ge M: Effects of endomorphin-1 postconditioning on myocardial ischemia/reperfusion injury and myocardial cell apoptosis in a rat model. *Mol Med Rep* 2016;14:3992–3998.
- 10 Surette ME: The science behind dietary omega-3 fatty acids. *CMAJ* 2008;178:177–180.
- 11 Duda MK, O'Shea KM, Stanley WC: Omega-3 polyunsaturated fatty acid supplementation for the treatment of heart failure: mechanisms and clinical potential. *Cardiovasc Res* 2009;84:33–41.
- 12 Herrera EA, Farías JG, González-Candia A, Short SE, Carrasco-Pozo C, Castillo RL: Ω 3 supplementation and intermittent hypobaric hypoxia induce cardioprotection enhancing antioxidant mechanisms in adult rats. *Mar Drugs* 2015;13:838–860.
- 13 Coquerel D, Kušíková E, Mulder P, Coëffier M, Renet S, Dechelotte P, Richard V, Thuillel C, Tamion F: Omega-3 polyunsaturated fatty acids delay the progression of endotoxic shock-induced myocardial dysfunction. *Inflammation* 2013;36:932–940.
- 14 Nestel P, Clifton P, Colquhoun D, Noakes M, Mori TA, Sullivan D, Thomas B: Indications for omega-3 long chain polyunsaturated fatty acid in the prevention and treatment of cardiovascular disease. *Heart Lung Circ* 2015;24:769–779.
- 15 Jump DB, Depner CM, Tripathy S: Omega-3 fatty acid supplementation and cardiovascular disease. *J Lipid Res* 2012;53:2525–2545.
- 16 Moreno C, Macías A, Prieto A, de la Cruz A, González T, Valenzuela C: Effects of *n*-3 polyunsaturated fatty acids on cardiac ion channels. *Front Physiol* 2012;3:245.
- 17 Ghule AE, Kandhare AD, Jadhav SS, Zangwar AA, Bodhankar SL: Omega-3 fatty acid adds to the protective effect of flax lignan concentrate in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis. *Int Immunopharmacol* 2015;28:751–763.
- 18 McGuinness J, Neilan TG, Sharkasi A, Bouchier-Hayes D, Redmond JM: Myocardial protection using an omega-3 fatty acid infusion: quantification and mechanism of action. *J Thorac Cardiovasc Surg* 2006;132:72–79.
- 19 Frangogiannis NG: Pathophysiology of myocardial infarction; in Pollock DM (ed): *Comprehensive Physiology*. Hoboken, Wiley, 2015, pp 1841–1875.
- 20 Kurian GA, Rajagopal R, Vedantham S, Rajesh M: The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: revisited. *Oxid Med Cell Longev* 2016;2016:1–14.
- 21 Maddika S, Elimban V, Chapman D, Dhalla NS: Role of oxidative stress in ischemia-reperfusion-induced alterations in myofibrillar ATPase activities and gene expression in the heart. *Can J Physiol Pharmacol* 2009;87:120–129.
- 22 Suzuki K, Murtuza B, Sammut IA, Latif N, Jayakumar J, Smolenski RT, Kaneda Y, Sawa Y, Matsuda H, Yacoub MH: Heat shock protein 72 enhances manganese superoxide dismutase activity during myocardial ischemia-reperfusion injury, associated with mitochondrial protection and apoptosis reduction. *Circulation* 2002;106:1270–1276.
- 23 Halestrap AP, Pasdois P: The role of the mitochondrial permeability transition pore in heart disease. *Biochim Biophys Acta* 2009;1787:1402–1415.
- 24 Ong SB, Samangouei P, Kalkhoran SB, Hausenloy DJ: The mitochondrial permeability transition pore and its role in myocardial ischemia reperfusion injury. *J Mol Cell Cardiol* 2015;78:23–34.
- 25 Mączewski M, Duda M, Marciszek M, Kołodziejczyk J, Dobrzyń P, Dobrzyń A, Mackiewicz U: Omega-3 Fatty acids do not protect against arrhythmias in acute nonreperused myocardial infarction despite some antiarrhythmic effects. *J Cell Biochem* 2016;117:2570–2582.
- 26 Madingou N, Gilbert K, Tomaro L, Prud'homme Touchette C, Trudeau F, Fortin S, Rousseau G: Comparison of the effects of EPA and DHA alone or in combination in a murine model of myocardial infarction. *Prostaglandins Leukot Essent Fatty Acids* 2016;111:11–16.
- 27 Van de Velde M, Wouters PF, Rolf N, Van Aken H, Flameng W, Vandermeersch E: Long-chain triglycerides improve recovery from myocardial stunning in conscious dogs. *Cardiovasc Res* 1996;32:1008–1015.
- 28 Altunkaynak HO, Ozelikay AT: Cardioprotective effect of postconditioning against ischemia-reperfusion injury is lost in heart of 8-week diabetic rat. *Gen Physiol Biophys* 2016;35:63–69.
- 29 Khan AR, Binabdulhak AA, Alastal Y, Khan S, Faricy-Beredo BM, Luni FK, Lee WM, Khuder S, Tinkel J: Cardioprotective role of ischemic postconditioning in acute myocardial infarction: a systematic review and meta-analysis. *Am Heart J* 2014;168:512–521.e4.
- 30 Zaman J, Jeddi S, Daneshpour MS, Zarkesh M, Daneshian Z, Ghasemi A: Ischemic postconditioning provides cardioprotective and antiapoptotic effects against ischemia-reperfusion injury through iNOS inhibition in hyperthyroid rats. *Gene* 2015;570:185–190.