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Review

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

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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 fulfil 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,[1] 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.[2-5] Therefore, ischemia and reperfusion (I/R)-induced myocardial cell death is a major risk fac-

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tor for heart failure and become the leading cause of adult death in U.S.[6,7]

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 0.4~4% of the consumed oxygen in the mitochondria is released as reactive oxygen species (ROS).[8] However, their levels vastly elevate during an I/R episode and contribute to I/R injury, which can lead to myocardial cell death.[1,9-14] 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[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, H_2O_2; hydroxyl radical, $^{\cdot}OH$; and peroxynitrite, $ONOO^{-}$.[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.[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,[22] resulting in mitochondrial dysfunction.[23-25] Moreover, recent evidence has shown that oxidative stress is responsible for opening mitochondrial permeability transition pore (mPTP),[26,27] leading to myocardial injuries and cell death.[27,28]

Mitochondrial 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.[29,30] For this reason, protection of mitochondria against oxidative stress *via* mitochondrial antioxidants 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 defence 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 (H_2O_2) and oxygen:

$$2O_2^{\cdot-} + 2H^+ + \text{MnSOD} \rightarrow H_2O_2 + O_2$$

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

Removal of H_2O_2

Relatively stable H_2O_2 produced from the process of dismutation of superoxide radicals by MnSOD in the mitochondria is considered to be potentially harmful because it can becomea highly reactive hydroxyl radical ($^{\cdot}OH$) in the presence of Fe^{2+} *via* Fenton reaction:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + {}^{\cdot}OH + OH^{-}$$

In fact, H_2O_2compared to charged superoxide radicals can be freely diffused across membranes and become a source of hydroxyl radicals. Recent evidence indicates that H_2O_2 not only causes a collapse of mitochondrial membrane potential by opening MPTP but also induces protein oxidation of sarcoplasmic reticulum Ca^{2+} ATPase, potentially leading to mitochondrial Ca^{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 H_2O_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 H_2O_2. Briefly, GPX is thiol-containing peroxidase and uses glutathione (GSH) as a reducing equivalent to reduce H_2O_2 to form oxidized glutathione (GSSG) and water. Similarly, a heme-containing homotetrametic enzyme, CAT, converts H_2O_2 to water and oxygen. A recent study has shown that mitochondria-targeted CAT in a mouse experimental model significantly increases life span,[35] whereas mutation of this enzyme exhibits more susceptibility to oxidative damage.[36] Recently, PRX III have received special attention because this enzyme exerts potent antioxidative roles in the cells, ranging from degradation of H_2O_2 and repair of membrane lipid to modification of apoptosis induction.[37] Indeed, it has been reported that PRXIII is the most abundant and efficient antioxidant enzyme targeting H_2O_2 in mitochondria.[38] PRX III neutralizes H_2O_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).[39-41]

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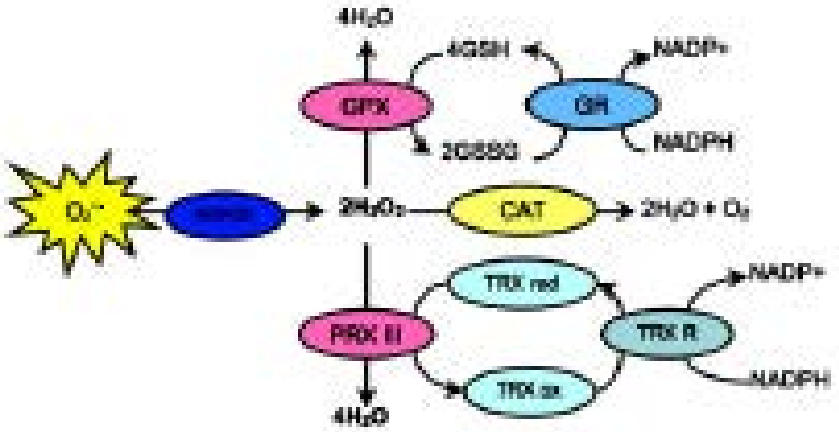
Figure 1. ***Simplistic Overview of the Interaction of Antioxidant Enzyme Network in Mitochondria:*** *A super oxide radical (O_2^-) produced from mitochondria is converted into hydrogen peroxide (H_2O_2), which is then detoxified by several antioxidant enzymes such as glutathione peroxidase (GPX), catalase (CAT), and peroxiredoxin III (PRX III), resulting in the conversion of H_2O_2 into water molecules. Importantly, GPX and PRX III utilize reducing molecules such as glutathione and reduced thioredoxin (TRX red), respectively to neutralize H_2O_2. After donating thiol groups, oxidized glutathione (GSSG) and thioredoxin (TRX ox) become reduced by glutathione reductase (GR) and thioredoxin reductase (TRX R), respectively, using NADPH as a reducing equivalent*



EXERCISE-INDUCED CARDIOPROTECTION AGAINST AN I/R INSULT: ROLE OF MNSOD

Endurance exercise has been demonstrated to reduce myocardial injury against I/R injuries including contractile function and myocardial infarction size.[42-46] 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 antioxidant 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 cardioprotection.[24,42,43,47,48] This crucial role of MnSOD in cardioprotection was strongly supported by a recent study in which knockdown of exercise-induced MnSOD expression *via in vivo* administration of an antisense oligodeoxyribonucleotide against MnSOD significantly diminished exercise-mediated cardioprotection.[47] Despite this clear association of increased MnSOD with cardioprotection, mechanisms of how endurance exercise increases MnSOD has not been clearly elucidated yet.

