

Original Article

The protective effect of omega-3 fatty acids on cardiac injury in lipopolysaccharide-induced acute sepsis rats model

Huaisheng Chen¹, Chengying Hong¹, Wei Li¹, Jing Cao¹, Yuluo Du¹, Wei Wang²

¹Department of Critical Care Medicine, ²Department of Endocrinology, The Second Clinical Hospital of Jinan University, Shenzhen People's Hospital, Shenzhen, Guangdong, China

Received July 19, 2017; Accepted September 10, 2018; Epub October 15, 2018; Published October 30, 2018

Abstract: Sepsis is a systemic inflammatory response to a local infection induced by microbes and endotoxins. Severe sepsis can lead to vital organ dysfunction and corresponds with a high mortality. Despite intensive treatment modalities, such as antibiotics, low molecular weight heparin therapy, and so on, there is still no highly effective therapeutic approach for sepsis. Recently, the combination of antibiotics with drug molecules has attracted attention. The search for new potentially potent medicines and the elucidation of the underlying mechanisms of sepsis pathology are vital for the advancement of sepsis therapy. A lipopolysaccharide (LPS)-induced sepsis rat model was established, the cardiac muscle tissues were stained with Hematoxylin & Eosin (HE), and the changes in cardiac tissue morphology were examined under a transmission electron microscope. The protein expression levels of protein phosphokinase I (PIIC), the inner mitochondrial membrane protein (ANT), and phospholamban (PLN) were analyzed by Western blot. Next, the mRNA expression levels of calsequestrin (CASQ1), Na⁺-Ca²⁺ exchanger (NCX), sarcoplasmic reticulum Ca²⁺ATPase (SERCA2a), ryanodine receptor (RyR2), protein phosphatase type 1α (PPP1CA), mitochondrial carrier, adenine nucleotide translocator 25, member 4 (Slc25a4), and PLN were all analyzed using real-time quantitative PCR. We found a lesion in the mitochondria of the lipopolysaccharide (LPS)-induced sepsis model. After treatment with omega-3 fish oil, the cardiac muscle cell morphology was recovered and the ultrastructural lesions were alleviated. Furthermore, we identified the involvement of genes expressed in cardiac muscle cell plasma, as well as mitochondria related enzymes, among the beneficial effects of fish oil treatment on sepsis. In conclusion, Omega-3 fish oil alleviates mitochondria lesions in our lipopolysaccharide-induced sepsis rat model. This work provides a potential molecular mechanism underlying sepsis treatment with fish oil fatty acids, indicating its possible application in sepsis therapy.

Keywords: Sepsis, omega-3 fish oil fatty acid, mitochondria, cardiac muscle cells

Introduction

Sepsis is a systemic inflammation with extremely high mortality. It is the result of the body's response to infection with microbes and endotoxins, and results in organ dysfunction [1]. The annual incidence of sepsis is about 200-1000 cases per 100,000 people in Sweden [2]. The vital organs, including the kidneys, lungs, and liver, are especially vulnerable to damage and subsequent functional impairment during sepsis. This is known as multi-organ dysfunction syndrome (MODS), the development of which is one of the key markers of sepsis [3]. Despite the rapid increase in sepsis incidence, most sepsis complications remain refractory to treat-

ment, since the underlying pathological mechanism is still elusive. The most widely used treatment is antibiotic usage, and recently, combinatorial therapy has become more popular due to its unique therapeutic effect [4, 5]. Owing to a lack of effective treatment approaches, sepsis is considered a life-threatening clinical syndrome worldwide. Thus, it is urgent to identify new candidate drug molecules.

The omega-3 fatty acids found in fish oils are widely considered to be beneficial for cardiovascular health and certain diseases related to inflammation because of their anti-inflammatory properties [6]. Dietary omega-3 fish oil has been shown to have a beneficial effect on con-

trolling the progression of sepsis [7]; a short term high dose of omega-3 fish oil therapy is safe and has been associated with promising effects on the inflammatory cascade and may therefore play a key role in treatment of patients with sepsis [8]. It is considered to have anti-inflammatory properties [9], and may partially inhibit a number of inflammatory mechanisms, including leucocyte chemotaxis, the expression of adhesion molecules, and leucocyte-endothelial adhesive interactions [10]. In addition, omega-3 fish oil may alleviate acute lung injury and reduce inflammation in sepsis rat models [11]. Its immunomodulatory role in sepsis depends on multiple things, such as the inhibition of inflammation factor production, blockage of the nuclear factor NF-kappa B pathway, and inhibition of Toll-like receptor signal transduction. Omega-3 fish oil has a protective effect on the cardiovascular system, which may correlate with its protection of mitochondria. Patch clamp studies have indicated that omega-3 fish oil can elongate the action potential of cardiac muscle cells, which is likely to protect against arrhythmia [12]. This effect is probably due to its regulation of ion channels in cardiac cells. Omega-3 fish oil has been reported to affect the $\text{Na}^{2+}/\text{Ca}^{2+}$ exchange, and maintain an intracellular Ca^{2+} concentration at resting potential, thereby reducing the occurrence of arrhythmia induced by Ca^{2+} overload. These studies directly prove that omega-3 fatty acids are a potential candidate drug molecule in combinatorial sepsis therapy. Nevertheless, the underlying mechanisms of these beneficial effects are not completely understood. One explanation is that omega-3 fatty acid affects the immune system through altering CD^{4+} T cell homeostasis [13], and Shinto, *et al.*, found that they modulate the immune cell production of matrix metalloproteinase-9 (MMP-9) to benefit multiple sclerosis patients [14]. In addition, fish oil fatty acids inhibit Toll-like receptor 4 (TLR4) and its downstream expression, protecting vital organs from inflammation [15, 16]. Fish oil fatty acids have also been reported to have a protective effect in cardiac surgery [17], a process that is likely related to mitochondrial function [18]. However, it is still not completely clear whether fish omega-3 fatty acids can prevent the cardiac lesions in sepsis.

