Review

Mitochondrial Antioxidant Enzymes and Endurance Exercise-induced Cardioprotection against Ischemia-Reperfusion Injury

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Article information

Received: March 8th, 2018; Revised: March 20th, 2018; Accepted: March 21st, 2018; Published: March 21st, 2018

Cite this article


ABSTRACT

Coronary artery disease (CAD) is the most common cause of myocardial injuries induced by prolonged cessation of blood flow (ischemia) to cardiac myocytes due to atherosclerosis. For several decades, many clinical trials have been applied to protect hearts against ischemia and reperfusion (I/R) injuries, but failed to show significant improvement in the restoration of cardiac function. By contrast, growing evidence has shown that a non-pharmacological strategy, endurance exercise, provides cardioprotection against ischemic myocardial injuries. Despite the prominent cardioprotective benefit; however, the exact molecular and cellular protective mechanisms remain an exciting issue. Nonetheless, given that excess production of reactive oxygen species (ROS) is a primary mediator of cardiac injuries caused by an I/R insult, improved myocardial antioxidant capacity in response to endurance exercise has been suggested to be a key mechanism against I/R injuries, in particular. Therefore, this review will focus the role of endurance exercise-induced improvement in myocardial antioxidants in cardioprotection against I/R induced myocardial infarction.

Keywords

Endurance exercise; Mitochondria; Antioxidant enzymes; Ischemia reperfusion; Cardioprotection.

Abbreviations

CAD: Coronary Artery Diseases; mPTP: mitochondrial Permeability Transition Pore; AIF: Apoptosis Inducing Factor; GPX: Glutathione Peroxidase; CAT: Catalase; PRX III: Peroxiredoxin III; ROS: Reactive Oxygen Species; GSH: Glutathione; GSSG: Oxidized Glutathione; TRX II: Thioredoxin II; NRF2: Nuclear Erythroid-2 Like Factor-2.

INTRODUCTION

The heart is one of the most dynamic organs in our body since it constantly pumps the blood through the whole body. To continuously fulfill this critical task, cardiac myocytes should receive suitable amounts of oxygen and nutrients through coronary arteries; however, if blood flow to coronary arteries is significantly obstructed due to atherosclerosis (a disease of the arteries caused by an increase in the deposition of plaques of fatty material on the inner walls of arteries), cardiomyocytes undergo ischemia, leading to myocardial infarction. Indeed, prolonged blockage of the blood flow (chronic ischemia) due to coronary artery diseases (CAD) causes the massive death of cardiac myocytes.

The degree of myocardial injuries varies depending upon the duration of ischemia, but beyond 20 minutes results in irreversible myocyte damage, but a timely restoration of the obstructed blood flow (reperfusion) can ameliorate levels of cell death. Nevertheless, this salvage procedure (i.e., reperfusion by angioplasty) still contributes to significant cell death and to formation of fibrosis, thus gradually leading to heart failure. Therefore, ischemia and reperfusion (I/R)-induced myocardial cell death is a major risk fac-
tor for heart failure and become the leading cause of adult death in U.S.\textsuperscript{6}\textsuperscript{,7}

Despite three decades of incessant pharmacological trials to mitigate I/R-induced myocardial injuries in the clinical setting, currently, satisfactory therapy is still greatly lacking, and thus there is an urgent need to devise potent therapeutic strategies. In this regard, endurance exercise has been suggested to remarkably reduce I/R-induced myocardial infarction. However, exact mechanisms responsible for exercise-induced cardioprotection against an I/R insult remain poorly understood and elusive.

In normal resting mammalian cells, about 0.4\textendash{}4% of the consumed oxygen in the mitochondria is released as reactive oxygen species (ROS).\textsuperscript{8} However, their levels vastly elevate during an I/R episode and contribute to I/R injury, which can lead to myocardial cell death.\textsuperscript{9,10} Given that regular endurance exercise has been reported to improve antioxidative capacity, it seems reasonable to presume that the enhanced antioxidative capacity may be an essential element for cardioprotection. Therefore, this review will provide basic information about how ROS causes cellular damages during an I/R insult, describe current molecular mechanisms of antioxidative network systems working against ROS, and present cardioprotective roles of endurance exercise-induced improvement of antioxidant capacity.