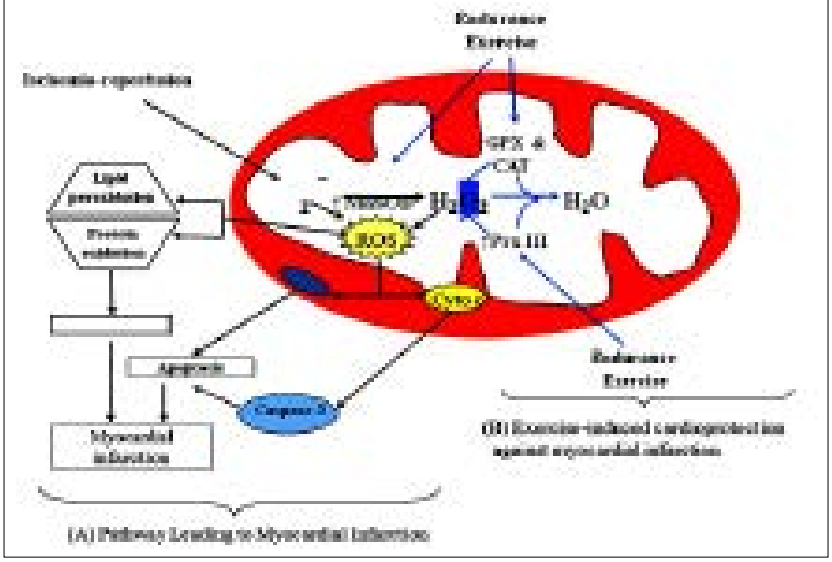
Nonetheless, according to recent research, a transcription factor, cAMP-responsive element binding protein (CREB), is linked to regulate MnSOD expression.[49,50] Also, another transcription factor, tumor necrosis factor-α (TNF-α), has been reported to induce MnSOD gene expression and plays an important role in cardioprotective role against an IR insult, as the cardioprotection was abolished when TNF-α was absent.[51] This observation appears to indicate that a TNF-α signaling may be a potential mechanism of exercise-induced MnSOD upregulation; however, given that endurance exercise rather reduces inflammatory cytokines including TNF-α,[52,53] this signaling pathway is less likely to be the case. Another potential mechanism involved in enhancing MnSOD activities is the nuclear erythroid-2 like factor-2 (NRF2), known as a master transcription regulator of various antioxidant enzymes.[54] In support of this notion, recent studies have reported that NRF2-mediated heme oxygenase-1 (HO-1) upregulation in the heart enhances MnSOD activities *via* carbon monoxide production upon HO-1 potentiation.[55] Currently, whether this notion is applicable to endurance, exercise-induced cardioprotection remain enigmatic because long-term endurance exercise impairs NRF2 signaling, resulting in cardiac dysfunction,[56] whereas moderate intensity endurance exercise improves cardiac oxidative stress *via* upregulation of NRF2 expression.[57] Therefore, further mechanistic studies are needed to determine the functional role of NRF2 in MnSOD regulation and involvement in exercise-induced cardioprotection.

EXERCISE-INDUCED CARDIOPROTECTION AGAINST AN I/R INSULT: ROLE OF GPX, CAT, AND PRX III

As previously described, removal of superoxide radicals by MnSOD results in the production of another form of ROS, H_2O_2. Thus, increased MnSOD expression or activities in response to endurance exercise can cause a potential source of oxidative stress in mitochondria. Regarding this notion, recent studies have shown that endurance exercise upregulates GPX in the mitochondria in parallel with reduced H_2O_2 production and mitochondrial lipid peroxidation.[24] Interestingly, Starnes et al did not observe an increase in GPX, but showed that CAT was increased in response to 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.[59] 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]

It is surprising that despite a potent antioxidative role

Figure 2. ***Graphic Illustration of Mitochondria-Mediated Reactive Oxygen Species (ROS) Production, Associated Antioxidant Enzymes Detoxifying ROS, and a Potent Role of Endurance Exercise-Induced Antioxidant Capacity in Preventing ROS-Pnduced Cell Death:*** *Ischemia/reperfusionpromotes generation of superoxide anions (O_2^-), which can damage mitochondrial proteins, lipids, and DNA. This superoxide radicals can be neutralized by manganese superoxide dismutase (MnSOD) by converting O_2^- to hydrogen peroxide (H_2O_2); however, since H_2O_2can be a potential source of another type of ROS (e.g., hydroxyl radicals), other antioxidant enzymes (e.g., GPX, CAT, PRX III) are needed to transform $H_2O_2$to water. Regular endurance exercise enhances antioxidant capacity by increasing MnSOD and GPX or CAT, or PRX III and thus reduces cellular oxidative damage and cell death against I/R injuries*



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,[61] 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 mitochondrial 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_2O_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 pharmacological therapeutic strategy against myocardial infarction.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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