In this study, we used a lipopolysaccharide (LPS)-induced sepsis rat model to evaluate the effects of omega-3 fish oil on cardiac muscle

cells under septic attack. We elucidated the ultrastructural alteration in mitochondria that was induced by treatment with omega-3 fish oil. In addition, we found that this effect was correlated with the altered expression of key genes in cardiac muscle cell plasma, as well as mitochondria related proteins. CASQ1, RyR2, NCX, PLN, SERCA2, PPP1CA and PIIC are molecules related with cardiac cell plasma, Slc25a4, and ANT are mitochondria related enzymes. These molecules are important regulators of myocardial function, mitochondria and the sarcoplasmic reticulum are very important organelles for the regulation of energy metabolism and Ca^{2+} transport in the myocardium, which plays an important role in the pathogenesis of sepsis-induced myocardial injury. This work provides a medical basis for the treatment of sepsis with omega 3 fish oil and makes the combinatorial strategy more optimal.

Materials and methods

Ethical statement

All animal handling procedures were carried out in accordance with the protocols approved by the Institutional Animal Care and Use Committee of Shenzhen People's Hospital.

Animals

Forty male Sprague-Dawley rats (5-6 weeks old, 150-180 g) were obtained from the Animal Center of Shenzhen University (Shenzhen, China). The animals were kept in a temperature controlled room at $22^{\circ}\text{C}\pm 2^{\circ}\text{C}$ in a single cage with a relative humidity of 45%-55% under a 12 hr/12 hr light and dark cycle. Throughout the study, all animals were allowed free access to food and water.

Lipopolysaccharides (LPS)-induced sepsis model and grouping

The animals were randomly divided into four groups ($n=10$ per group), including the control, sepsis, pretreatment, and treatment groups. The rats in the control group were intravenously injected with 3 ml saline for two days. The sepsis model was established according to a previous study [19]; rats were intravenously injected with 15 mg/kg LPS (from *Escherichia coli* D55:B5) (Sigma, USA) dissolved in 3 ml phosphate buffered saline (PBS) once a day for two days. Rats in the pretreatment group were fed

300 mg omega-3 fish oil (containing 180 mg EPA and 120 mg DHA) for 15 days before undergoing injection with 15 mg/kg LPS. In the treatment group, after LPS injection, sepsis models were immediately injected with 2 ml of 10% omega-3 fish oil fatty acid (containing 0.2 g fish oil) and 20% long-chain fatty acid (containing 0.2 g fatty acid) twice a day for two days.

H&E staining

All animals were euthanized with 300 mg/kg sodium barbital on day 3. The cardiac muscle tissues were dissected and cut into 1 mm³ pieces. Sections were deparaffinized in xylene and dehydrated in alcohol. They were then stained in Harris hematoxylin solution for 8 minutes before bluing in 0.2% ammonia water or saturation lithium carbonate solution for 1 minute. After rinsing in 95% alcohol, sections were counterstained in eosin-phloxine solution for 1 minute. Finally, sections were mounted with a xylene based mounting medium. All images were captured with a Nikon microscope (Tokyo, Japan). Image J was analyzed by ImageJ software. The degree of cardiac injury was assessed by myocardial necrosis, hemorrhage, interstitial edema and neutrophil infiltration, the scores of injury was graded as: 0 (normal), 1 (the proportion of the lesion area $\leq 1/4$), 2 ($1/4 < \text{the proportion of the lesion area} < 1/2$), 3 (the proportion of the lesion area $\geq 1/2$), the final score was simply the sum of each item score.

Transmission electron microscope

Cardiac tissues were sequentially fixed by 3% glutaraldehyde, 1.5% paraformaldehyde, and 1% osmic acid-1.5% potassium ferrocyanide. They were then dehydrated with ethanol-acetone and embedded in ethoxyline resin. Sections were stained for 20 min with 2% (w/v) aqueous uranyl acetate, followed by 6 min with Reynolds lead citrate (Sigma-Aldrich, USA), and were later examined in a Philips EM208 transmission electron microscope (Philips, Eindhoven, The Netherlands). The damage was scored according to Flameng method [20].

Western blotting

Cardiac tissue lysate was loaded onto a polyacrylamide gel, then blotted onto a polyvinylidene difluoride (PVDF) membrane. After block-

ing with PBST containing 5% nonfat dry milk, the membrane was incubated with antibodies against PIIC, ANT, PLN, and GAPDH (purchased from Cell Signaling, NY, USA). Peroxidase-linked IgG (Abcam, IL, USA) was used as a secondary antibody. These proteins were visualized using an ECL western blotting detection kit (Amersham Biosciences, USA). All images were analyzed with Image J software (National Institute of Health, MD, USA).