**MITOCHONDRIAL ROS AND APOPTOSIS**

Free radicals are chemically reactive molecules due to an unpaired electron in the outer orbital\textsuperscript{11} and thus become origins of ROS. For example, a superoxide anion is an oxygen-driven radical produced as a result of the univalent reduction of molecular oxygen. It's production leads to the formation of many other ROS including hydrogen peroxide, \( \text{H}_2\text{O}_2 \), hydroxyl radical, \( \cdot\text{OH} \), and peroxynitrite, ONOO\textsuperscript{−}.\textsuperscript{11,15,16} It has been reported that mitochondrion in mammalian cells is the main locus that generates superoxide anions due to an electron leaked from complex I and III of mitochondrial electron transport chain.\textsuperscript{17-21} Since superoxide radicals are charged molecules, they have less chance to cross the mitochondrial membranes; thus, if not scavenged, superoxide radicals cause mitochondrial membrane lipid peroxidation and protein oxidation in electron transport chain complexes as well as Krebs cycle enzymes,\textsuperscript{22} resulting in mitochondrial dysfunction.\textsuperscript{23,24} Moreover, recent evidence has shown that oxidative stress is responsible for opening mitochondrial permeability transition pore (mPTP),\textsuperscript{25,26} leading to myocardial injuries and cell death.\textsuperscript{27,28}

Mitochondrion has been known to mediate cell death upon oxidative damages via a series of apoptotic signaling cascades by releasing cytochrome C and/or apoptosis inducing factor (AIF) from mitochondria. This triggers caspase-dependent and/or -independent apoptosis, respectively.\textsuperscript{29,30} For this reason, protection of mitochondria against oxidative stress via mitochondrial antioxidant has been suggested to be a key countermeasure against I/R-induced myocardial injury owing to the massive production of ROS during I/R.

Two major antioxidative defense systems in mitochondria exist to work as a unit to eliminate oxidative stress: 1) manganese superoxide dismutase (MnSOD) and 2) glutathione peroxidase (GPX), catalase (CAT), and peroxiredoxin III (PRX III).

**Removal of Superoxide Radicals**

MnSOD detoxifies superoxide radicals by converting them into hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and oxygen:

\[
\text{O}_2^- + 2\text{H}^+ + \text{MnSOD} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

Thus, in mammalian cells, MnSOD has been considered as an essential antioxidant enzyme responsible for cardioprotection.\textsuperscript{31} Indeed, multilayers of evidence have demonstrated that partial downregulation or complete knockdown of MnSOD accelerates myocardial injuries under oxidative stress,\textsuperscript{32,33} while upregulation of MnSOD minimizes infarct size of the heart undergoing an I/R insult.\textsuperscript{34}

**Removal of \( \text{H}_2\text{O}_2 \)**

Relatively stable \( \text{H}_2\text{O}_2 \) produced from the process of dismutation of superoxide radicals by MnSOD in the mitochondria is considered to be potentially harmful because it can become highly reactive hydroxyl radical (\( \cdot\text{OH} \)) in the presence of Fe\textsuperscript{2+} via Fenton reaction:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-
\]

In fact, \( \text{H}_2\text{O}_2 \) compared to charged superoxide radicals can be freely diffused across membranes and become a source of hydroxyl radicals. Recent evidence indicates that \( \text{H}_2\text{O}_2 \) not only causes a collapse of mitochondrial membrane potential by opening MPTP but also induces protein oxidation of sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase, potentially leading to mitochondrial Ca\textsuperscript{2+} overload. Therefore, this oxidant can also initiate apoptosis. Due to this deleterious effect, endogenous antioxidants (e.g., GPX, CAT, and PRX III) specifically targeting \( \text{H}_2\text{O}_2 \) in mitochondria exist.

