Review

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Review

Involvement of Oxidative Stress and Oxidants in Modification of Cardiac Dysfunction Due to Ischemia-Reperfusion Injury

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Abstract: Delayed reperfusion of the ischemic heart (I/R) is known to impair recovery of cardiac function and produce a wide variety of myocardial defects including ultrastructural damage, metabolic alterations, subcellular Ca^{2+}-handling abnormalities, activation of proteases and changes in cardiac gene expression. Although I/R-injury has been reported to induce the formation of reactive oxygen species (ROS), inflammation and intracellular Ca^{2+}-overload, generation of oxidative stress is considered to play a critical role in the development of cardiac dysfunction. There occurs an increase in the production of superoxide and hydroxyl radicals as well as oxidants such as hydrogen peroxide and hypochlorous acid in hearts subjected to I/R-injury. In fact, mitochondria are a major source of excessive production of ROS in I/R hearts due to impairment in the electron transport system as well as activation of xanthine oxidase, monoamine oxidase and NADPH oxidase. Nitric oxide synthase, mainly present in the endothelium, is also activated due to I/R injury to produce nitric oxide, which upon combination with superoxide radicals, generates nitrosative stress. Alterations in cardiac function, sarcolemma and sarcoplasmic reticulum Ca^{2+}-handling activities, and mitochondrial oxidative phosphorylation as well as protease activation due to I/R-injury are simulated upon exposing the heart to oxyradical generating system (xanthine plus xanthine oxidase) or H_2O_2. On the other hand, the activation of endogenous antioxidants such as superoxide dismutase, catalase and glutathione peroxidase as well as the concentration of a transcription factor (Nrf2), which modulates the expression of various endogenous antioxidants, are depressed due to I/R injury in hearts. Furthermore, pretreatment of hearts with antioxidants such as catalase plus superoxide dismutase, N-acetylcysteine and mercaptopropionylglycerine was observed to attenuate the I/R-induced subcellular Ca^{2+}-handling and changes in Ca^{2+}-regulatory activities as well as depress protease activation and improve the recovery of cardiac function. These observations indicate that oxidative stress is intimately involved in the pathological effects of I/R-injury and different antioxidants attenuate I/R-induced subcellular alterations and improve the recovery of cardiac function. Thus, there is a challenge to develop safe and effective antioxidants as well as agents for upregulating the expression of endogenous antioxidants for the therapy of I/R-injury.

Keywords: ischemia-reperfusion injury; cardiac dysfunction; oxidative stress; antioxidants; subcellular defects; Ca^{2+}-handling abnormalities

1. Introduction

It is now well known that myocardial ischemia induces marked alterations in contractile function, cellular metabolism and cardiac ultrastructure due to the lack of both oxygen and

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substrates. Although reperfusion of the ischemic myocardium is beneficial in improving cardiac function and myocardial changes, delayed reperfusion, after a certain critical time of ischemic insult has been shown to impair the recovery of cardiac function and exacerbate metabolic alterations, induce Ca^{2+}-handling abnormalities and promote damage to the myocardial structure [1–15]. These defects due to reperfusion of the ischemic heart are termed as ischemia-reperfusion (I/R)-injury, which is commonly seen in clinical conditions such as acute coronary syndrome, angioplasty, thrombolysis, coronary bypass surgery, cardiac transplantation and stroke. Extensive studies regarding the pathophysiology of I/R-injury to the heart have been carried out to understand its mechanisms involving different factors, signal transduction pathways and mitochondrial alterations [16–35]. However, it appears that the mechanisms of I/R-injury induced cardiac dysfunction are similar to those for hypoxia-reoxygenation induced alterations, but are of complex nature because the effects of reperfusion are superimposed upon those for myocardial ischemia. It should be mentioned that I/R-injury also includes a series of events such as reperfusion arrhythmias, myocardial stunning, microvascular damage and cell death, which occur during the development of cardiac dysfunction.

Cardiac dysfunction in hearts subjected to I/R-injury has also been shown to involve oxidative stress, inflammation, endoplasmic reticulum stress, intracellular Ca^{2+}- overload and defects in subcellular organelles such as sarcolemma (SL), sarcoplasmic reticulum (SR), mitochondria (MT) and myofibrils (MF) [36–51]. However, oxidative stress is considered to play a critical role in the development of impaired cardiac performance due to I/R- injury because several defects associated with this pathological condition are elicited as its consequence. Furthermore, different antioxidants have been reported to attenuate the I/R-induced alterations in the heart [7,11,15,36,41,44,48,50–52]. It is therefore the objective of this article to provide an updated comprehensive review of various sources for the generation of oxidative stress as well as the role of endogenous antioxidants during the development of I/R-injury. It is also planned to discuss the implications of oxidative stress in inducing cardiac dysfunction due to I/R-injury. Furthermore, this article is intended to include evidence for the involvement of oxidative stress in the I/R-induced subcellular alterations and subsequent impairment of cardiac performance. In addition, the pharmacotherapy of I/R-injury with both endogenous and exogenous antioxidant systems will be described with respect to improving cardiac function in pathological conditions.