RNA extraction and real-time quantitative PCR

Total RNA extraction was performed using TRIzol reagent (Life Technologies, USA), according to the manufacturer's instructions. Two μg of total extracted RNA was subjected to reverse transcription. The cDNA synthesis was performed with a one-step RT-PCR kit from Takara (Dalian, China). SYBR Green (Toyobo, Japan) RT-PCR amplification and real time fluorescence detection were performed using an ABI 7300 real-time PCR thermal cycle instrument (ABI, USA), according to the supplied protocol. The relative gene expression levels were calculated with the $2^{-\Delta\Delta C_t}$ method. The primers used were as follows: CASQ1, (forward) 5'-GGCTT-GTTGGGGTCTGTATG-3' and (reverse) 5'-TGAA-GGGAGTGACGGAAGAA-3'; NCX, (forward) 5'-TT-GGCTGCACCATTTGGTCTGAAAG-3' and (reverse) 5'-ACACTTTGAACTGTTCCCGTTGG-3'; SERCA-2a, (reverse) 5'-GCACCGAGATTGGGAAGA-3' and (reverse) 5'-ACGCAGATGAGGGAGATGAC-3'; RyR2, (forward) 5'-AAG TGC CAG AGT CAG CAT TC-3', and (reverse) 5'-AGT AGT ATC CAA TGA TGC AG-3'; PPP1CA, (forward) 5'-AGGAGAGC-CAGGCCGGAAGG-3' and (reverse) 5'-TGATGT-GCTCTGCAGATGAGGTCC-3'; Slc25a4, (forward) 5'-TAAGAA GCT ACA CAC AGC CTA C-3' and (reverse) 5'-ATC AGA AAC TGT CTG CTC AGA C-3'; PLN, (forward) 5'-TGAGGACAAACAGACT-GGAC-3' and (reverse) 5'-AGTCTGCCTTTGAGC-CTAGC-3'; The relative expression levels were normalized to the expression of endogenous GAPDH (forward) (5'-GGTATCGTGAAGGACTC-ATGAC-3' and (reverse) 5'-ATGCCAGTGAGCTT-CCCGT TCAGC-3').

Statistical analysis

All of the statistical analysis data was analyzed with SPSS 19.0 software (SPSS Inc. Chicago, IL, USA). Data are presented as mean \pm SEM and were analyzed with the two-tail Student's t-test and analysis of variance (one way ANOVA

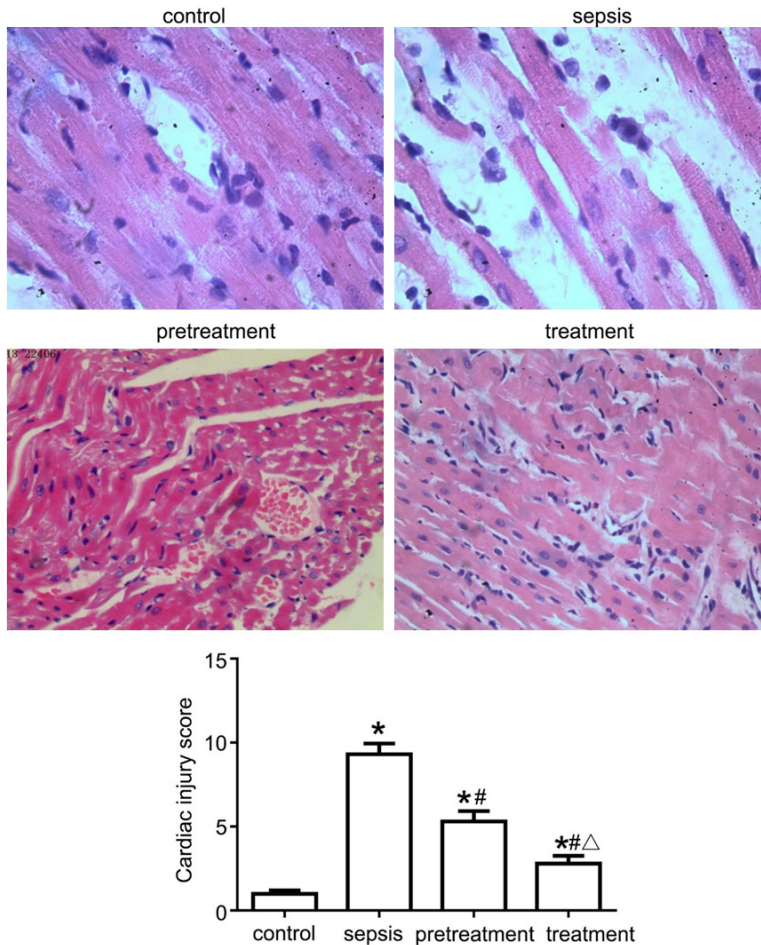


Figure 1. HE staining of cardiac muscle tissues in rats from the control, sepsis, fish oil pretreatment, and fish oil treatment after sepsis occurrence. The images were analyzed and quantitated by ImageJ software. Omega-3 fish oil fatty acid prevented cell death in cardiac muscle. * $P < 0.05$ vs. control; # $P < 0.05$ vs. sepsis; Δ $P < 0.05$ vs. pretreatment.

with Turkey test). A p value less than 0.05 was considered to be statistically significant.

Results

Omega-3 fish oil prevents cardiac muscle cell death in sepsis

First, we determined whether fish oil fatty acid could exert a protective effect on cardiac muscle cells from sepsis attack. In this established rat sepsis model, cardiac muscle cells showed increased volume in the cell bodies and a misalignment of the cardiac muscles (**Figure 1**, sepsis), which are typical characteristics of cardiac cell death or apoptosis, compared to the saline control with a normal cell morphology and well-characterized muscle alignment

(**Figure 1**, control). In contrast, animals with both the fish oil pretreatment before LPS injection and the fish oil treatment after LPS injection showed beneficial effects on the prevention of cell death (**Figure 1**, pretreatment and treatment). Both the pretreatment and treatment groups demonstrated at least partial recovery of cell morphology, as well as a normal alignment of cardiac muscles, which showed to be more therapeutically effective in the group treated with fish oil after the occurrence of sepsis. In addition, the cardiac injury score was shown in **Figure 1**. The injury score in sepsis group was significantly increased compared with control group, the injury scores in pretreatment and treatment groups were significantly decreased compared with sepsis group, which indicate that fish oil could reduce cardiac injury either used before or after the occurrence of sepsis. Collectively, these data show that Omega-3 fish oil may have a therapeutic effect on sepsis-induced cardiac muscle cell injury.