**GPX, CAT, and PRX III**

As shown in Figure 1, GPX, CAT, and PRX III function as a unit to remove \( \text{H}_2\text{O}_2 \). Briefly, GPX is thiol-containing peroxidase and uses glutathione (GSH) as a reducing equivalent to reduce \( \text{H}_2\text{O}_2 \) to form oxidized glutathione (GSSG) and water. Similarly, a heme-containing homotetrameric enzyme, CAT, converts \( \text{H}_2\text{O}_2 \) to water and oxygen. A recent study has shown that mitochondria-targeted CAT in a mouse experimental model significantly increases life span,\textsuperscript{35} whereas mutation of this enzyme exhibits more susceptibility to oxidative damage.\textsuperscript{36} Recently, PRX III have received special attention because this enzyme exerts potent antioxidative roles in the cells, ranging from degradation of \( \text{H}_2\text{O}_2 \) and repair of membrane lipid to modification of apoptosis induction.\textsuperscript{37} Indeed, it has been reported that PRXIII is the most abundant and efficient antioxidant enzyme targeting \( \text{H}_2\text{O}_2 \) in mitochondria.\textsuperscript{38} PRX III neutralizes \( \text{H}_2\text{O}_2 \) produced in mitochondria through their peroxidase activity with the use of electrons provided by thioredoxin II (TRX II) that is reduced by NADPH via thioredoxin reductase (TRX R II).\textsuperscript{39,40}
Endurance exercise has been demonstrated to reduce myocardial injury against I/R injuries including contractile function and myocardial infarction size. While several cardioprotective mechanisms induced by endurance exercise have been proposed (eg, reduced calcium overload, heat shock proteins, and increased ATP-dependent potassium channels), an increase in antioxidative capacity has been recognized as a key mechanism. Indeed, given that I/R contribute to massive ROS production, the notion that enhanced antioxidative capacity in response to exercise is associated with cardioprotection is not surprising. Growing evidence has shown that increased activities of manganese MnSOD are linked to exercise-induced MnSOD upregulation; however, given that TNF-α was absent. This observation appears to indicate that increased MnSOD expression or activities in response to endurance exercise increases MnSOD has not been clearly elucidated yet.

Nonetheless, according to recent research, a transcription factor, cAMP-responsive element binding protein (CREB), is linked to regulate MnSOD expression. Also, another transcription factor, tumor necrosis factor-α (TNF-α), has been reported to induce MnSOD gene expression and plays an important role in cardioprotective role against an I/R insult, as the cardioprotection to end short-term endurance exercise.58 Consistent with this study, a study by Moran et al also showed that 12 weeks of endurance training does not modulate GPX in rat’s myocardium. Currently, it remains unknown why GPX responds differently to exercise training; but a study led by Ji group seems to provide a potential answer. His research group showed that modulation of antioxidant levels in response to endurance exercise appears to be tissue-specific, with highly oxidative tissues such as soleus and heart showing no significant alteration and rather reduction in some cases.60

As previously described, removal of superoxide radicals by MnSOD results in the production of another form of ROS, H2O2. As previously described, removal of superoxide radicals by MnSOD results in the production of another form of ROS, H2O2. Therefore, further mechanistic studies are needed to determine the functional role of NRF2 in MnSOD regulation and involvement in exercise-induced cardioprotection.
of PRX III against oxidative stress, very little studies have been conducted. Currently, only one study is available, demonstrating that PRX III levels were elevated in mitochondria by endurance exercise, suggesting that this phenotypic change may be linked to cardiac protection against oxidative stress.

**SUMMARY**

It is well known that oxidative stress during an I/R episode contributes to myocardial infarction, and thus improved antioxidative capacity has been suggested to reduce myocardial infarction. Similarly, a non-pharmacological intervention, endurance exercise, has been reported to improve endogenous antioxidant capacity, leading to cardioprotection against an I/R insult. Given that mitochondria are major sources of ROS production and become a potent initiator of cell death under stressed conditions such as an I/R insult, mitochondria-specific antioxidant enzymes have emerged as a potential strategy that reduces oxidative stress and infarction. In mitochondria, MnSOD converts superoxide into weak oxidant H$_2$O$_2$, which is then detoxified by GPX or CAT, or PRX III, resulting in the production of oxygen and water. Both classical and recent studies have shown that endurance exercise-induced improvement in MnSOD, GPX, CAT, and PRX III is associated with cardioprotection against I/R injuries by reducing both apoptosis and necrosis (Figure 2). However, how regular endurance exercise upregulates these antioxidant enzymes has not been clearly elucidated yet although some transcription factors (e.g., CREB, TNF-α, and NRF2) has been indicated as plausible candidates. Therefore, identification of clear signaling pathways of exercise-induced antioxidant upregulation will provide key insight into developing a pharmaceutical therapeutic strategy against myocardial infarction.

**ACKNOWLEDGEMENT**

This project was supported by a research grant from the University of West Florida though Office of Research and Sponsored Programs (R0062) and UWF Florida Research Fellowship to YL (CF6672 and CR0070).

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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