2. Generation of Oxidative Stress and Status of Antioxidant Systems in I/R Hearts

Oxidative stress is associated with an increase in the production of reactive oxygen species (ROS) and/or a decrease in the activities of antioxidant defense systems due to I/R- injury in the heart [8,9,14,15,43,44,51]. There are five major types of ROS such as superoxide radicals, hydroxyl radicals, hydrogen peroxide (H_2O_2), hypochlorous acid and peroxynitrite, which are increased in the heart upon inducing I/R injury. ROS are mainly generated by impairment of electron transport in mitochondria, xanthine + xanthine oxidase reaction, arachidonic acid metabolism, as well as the activation of monoamine oxidase, NADPH oxidase, the endothelium and neutrophils. It should be mentioned that superoxide radicals are rapidly converted into H_2O_2, which is a precursor of hydroxyl ions in the presence of iron and copper, as well as hypochlorous acid (HOCl) in the presence of myeloperoxidases and chloride ions. Superoxide radicals also react with nitric oxide (produced upon the activation of endothelium) to form peroxynitrite. Although all members of the ROS family are inter-related, hydroxyl radicals are considered to be the most reactive with a very short half-life. It should also be pointed out that low concentrations of ROS produce beneficial effects on the heart by activating different redox-sensitive signal transduction pathways whereas high concentrations of ROS (oxidative stress) produce harmful effects by reacting with cellular proteins, lipids, carbohydrates and DNA. Thus, the I/R injury produces changes in membrane permeability, and protein thiol group oxidation as well as lipid peroxidation, which are considered to be mediated through the generation of oxidative stress.

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Excessive generation of ROS by mitochondrial defects and other sources have been demonstrated to be associated with activation of transient receptor potential melastatin 2 (TRPM2), production of endoplasmic reticulum stress (ER stress), DNA damage as well as upregulation of NADPH oxidase, hemeoxygenases and cyclooxygenases [53–66]. ROS have also been shown to produce ventricular arrhythmias, alter apelinergic system and ubiquitin-proteosome axis in addition to inducing different types of cell death such as necrosis, apoptosis, necroptosis, pyroptosis and ferrotosis [67–78]. Different endogenous proteins such as perilipin, SESTRIN2, heat shock protein 22 and humanin as well as exosomes and microRNA with antioxidant properties have been reported to protect the harmful effects of ROS in the heart [79–85]. Various enzymes including superoxide dismutase (SOD), catalase and glutathione peroxidases as well as vitamins such as vitamin E, vitamin A and vitamin C are known to serve as endogenous antioxidants [8,44,49–51]. These antioxidants are considered to act through mechanisms involved in either scavenging ROS or inhibiting the generation of ROS in the myocardium. The levels of these antioxidants are not only decreased upon reperfusing the ischemic heart, but these interventions are also effective in attenuating I/R-injury to the heart upon pretreatment. These observations support the view that generation of ROS and development of oxidative stress are intimately involved in the pathogenesis of I/R-injury to the heart [8,49–51,86,87].

3. Mechanisms of I/R-Injury Induced Subcellular Defects and Cardiac Dysfunction

Since the status of cardiac function is determined by the coordinated Ca^{2+}-handling activities of different subcellular organelles including SL, SR and MT as well as Ca^{2+}-regulated activity of MF [11,88–90], it has been suggested that abnormalities in subcellular function due to oxidative stress play an important role in the development of cardiac dysfunction as a consequence of I/R-injury [8,41,51]. Oxidative stress is known to induce Ca^{2+}-handling abnormalities in SL, SR and MT in addition to producing a loss of Ca^{2+}-sensitivity in MF and thus leads to the development of cardiac dysfunction. It should be pointed out that myocardial ischemia upon occluding the coronary arteries is associated with the lack of oxygen, inability of MT to oxidize substrate and accumulation of hydrogen in cardiomyocytes; these alterations result in increasing the intracellular concentration of Ca^{2+} upon stimulating SL Na^{+}-H^{+} exchange and SL Na^{+}- Ca^{2+}-exchange systems. The increased levels of Ca^{2+} in the ischemic myocardium activate xanthine oxidase and produce ROS through the xanthine plus xanthine oxidase reaction. Furthermore, the inability of MT to oxidize substrate in the ischemic heart is known to depress oxidative phosphorylation, and impair the MT electron transport system, generate ROS and produce cessation of contractile activity. All these changes due to myocardial ischemia are reversible upon reperfusion; however, delayed reperfusion results in marked defects in cardiomyocytes, myocardial interstitium, endothelium and coronary vasculature for the induction of ultrastructural abnormalities as well as impairment of contractile function recovery as a consequence of ROS production [1–11,41,43,51]. Such alterations in cardiomyocytes due to reperfusion of the ischemic heart are associated with increased entry of Ca^{2+} due to increase in membrane permeability as well as depressions in SL Na^{+}-K^{+} ATPase and SL Na^{+}- Ca^{2+}-exchange activities. In addition, there occurs a depression in the SR Ca^{2+}-pump activity and leakage of Ca^{2+} from the SR tubular system due to ROS-induced alterations in SR Ca^{2+}-release channels. These Ca^{2+}-handling abnormalities in SL and SR lead to the development of MT Ca^{2+}-overload and further defects in MT electron transport system, oxidative phosphorylation activity and energy production, in addition to generation of oxidative stress, opening of MT pores and release of cytotoxic substances for the induction of programmed cell death.