Omega-3 fish oil alleviated the sepsis-induced ultrastructural alteration in cardiac muscle cells

Next, we detected whether the omega-3 fish oil could alter the ultrastructure of cardiac muscle cells. And Flameng score was applied to analyze the degree of mitochondrial damage. In the control rats, the cells were aligned with regular shapes (**Figure 2**, control). However, LPS injection induced cell misalignment and an irregular shape of cardiac cells in the rat models (**Figure 2**, sepsis). Meanwhile, severe mitochondrial lesions were observed in the sepsis model. In contrast, treatment with omega-3 fish oil recovered the ultrastructural damage in sepsis models, and alleviated the mitochondrial damage in cardiac muscle cells, whether given before or

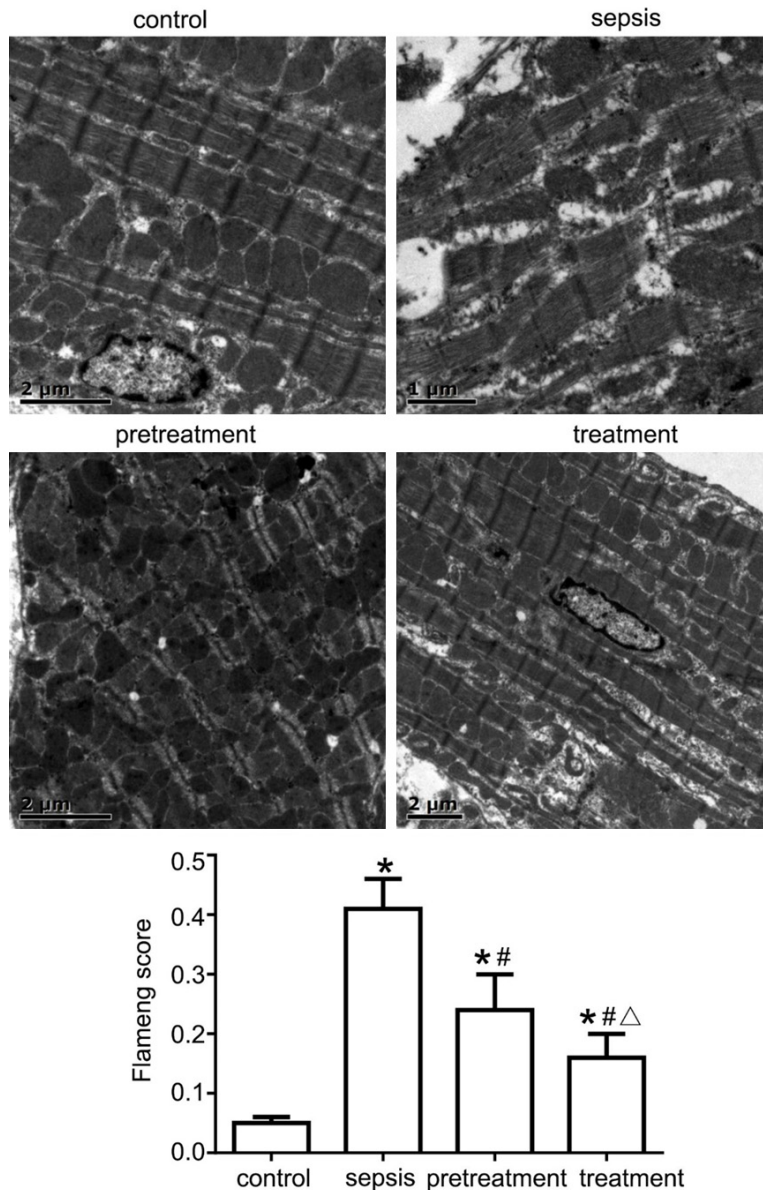


Figure 2. Transmission electron microscopy. Ultrastructural alteration in cardiac muscle cells after treatment with fish oil fatty acid. Flameng score was applied to analyze the degree of mitochondrial damage. Scale bars in the control, pretreatment, and treatment groups are 2 μm , and 1 μm in the sepsis group. * $P < 0.05$ vs. control; # $P < 0.05$ vs. sepsis; $\Delta P < 0.05$ vs. pretreatment.

after sepsis occurrence (**Figure 2**, pretreatment and treatment). This suggests that omega-3 fish oil has the capacity to reverse the ultrastructural alteration induced by sepsis.

Omega-3 fish oil altered the expression levels of key genes in cardiac muscle cells

Since omega-3 fish oil improved the morphology of cardiac muscle cells, we tried to elucidate the underlying mechanism involved in this pro-

cess. We found that treatment with fish oil changed the mRNA expression level of genes expressed in cardiac muscle cell plasma and mitochondria related enzymes. As shown in **Figure 3**, the expression level of PLN was significantly increased in the sepsis models, compared to the control (* $P < 0.05$), and either pretreatment or treatment with omega-3 fish oil significantly increased the PLN expression level (# $P < 0.05$, compared with the sepsis group). Nevertheless, we did not observe a significant change in the expression levels of CASQ1, RyR2, or slc25a4 after sepsis attack. In the omega-3 fish oil-pretreated model, we found that, compared with the sepsis group, there were significant alterations in the expressions of RyR2, PPP1CA, slc25a4, PLN, and SERCA2 (# $P < 0.05$), but not in other genes. In addition, in the fish oil-treated sepsis models, the expression levels of NCX, RyR2, slc25a4, and PLN were increased drastically (# $P < 0.05$). Similar data was obtained with western blotting analysis. Treatment with omega-3 fish oil induced increased expression levels of PIIC and PLN, while the expression level of ANT was only increased in the group treated with fish oil after the occurrence of sepsis, but not in the pretreatment group (**Figure 4**).

Collectively, these data demonstrate that fish oil fatty acid treatment in a rat sepsis model impacted the expression levels of cardiac cell plasma genes and mitochondria related enzymes.

Discussion

Sepsis is a systemic inflammatory response to infection with microbes and endotoxins. Severe sepsis can lead to vital organ dysfunction and

Protective effect of omega-3 fish oil on sepsis

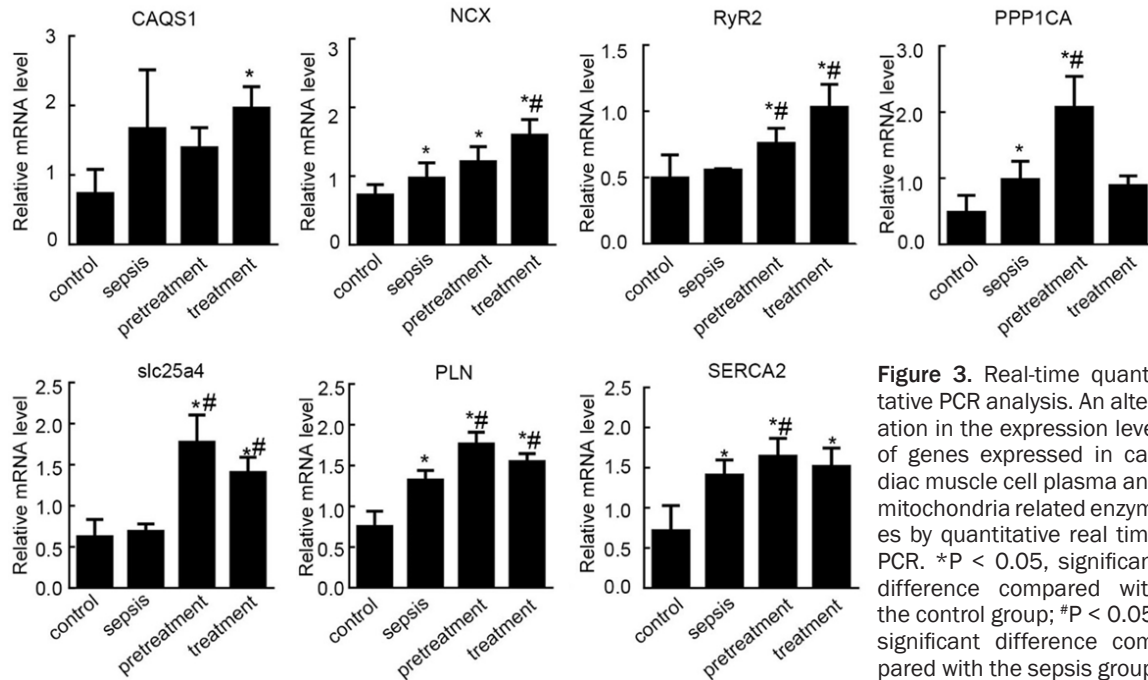


Figure 3. Real-time quantitative PCR analysis. An alteration in the expression level of genes expressed in cardiac muscle cell plasma and mitochondria related enzymes by quantitative real time PCR. *P < 0.05, significant difference compared with the control group; #P < 0.05, significant difference compared with the sepsis group.

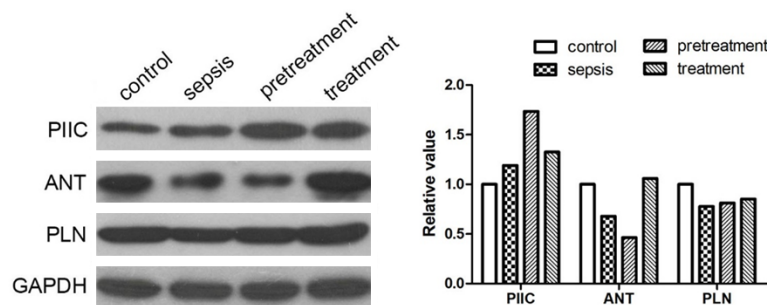


Figure 4. Western blot. Alteration in the expression levels of PIIC, ANT, and PLN expressed in cardiac muscle cell plasma. Treatment with omega-3 fish oil induced the increased expression levels of PIIC and PLN, while the expression level of ANT only increased in the treatment group after the occurrence of sepsis, but not in the pretreatment group.

result in high mortality. Recently, the combination of antibiotics with drug molecules to treat sepsis has attracted more attention. In this study, we established LPS-induced rat sepsis models to evaluate the potential protective effects of omega-3 fish oil on sepsis. Our results indicate that omega-3 fish oil can alleviate mitochondrial lesions in the sepsis model. This process is likely to be related with the altered expressions of genes expressed in cardiac cell plasma and mitochondria related enzymes. This work will facilitate the further understanding of the pathological mechanism underlying the role of mitochondria and the sarcoplasmic reticulum in sepsis.