Although oxidative stress is considered to be mainly generated by MT, upon inducing I/R injury, the involvement of other systems such as Ca^{2+}-handling abnormalities in SL and SR for the occurrence of MT Ca^{2+}-overload, endothelium nitric oxide synthase for the production of nitric oxide and peroxynitrite, macrophages and leukocytes for the release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) as well as interleukins (IL-1β and IL-6) as well as long noncoding

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RNA-RNA axis and thioredoxin-interacting protein for the generation of oxidative stress during the development of I/R-injury cannot be overlooked [91–99]. Furthermore, the activation of both SL and MT associated NADPH oxidases, as well as MT monoamine oxidase (MAO), glucose homeostasis and oxidation of free fatty acids, and arachidonic acid metabolic pathways have been demonstrated to play a critical role for the development of oxidative stress during the initiation and progression of I/R-injury to the heart [20,21,51,100]. It is also noteworthy that the concentrations of different ROS and oxidants are increased whereas those for endogenous antioxidant enzymes are decreased in the heart upon inducing I/R injury [49–51,101–103]; these observations provide strong evidence for the occurrence of oxidative stress during the development of oxidative stress. Several studies have also shown that transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1), which have been shown to modulate the activities of various endogenous antioxidants, protect the occurrence of intracellular Ca^{2+}-overload, inflammation and oxidative stress during the induction of I/R-injury in the heart [104–112]. Accordingly, it is evident that different subcellular organelles are not only involved in the generation of oxidative stress and intracellular Ca^{2+}-overload in cardiomyocytes, but are also adversely affected by these pathogenic factors leading to the development of cardiac dysfunction due to I/R-injury.

4. Pharmacotherapy and Cardioprotection of I/R-Induced Injury

Extensive experimental work in the area of pharmacotherapy and cardioprotection has been carried out to investigate the beneficial effects of several interventions in preventing the I/R injury in the heart [8,44,51,113]. Different pharmacologic agents such as hypoglycemic drugs [114,115], aldosterone inhibitors [116], regulatory interventions including SIRT1 (regulator of autophagy), STING (stimulator of interferon genes) and AMP-activated protein kinase [117–119] as well as nanomedicines [120] were found to attenuate the I/R-injury. Likewise, some interventions such as nitric oxide, heme oxygenase 1, propofal and adiponectin, which affects diverse molecular targets, were observed to partially prevent the I/R-injury in the heart [121–124]. Furthermore, different types of RNAs (circular RNA, noncoding RNA and micro RNA) have also been reported to exert beneficial effects in attenuating the I/R-injury through some complex mechanisms [125–127]. Some phytochemicals, natural products and micronutrients have been claimed to protect I/R-injury upon improving the antioxidant status [128–130]. Although it may well be that all these interventions indicated in this section may partially depress the I/R-injury by reducing the formation of ROS or increasing the concentrations of different antioxidants; however, the involvement of other mechanisms such as alterations in inflammation, reduction in intracellular Ca^{2+}-overload and inhibition of protease activation cannot be ruled out with any certainty.

In view of the depressed endogenous antioxidant levels in the I/R hearts, several investigators have used redox sensitive therapy for preventing the I/R-injury. In this regard, supplementation with exogenous antioxidant preparations [51,52,126,131,132] and administration of interventions which up-regulate the transcription factor, Nrf2, [107,110,111] for increasing the antioxidant status were shown to exert beneficial effects against I/R-injury. Various proteins such as humanin, berberine, sestrin, taurine, fisetin, quercetin and polydatin with antioxidant properties were also observed to attenuate I/R- injury in the heart [81,122,133–137]. Different vitamins such as vitamins A, C and E with antioxidant activities have also been shown to exert beneficial effects against I/R injury [138,139]. Some gases such as molecular hydrogen and hydrogen sulfide [109,131,140–143] as well as ischemic preconditioning [90,144] have also been reported to exert beneficial actions. All these observations showing cardioprotective effects of various interventions in I/R hearts due to their antioxidant activities support the view that oxidative stress plays a pivotal role in the development of I/R-injury.

5. Evidence for the Involvement of Oxidative Stress in I/R-Induced Cardiac Dysfunction

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In order to provide further evidence for the occurrence of oxidative stress in the impairment of cardiac function recovery upon reperfusion of the ischemic heart, we have analyzed some information from some experiments, which were carried out for inducing I/R-injury in the isolated perfused hearts pretreated in the absence and presence of some antioxidants. It should be mentioned that isolated heats were subjected to 30 min of global ischemia before inducing reperfusion for 30 to 60 min. Furthermore, the effects of I/R-induced injury were compared with those obtained from hearts perfused with or without oxyradical generating system (xanthine plus xanthine oxidase) as well as H_2O_2, a well-known oxidant, for 30 min.

The data [87] in Figure 1 show that the left ventricular developed pressure (LVDP) (A) was depressed whereas the left ventricular end diastolic pressure (LVEDP) (B) was markedly increased upon reperfusion indicating the impairment of cardiac function due to I/R-injury. Furthermore, the biomarkers of oxidative stress (H_2O_2 content and MDA content) were also increased in the I/R hearts (Figure 1 C and D). Treatment of hearts with an antioxidant mixture (SOD plus catalase) was observed to attenuate the I/R-induced changes in cardiac function and biomarkers of oxidative stress. It is also pointed out myocardial Ca^{2+}-content in control, I/R and SOD plus catalase treated I/R hearts were 8.4±1.2, 22.6±2.9 and 9.8±1.6 μmol/g dry wt., respectively [87].

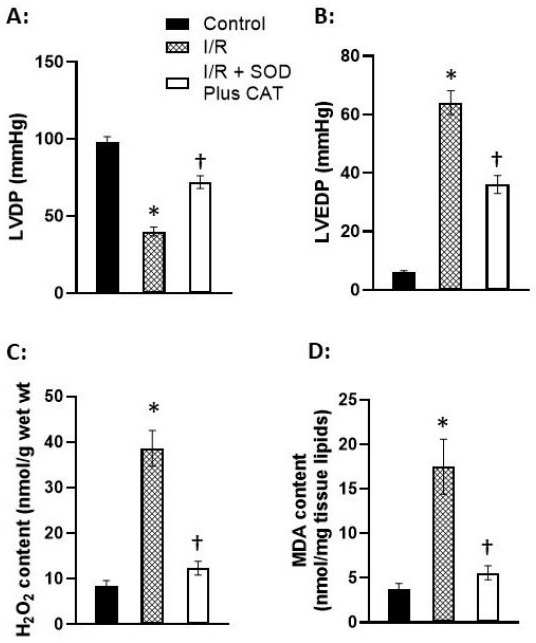

Figure 1. Effect of ischemia-reperfusion with or without oxyradical scavenger mixture (SOD plus CAT) on cardiac function (A and B) and myocardial markers for oxidative stress (C and D). Hearts were subjected to 30 min global ischemia followed by 60 min reperfusion (I/R) in the absence or presence of 80 μg/mL superoxide dismutase (SOD) plus 10 μg/mL catalase (CAT). Control hearts in each experiment were perfused with normal medium for appropriate time. The data are based on the analysis of information in Dhalla et al. [87]. LV = left ventricle; DP= developed pressure; EDP= end diastolic pressure; MDA = malondialdehyde, * $p < 0.05$ vs. respective control value, † $p < 0.05$ vs. respective I/R value.

Figure 2 shows that depressed LV function in I/R hearts was associated with markedly decreased SL Na^+-K^+ ATPase (Figure 2A) activity as well as increased protease activities for matrix metalloproteinase (MMP) (Figure 2B) and calpain (Figure 2C) [145]. These effects of I/R-injury were attenuated when reperfusion of the ischemic hearts was carried out in the presence of well-known antioxidants namely N-acetylcysteine (NAC) or mercaptopropionyl glycine (MGP) (Figure 2). The depressed LVDP was shown to be associated with reduced Na^+-K^+ ATPase activities in hearts perfused with xanthine plus xanthine oxidase (X+XO) (Figure 2D) and was also observed to be associated with increased activities of both MMP and calpain (Figures 2E and F). The data in Figure 3 show that Na+-Ca^{2+} exchange (A), ATP-dependent Ca^{2+}-uptake (B) and Ca^{2+}-stimulated ATP

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activities (C) were depressed upon reperfusing the ischemic hearts (I/R hearts). These alterations due to I/R were attenuated when the I/R was carried out in the presence of SOD plus catalase [146–148].