Cardiac dysfunction is one of the major hallmarks of sepsis, and is characterized by changes in contractility, ventricular response to fluid therapy, and ventricular dilatation [21]. Several factors have been reported to affect cardiac dysfunction induced by sepsis, including IκB Kinase [22], NLRP3 inflammasome [23], sarcoplasmic reticulum calcium [24], etc. These studies indicate that the immune response maybe critical in sepsis induced cardiac dysfunction.

In addition, lesions in cellular organelles are another signature of organ dysfunction. In this study, we found that mitochondria from cardiac cells were targeted in the LPS-induced sepsis model with the occurrence of ultrastructural lesions. This is consistent with findings in other cell types, including hippocampus neurons [25], hepatocytes [26], skeletal cells [27], etc. This demonstrates that sepsis leads to mitochondria lesions in many tissues, implying the successful establishment of a sepsis model in rats. Mitochondria and the sarcoplasmic reticulum are very important organelles for the regulation of energy metabolism and Ca^{2+} transport in the myocardium, which plays an

important role in the pathogenesis of sepsis-induced myocardial injury [28]. In this study, we found that treatment with omega-3 fish oil could change the mRNA level of genes expressed in the cardiac muscle cell plasma, as well as mitochondria related enzymes. The mRNA expression levels of NCX, PPP1CA, PLN, and SERCA2 were increased in the sepsis models. We also found significantly increased expressions of RyR2, PPP1A, slc25a, PLN and SERCA2 in the omega-3 fish oil pretreatment group. In the fish oil-treated sepsis models, the expression levels of NCX, RyR2, slc25a4 and PLN were drastically increased. The western blot results indicated that treatment with fish oil induced an increased protein expression of PIIC and PLN, while the expression level of ANT was only increased in the group treated after the occurrence of sepsis, but not in the pretreatment group.

Calsequestrin has been recognized as the main Ca^{2+} binding protein inside the sarcoplasmic reticulum, the organelle that stores and upon demand mobilizes Ca^{2+} for contractile activation of muscle. Cardiac muscle contraction requires sarcoplasmic reticulum Ca^{2+} release mediated by the quaternary complex comprising the ryanodine receptor 2 (RyR2), calsequestrin 2 (CSQ2), junctin and triadin, it is reported that a direct interaction exists between RyR2 and CSQ2 [29]. The Ca^{2+} concentration in the sarcoplasm is regulated by the functional calcium pump protein in sarcoplasmic reticulum membranes. As a major Ca^{2+} pump in the sarcoplasmic reticulum of cardiomyocytes, SERCA2 controls the relaxation and contraction of the cardiomyocyte, transporting Ca^{2+} ions inside the cytoplasm. SERCA2 is a sarcoplasmic reticulum transmembrane protein that mediates calcium re-uptake from the myoplasm and is expressed at a high level in cardiac myocytes [30]. Dephosphorylated PLN is an inhibitor of SERCA2a and is phosphorylated by protein kinase A (PKA). Therefore, the activity of SERCA2a is regulated by PLN. Heinis, *et al.*, [31] reported that cardiac function was maintained at a normal level in a novel genetic mouse model of inducible severe and progressive SERCA2 deficiency, and that this might be due to the regulation of PLN28. SERCA2a/PLN complexes are important regulators of myocardial function and play key roles in cardiac insufficiency caused by sepsis. PLN can be dephosphorylated by protein phosphatase 1 (PPI),

whereas a dephosphorylated form of PLN inhibits the apparent affinity of SERCA2 for Ca^{2+} [32]. Li, *et al.*, [33] developed a SERCA2 knockout in mice for seven-weeks, demonstrating that the elevated Na^+ was due to an increased influx of Na^+ through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and the Na^+/H^+ exchanger, with the latter being exacerbated by intracellular acidosis. Furthermore, the upregulation of NCX resulted in increased ATP consumption for ion transport, suggesting that heart failure may be characterized by metabolic abnormalities. In this study, we found an abnormal expression of PPP1CA in myocardial cells from LPS-induced sepsis rats. Therefore, mitochondrial lesions are a potential target for sepsis treatment.

Omega-3 fish oil has been shown to have anti-inflammatory properties, counteracting the inflammation induced by sepsis. Furthermore, this work indicates that fish oil also alleviates mitochondria lesions caused by sepsis. This is further confirmed by the finding that the expression levels of critical mitochondria-related genes are altered after fish oil treatment in the sepsis model. This effect is potentially due to its anti-inflammatory property, since inflammation is a major cause of mitochondria damage [34]. The potential targets include Toll-like receptor 2 [35] and interleukin-6 [36]. Consistent with the current body of work, our studies found a beneficial effect of omega-3 fatty acids on cardiac tissues. Langlois, *et al.*, found that omega-3 polyunsaturated fatty acids are protective in cardiac surgery patients [37, 38]. Meanwhile, dietary fish oil can improve cardiac efficiency [39]. All of these findings together demonstrate a direct correlation between omega-3 fish oil and a therapeutic effect on cardiac diseases. Mechanistically, it is not fully understood which molecules or signaling pathways control or modulate this process, but the clinical efficacy of dietary fish oil is due to the intrinsic modulation of intracellular Ca^{2+} handling [40].

Conclusions

In conclusion, this study expands the knowledge of the effect of omega-3 fish oil fatty acids on cardiac muscle cells on an ultrastructural level. We elucidated the involvement of mitochondria and pinpointed the key genes participating in this process. This work provided mechanistic evidence to support the known beneficial role of fish oil in cardiac dis-

ease treatment. Future work will focus on the mitochondria-dependent apoptotic pathway, in order to elucidate the equilibrium in cell turnover when fish oil exerts its effects on the cardiac muscle.