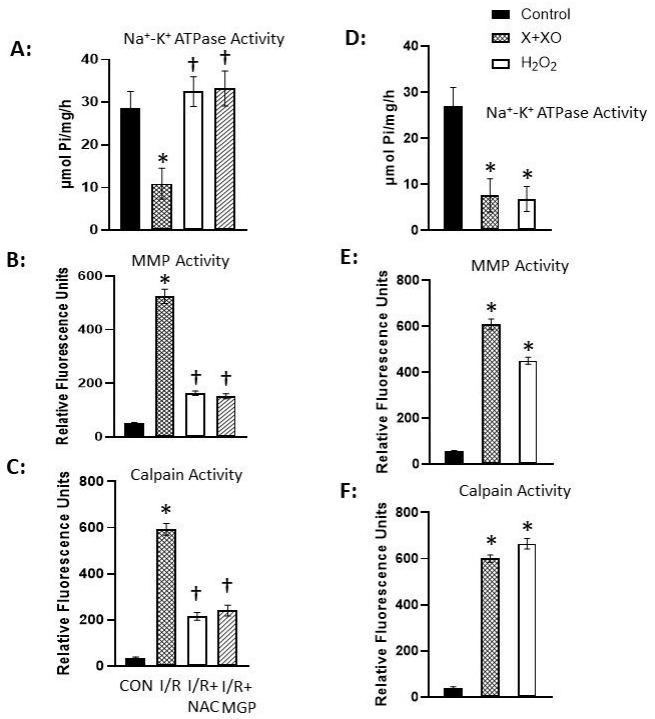

Figure 2. Effect of ischemia-reperfusion (I/R) with or without some antioxidants (A, B and C) as well as perfusion with xanthine plus xanthine oxidase (X + XO) or H_2O_2 on sarcolemmal Na^+-K+ ATPase activity (D) and protease activities (E and F) in isolated perfused hearts. Hearts were subjected to 30 min global ischemia followed by 30 min reperfusion (I/R) in the absence and presence of 100 µM N-acetylcysteine (NAC) or 300 µM mercaptopropionylglycine (MGP). Hearts were also perfused for 30 min with 2 mM xanthine (X) plus 60 mU/mL xanthine oxidase (XO) mixture or 100 mM H2O2 followed by 30 min reperfusion. Control hearts in each experiment were perfused with normal medium for 60 min. The data are based on the analysis of information in Singh et al. [145]. MMP = matrix metalloproteinase, *p < 0.05 vs. respective control value, †p < 0.05 vs. respective I/R value.

Likewise, Figure 3 D-F show depressions in the SL Na^+-Ca^{2+} exchange, ATP-dependent Ca^{2+}-uptake and Ca^{2+}-stimulated ATP activities, respectively, upon perfusion with X+XO. These changes due to ROS generating system were attenuated in the presence of SOD plus catalase [146–148]. It should also be noted that SR Ca^{2+}-uptake, Ca^{2+}-stimulated ATPase, Ca^{2+}-release and ryanodine binding activities were depressed in the I/R hearts (Figure 4), but when the reperfusion of the ischemic hearts was carried out in the presence of SOD plus catalase, these alterations (except that for Ca^{2+}-stimulated ATPase) were attenuated [149]. Furthermore, perfusion of hearts with X+XO or H_2O_2 [149] was also observed to depress SR Ca^{2+}-uptake, Ca^{2+}-stimulated ATPase, Ca^{2+}-release and ryanodine binding activities (Figure 5). These observations indicate that defects in both SL and SR organelles due to I/R-injury are not only prevented by antioxidants mixture, but are simulated by ROS generating systems.

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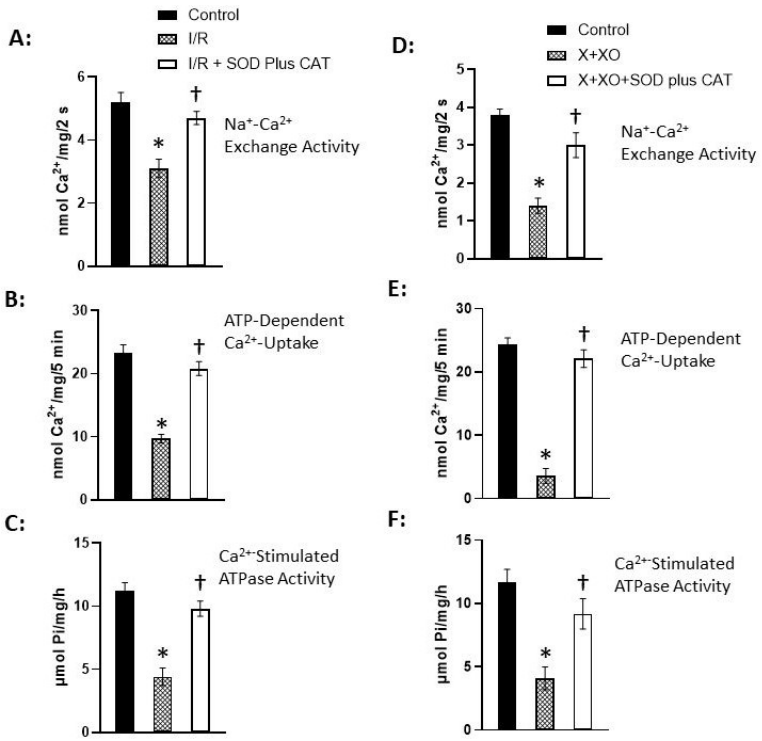

Figure 3. Influence of ischemia-reperfusion (I/R) with or without oxyradical scavenger (SOD plus CAT) as well as perfusion with xanthine plus xanthine oxidase (X + XO) or H_2O_2 on sarcolemmal Na^+-Ca^{2+} exchange, Ca^{2+}-uptake and Ca^{2+}-stimulated ATPase activities in isolated perfused hearts. Hearts were subjected to 30 min global ischemia followed by 5 min reperfusion (I/R) in the absence or presence of 50 U/mL superoxide dismutase (SOD) plus 50 U/mL catalase (CAT). Hearts were also perfused with 2 mM xanthine (X) plus 100 mU/mL xanthine oxidase for 20 min in the absence or presence of SOD plus CAT. Control hearts in each experiment were perfused with normal medium for appropriate period. The data are based on the analysis of information in our papers Dixon et al. [146], Matsubara and Dhalla [147] and Matsubara and Dhalla [148]. * $p < 0.05$ vs. respective control, † $p < 0.05$ vs. respective I/R or X + XO group.

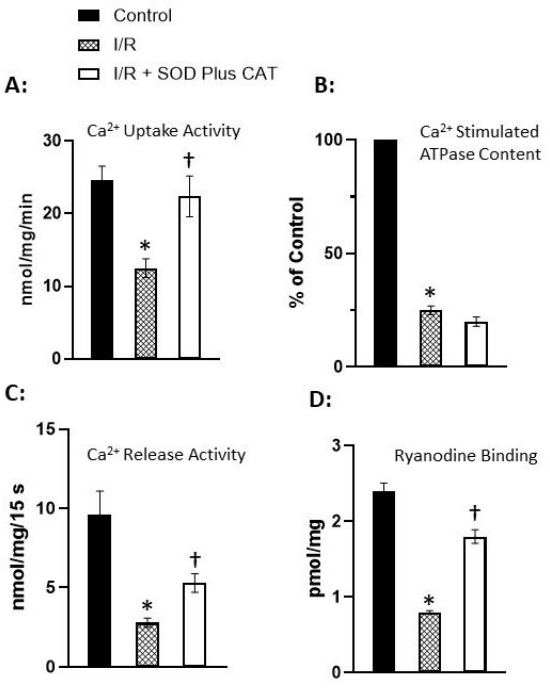

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Figure 4. Effect of ischemia-reperfusion (I/R) with or without oxyradical scavenger (SOD plus CAT) on sarcoplasmic reticular Ca^{2+}-uptake and Ca^{2+}-release activities and ryanodine binding in isolated perfused hearts. Hearts were subjected to 30 min global ischemia followed by 60 min reperfusion (I/R) in the absence or presence of 50 U/mL superoxide dismutase (SOD) and 75 U/mL catalase. Control hearts in each experiment were perfused with normal medium for appropriate time period. The data are based on the analysis of information in our paper Temsah et al. [149]. * $p < 0.05$ vs. respective control value, † $p < 0.05$ vs. respective I/R value.