Acknowledgements

This study was supported by Shenzhen Science and Technology Innovation Committee (JC-YJ20140416122811971).

Disclosure of conflict of interest

None.

Abbreviations

PIIC, protein phosphokinase I; ANT, the inner mitochondrial membrane protein; PLN, phospholamban; CASQ1, calsequestrin 1; NCX, Na⁺-Ca²⁺ Exchanger; SERCA2, sarcoplasmic reticulum Ca²⁺ATPase; RyR2, ryanodine receptor 2; PPP1CA, protein phosphatase type 1α; Slc-25a4, mitochondrial carrier, adenine nucleotide translocator 25, member 4; MMP-9, matrix metalloproteinase-9; TLR4, Toll-like receptor 4.

Address correspondence to: Huaisheng Chen and Wei Wang, The Second Clinical Hospital of Jinan University, Shenzhen People's Hospital, No. 1017, Dong Men North Road, Luohu District, Shenzhen 518020, Guangdong, China. Tel: +86136325718-54; Fax: +86-25533497; E-mail: sunshinic@hotmail.com (HSC); wangwszhen@yeah.net (WW).

References

- [1] Rossaint J and Zarbock A. Pathogenesis of multiple organ failure in sepsis. *Crit Rev Immunol* 2015; 35: 277-291.
- [2] Wilhelms SB, Huss FR, Granath G and Sjoberg F. Assessment of incidence of severe sepsis in Sweden using different ways of abstracting international classification of diseases codes: difficulties with methods and interpretation of results. *Crit Care Med* 2010; 38: 1442-1449.
- [3] Ramirez M. Multiple organ dysfunction syndrome. *Curr Probl Pediatr Adolesc Health Care* 2013; 43: 273-277.
- [4] Aydin S, Caylan R, Aydin K, Yulug E, Yenilmez E and Koksai I. The influence of G-CSF addition to antibiotic treatment of experimental sepsis on pulmonary tissue. *J Natl Med Assoc* 2005; 97: 1489-1495.
- [5] Fei Y, Wang W, Kwiecinski J, Josefsson E, Pullerits R, Jonsson IM, Magnusson M and Jin T. The combination of a tumor necrosis factor inhibitor and antibiotic alleviates staphylococcal arthritis and sepsis in mice. *J Infect Dis* 2011; 204: 348-357.
- [6] Opperman M and Benade S. Analysis of the omega-3 fatty acid content of South African fish oil supplements: a follow-up study. *Cardiovasc J Afr* 2013; 24: 297-302.
- [7] Svahn SL, Ulleryd MA, Grahnmemo L, Ståhlman M, Borén J, Nilsson S, Jansson JO and Johansson ME. Dietary omega-3 fatty acids increase survival and decrease bacterial load in mice subjected to *S. aureus*-induced sepsis. *Infect Immun* 2016; 84: 1205-1213.
- [8] Hosny M, Nahas R, Ali S, Elshafei SA and Khaled H. Impact of oral omega-3 fatty acids supplementation in early sepsis on clinical outcome and immunomodulation. *Egyptian Journal of Critical Care Medicine* 2013; 1: 119-126.
- [9] Calder PC. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochim Biophys Acta* 2015; 1851: 469-484.
- [10] Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013; 75: 645-662.
- [11] Li X, Zhang X, Yang E, Zhang N, Cao S and Zhou Y. Fish oil-supplemented parenteral nutrition could alleviate acute lung injury, modulate immunity, and reduce inflammation in rats with abdominal sepsis. *Nutr Res* 2015; 35: 784-791.
- [12] Chung MK. Omega-3 fatty acid supplementation for the prevention of arrhythmias. *Curr Treat Options Cardiovasc Med* 2008; 10: 398-407.
- [13] Chang YF, Hou YC, Pai MH, Yeh SL and Liu JJ. Effects of ω-3 polyunsaturated fatty acids on the homeostasis of CD4⁺ T cells and lung injury in mice with polymicrobial sepsis. *JPEN J Parenter Enteral Nutr* 2017; 41: 805-814.
- [14] Shinto L, Marracci G, Bumgarner L and Yadav V. The effects of omega-3 Fatty acids on matrix metalloproteinase-9 production and cell migration in human immune cells: implications for multiple sclerosis. *Autoimmune Dis* 2011; 2011: 134592.
- [15] Chen F, Liu Y, Zhu H, Hong Y, Wu Z, Hou Y, Li Q, Ding B, Yi D and Chen H. Fish oil attenuates liver injury caused by LPS in weaned pigs associated with inhibition of TLR4 and nucleotide-binding oligomerization domain protein signaling pathways. *Innate Immunity* 2013; 19: 504.
- [16] Liu Y, Chen F, Li Q, Odle J, Lin X, Zhu H, Pi D, Hou Y, Hong Y and Shi H. Fish oil alleviates activation of hypothalamic-pituitary-adrenal axis associated with inhibition of TLR4 and NOD signaling pathways in weaned piglets after a lipopolysaccharide challenge. *J Nutr* 2013; 143: 1799-1807.