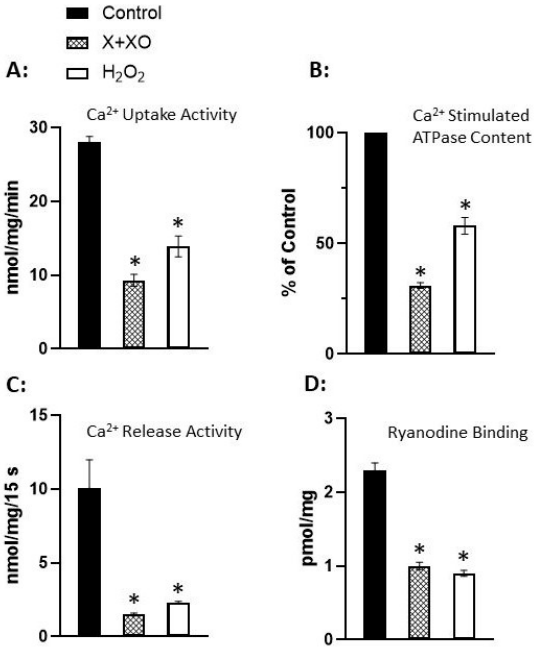

Figure 5. Outcome of perfusion with xanthine plus xanthine oxidase (X + XO) or H_2O_2 on sarcoplasmic reticular Ca^{2+}-uptake and Ca^{2+}-release activities and ryanodine binding in isolated perfused hearts. Hearts were also perfused for 20 min with 2 mM xanthine (X) plus 0.03 U/mL xanthine oxidase or 300 μM H_2O_2. Control hearts in each experiment were perfused with normal medium for appropriate time period. The data are based on the analysis of information in our paper Temsah et al. [149]. * $p < 0.05$ vs. respective control value.

The effects of I/R-injury and ROS generating system on MT and MF have also been examined. The data in Figure 6 show that MT state 3 respiration (A) and ADP/O ratio index (B) were depressed upon subjecting the heart to I/R-injury and these effects were attenuated by SOD plus catalase [150]. Furthermore, perfusing the hearts with X+XO or H_2O_2 [150] was found to depress MT state 3 respiration and ADP/O ratio index (Figure 6 C and D).

The data in Figure 7 indicate depression in MF Ca^{2+}-stimulated ATPase activity due to I/R was prevented in the presence of SOD plus catalase or N-acetylcysteine (Figure 7B) [151,152]. It should also be noted that MF Mg^{2+}- ATPase activity was increased whereas MF Ca^{2+}-stimulated ATPase activity was depressed by perfusing the hearts with X+XO or H_2O_2 (Figure 7 C and D). These observations suggest that both I/R injury and ROS generating system produce similar changes in the MT and MF and the effects of I/R-injury were attenuated by antioxidants.

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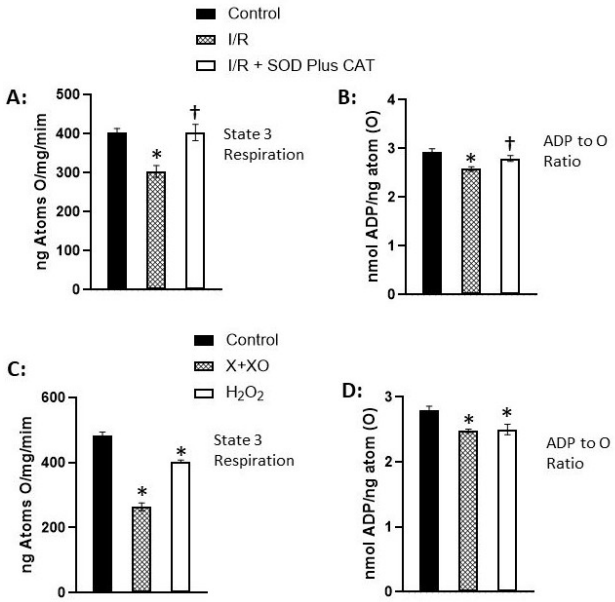

Figure 6. Effect of ischemia-reperfusion (I/R) in the absence or presence of oxyradical scavenger mixture (SOD plus CAT) as well as perfusion with oxyradical generating mixture (X plus XO) or H_2O_2 on mitochondrial function in isolated perfused hearts. Hearts were subjected to 30 min global ischemia followed by 30 min reperfusion in the absence or presence of 50 U/mL superoxide dismutase plus 75 U/mL catalase. Hearts were also perfused for 30 min with 2 mM xanthine (X) plus 60 mU/mL oxidase or 100 µM H_2O_2. Control hearts in each experiment were perfused with normal medium for appropriate time. The data are based on the analysis of information in our paper Makazan et al. [150]. * $p < 0.05$ vs. respective control value, † $p < 0.05$ vs. respective I/R value.

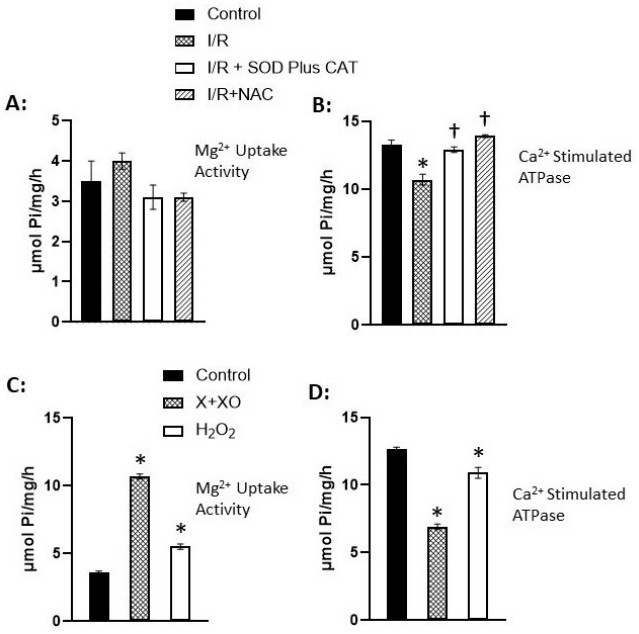

Figure 7. Influence of ischemia-reperfusion (I/R) with or without oxyradical scavenger and antioxidant as well as perfusion with xanthine plus xanthine oxidase (X + XO) or H_2O_2 on cardiac function (A) and myofibrillar ATPase activities in isolated perfused hearts. Hearts were subjected to 30 min global ischemia followed by 30 min reperfusion (I/R) in the absence and presence of 80 µg/mL superoxide dismutase (SOD) plus 10 µg/mL catalase (CAT) or 100 µM N-acetylcysteine (NAC). Hearts were also perfused for 30 min with 2 mM xanthine (X) plus 60 mU/mL xanthine oxidase or 100 µM H_2O_2 followed by 30 min reperfusion. Control hearts in each experiment were perfused with normal medium for appropriate period. The data are based on the analysis of

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information in our papers Maddika et al. [151] and Suzuki et al. [152]. * $p < 0.05$ vs. respective control value, † $p < 0.05$ vs. respective I/R value.

6. Conclusions

It is now well known that delayed reperfusion of the ischemic heart produces marked alterations in myocardial metabolism and cardiac ultrastructure in addition to impairing the recovery of cardiac function. Such changes in the ischemic-reperfused hearts (I/R-injury) are associated with increased formation of superoxide and hydroxyl radicals as well as oxidants including H_2O_2, hypochlorous acid and peroxynitrite, which are collectively termed as reactive oxygen species (ROS). It is noteworthy that MT are the major source of ROS production due to defects in the electron transport system as well as xanthine oxidase as a consequence of reperfusion of the ischemic hearts. There also occurs the activation of endothelium nitric oxide synthase and formation of nitric oxide, which combines with superoxide radicals to generate peroxynitrite. On the other hand, the concentrations of endogenous antioxidants including superoxide dismutase, catalase, and glutathione peroxidase (which antagonizes the actions of ROS) are decreased due to the induction of reperfusion. It should also be mentioned that the levels of transcription factor, Nrf2, which regulates the status of several antioxidants, are also depressed upon reperfusion. Thus, there occurs an imbalance between the excessive formation of ROS and reduced levels of antioxidants due to reperfusion, which leads to the generation of oxidative stress. Although various defects such as inflammation, subcellular alterations, intracellular Ca^{2+}-overload, activation of proteases and changes in cardiac gene expression occur in the ischemia-reperfused hearts, these abnormalities seem to be the consequence of oxidative stress generation during the development of I/R-injury.

Impaired recovery of cardiac function due to I/R-injury has been shown to be associated with depression in SL Na^+-K^+ ATPase, Na^+-Ca^{2+} exchange and ATP-dependent Ca^{2+}-pump activities. These SL alterations would increase the entry of Ca^{2+} into cardiomyocytes and elevate the intracellular concentration of Ca^{2+}. Furthermore, I/R-injury has been demonstrated to induce changes in the SR Ca^{2+}-pump activity and promote the leakage of Ca^{2+} to further raise the intracellular concentration of Ca^{2+} and cause the recurrence of MT Ca^{2+}-overload, depression in the oxidative phosphorylation and energy production, opening MT pores for the leakage of cytotoxic material and development of programmed cellular death. The occurrence of Ca^{2+}-handling abnormalities in the SL and SR organelles is also associated with the development of intracellular Ca^{2+}-overload, activation of proteases such as MMP and calpain, depression in the cardiac gene expression and loss of MF Ca^{2+}-sensitivity. Furthermore, treatment of the heart with different antioxidants has been shown to attenuate I/R-induced Ca^{2+}-handling defects in subcellular organelles, activation of proteases, loss of MF Ca^{2+} sensitivity and development of cardiac dysfunction. It is thus evident that oxidative stress plays an important role in impairing the recovery of cardiac function due to I/R-injury. Accordingly, it is suggested that efforts should be made to develop safe and effective interventions for the upregulation of endogenous antioxidants for improving cardiovascular abnormalities associated with I/R-injury.

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