- [17] Akintoye E, Wu JH, Hou T, Song X, Yang J, Hammock B and Mozaffarian D. Effect of fish oil on monoepoxides derived from fatty acids during cardiac surgery. *J Lipid Res* 2016; 57: 492-8.
- [18] Ting HC, Chao YJ and Hsu YH. Polyunsaturated fatty acids incorporation into cardiolipin in H9c2 cardiac myoblast. *J Nutr Biochem* 2015; 26: 769-775.
- [19] He ZB, Chen P, Peng ZY and Jin LY. Effect of corynoline isolated from *corydalis bungeana* turcz on lipopolysaccharides-induced sepsis in vivo and in vitro. *Tropical Journal of Pharmaceutical Research* 2014; 13: 81-86.
- [20] Vandeplasse G, Wouters L, Hermans C, Xhonneux R, Borgers M and Flameng W. An efficient method to evaluate protective drugs against regional ischemia of the heart. *Drug Development Research* 1986; 8: 417-424.
- [21] Zanotti-Cavazzoni SL and Hollenberg SM. Cardiac dysfunction in severe sepsis and septic shock. *Curr Opin Crit Care* 2009; 15: 392.
- [22] Chen J, Kieswich JE, Chiazza F, Moyes AJ, Gobetti T, Purvis GS, Salvatori DC, Patel NS, Perretti M and Hobbs AJ. I κ B kinase inhibitor attenuates sepsis-induced cardiac dysfunction in CKD. *J Am Soc Nephrol* 2017; 28: 94-105.
- [23] Kalbitz M, Fattahi F, Grailer JJ, Jajou L, Malan EA, Zetoune FS, Huber-Lang M, Russell MW and Ward PA. Complement-induced activation of the cardiac NLRP3 inflammasome in sepsis. *FASEB J* 2016; 30: 3997-4006.
- [24] Sepúlveda M, Gonano LA, Viotti M, Morell M, Blanco P, Alarcón ML, Ramos IP, Carvalho AB, Medei E and Vila PM. Calcium/calmodulin protein kinase II-dependent ryanodine receptor phosphorylation mediates cardiac contractile dysfunction associated with sepsis. *Crit Care Med* 2016; 1.
- [25] Jing W, Zhang M, Hao S, Ming J, Ji M, Qiu L, Sun X, Yang J and Li K. Mitochondria-targeted peptide reverses mitochondrial dysfunction and cognitive deficits in sepsis-associated encephalopathy. *Mol Neurobiol* 2015; 52: 783-791.
- [26] Kantrow SP, Taylor DE, Carraway MS and Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys* 1997; 345: 278-288.
- [27] Welty-Wolf KE, Simonson SG, Huang YC, Frasca PJ, Patterson JW and Piantadosi CA. Ultrastructural changes in skeletal muscle mitochondria in gram-negative sepsis. *Shock* 1996; 5: 378-384.
- [28] Pesaturo JA and Gwathmey JK. The role of mitochondria and sarcoplasmic reticulum calcium handling upon reoxygenation of hypoxic myocardium. *Circ Res* 1990; 66: 696-709.
- [29] Handhale A, Ormonde CE, Thomas NL, Bralesford C, Williams AJ, Lai FA and Zissimopoulos S. Calsequestrin interacts directly with the cardiac ryanodine receptor luminal domain. *J Cell Sci* 2016; 129: 3983-3988.
- [30] Greene AL, Lalli MJ, Ji Y, Babu GJ, Grupp I, Sussman M and Periasamy M. Overexpression of SERCA2b in the heart leads to an increase in sarcoplasmic reticulum calcium transport function and increased cardiac contractility. *J Biol Chem* 2000; 275: 24722-24727.
- [31] Heinis FI, Andersson KB, Christensen G and Metzger JM. Prominent heart organ-level performance deficits in a genetic model of targeted severe and progressive SERCA2 deficiency. *PLoS One* 2013; 8: e79609.
- [32] Nef HM, Möllmann H, Skwara W, Bölk B, Schwinger RH, Hamm Ch, Kostin S, Schaper J and Elsässer A. Reduced sarcoplasmic reticulum Ca²⁺-ATPase activity and dephosphorylated phospholamban contribute to contractile dysfunction in human hibernating myocardium. *Mol Cell Biochem* 2006; 282: 53-63.
- [33] Li L, Louch WE, Niederer SA, Aronsen JM, Christensen G, Sejersted OM and Smith NP. Sodium accumulation in SERCA knockout-induced heart failure. *Biophys J* 2012; 102: 2039-2048.
- [34] Contreras-Shannon V, Heart DL, Paredes RM, Navaira E, Catano G, Maffi SK and Walss-Bass C. Clozapine-induced mitochondria alterations and inflammation in brain and insulin-responsive cells. *PLoS One* 2013; 8: e59012.
- [35] Leone M and Moreau R. Leukocyte Toll-like receptor 2-mitochondria axis in sepsis: unraveling immune response sophistication. *Anesthesiology* 2014; 121: 1147-1149.
- [36] Lowes DA, Webster NR, Murphy MP and Galley HF. Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *Br J Anaesth* 2013; 110: 472-480.
- [37] Langlois PL, Hardy G and Manzanares W. Omega-3 polyunsaturated fatty acids in cardiac surgery patients. *JPEN J Parenter Enteral Nutr* 2017; 41: 152-154.
- [38] Langlois PL, Hardy G and Manzanares W. Omega-3 polyunsaturated fatty acids in cardiac surgery patients: an updated systematic review and meta-analysis. *Clin Nutr* 2017; 36: 737-746.
- [39] Loiselle DS, Han JC, Goo E, Chapman B, Barclay CJ, Hickey AJ and Taberner AJ. Thermodynamic analysis questions claims of improved cardiac efficiency by dietary fish oil. *J Gen Physiol* 2016; 148: 183-193.
- [40] McLennan PL. Cardiac physiology and clinical efficacy of dietary fish oil clarified through cellular mechanisms of omega-3 polyunsaturated fatty acids. *Eur J Appl Physiol* 2014; 114: 1333-1356.