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Reactive oxygen species: Their role in severe clinical conditions and regulation of redox-sensitive cell signaling transduction events

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ABSTRACT

So long as cellular energy generation by enzymatic systems, β-oxidation of fatty acids in the liver peroxisomes and phagocytosis stimulation by pathogens persist, there will be a continuous production of reactive oxygen species in the cell. Reactive oxygen species play important roles in regulating signal transduction processes in the cell. Their physiological or pathological relevance to the cell depends on their concentration, site of production, duration of exposure to cells, and the redox state of the cellular environment. This review examines the physiological role played by reactive oxygen species in cell signaling events, their involvement in the etiology of many diseases like cardiovascular disorders, cancer, oxidative stress and muscle wasting, as well as the mechanisms by which these reactive oxygen species exert their effects. The definition of the role of reactive oxygen species in the aforementioned pathologies may help open the way for opportunities in the development of new drugs targeted toward lowering their concentration and rate of production, or their immediate clearance from the system soon after they are produced.

Keywords: Sarco (endo) plasmic reticulum calcium–ATPase, ryanodine receptor, lipid peroxidation, reperfusion injury, oxidative stress, hypoxia inducible factor.

INTRODUCTION

Reactive oxygen species (ROS) are small, highly reactive molecules with unpaired valence shell electrons. The group includes reactive anions and/or molecules containing oxygen atoms which can generate free radicals. Examples include hydroxyl radical ($^{\cdot}OH$), superoxide ($O_2^{\cdot\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxinitrites ($ONOO^{\cdot\cdot}$). In general, ROS are

produced in cells as by-products of aerobic metabolism from various enzymatic systems including the mitochondrial electron transport chain, NADPH oxidase complex, xanthine oxidase and cytochrome P-450 [1]. Fatty liver cells harbor additional sites of ROS production. These include peroxisomes – host to β-oxidation of fatty acids, microsomes [2, 3, and 4], arginine metabolism and phagocytic action of macrophages stimulated by pathogens. The cell exhibits some defense or protective mechanisms against the toxic effects of these ROS. These include enzymes like super oxide dismutase (SOD), catalase and glutathione peroxidase. These enzymes are referred to as scavengers of free radicals species generated in the cell. The superoxide ion generated in this process is converted by mitochondrial manganese superoxide dismutase (Mn SOD) to H_2O_2. In the cytoplasm this reaction is catalyzed by Cupper-Zinc superoxide dismutase. H_2O_2 itself is toxic as are organic hydroperoxides and these can be transformed to water and oxygen by catalase or glutathione peroxidase, respectively. In some cases H_2O_2 is further reduced to the highly reactive and very destructive hydroxyl free radical. This reaction is catalyzed by transition metal ions like iron (Fe) and copper (Cu) [100]. The other defense mechanisms involve antioxidants like tochopherol (vitamin E), ascorbic acid (vitamin C), caroteinoids [108] and glutathione. The most potent of the antioxidants is vitamin C [107] and is found in great abundance in orange fruits and vegetables [109]. The combined defensive actions of these enzymes and vitamins coupled with a reduced intracellular redox environment help to keep in check excessive concentrations of the ROS and minimize oxidative damage that may be caused by ROS on macromolecules. Nevertheless, the redox mechanism of the cell defense system is not always maintained in its optimum. Imbalances between the amount of ROS generation and the rate with which they are cleared from the cell may lead to the accumulation of these ROS.

Effects of ROS on Ca^{2+} signaling in Muscle cells and their association with cardiovascular diseases:

Cardiac and skeletal muscles are endowed with important protein components, which actively participate in the regulation of calcium needed for contraction of muscle. The major players in calcium release mechanism include calcium ion channels like the ryanodine receptor protein (RyR), dihydropyridine receptor (DHPR), the sarco(endo)plasmic reticulum calcium-ATPase (SERCA) and the Na^+-Ca^{2+} exchange system.

The RyRs are intracellular calcium channels localized on the membrane of the sarco(endo)plasmic reticulum of muscles and non-muscle cells where they mediate calcium release from intracellular stores [5]. Three isoforms of RyR channels (RyR1, RyR2 and RyR3) have been identified in mammals. The RyR1 and RyR2 isoforms are predominantly expressed in the skeletal and cardiac muscles, respectively. RyR3 isoform is less abundantly expressed in the brain and other tissues like the heart and, skeletal muscles as well [6, 7].

Unlike in cardiac muscles where calcium release is triggered by an entry of small amount of Ca^{2+} through DHPR-sensitive L-type Ca^{2+} channel - a mechanism defined as calcium-induced-calcium release (CICR) [8], the release of Ca^{2+} in skeletal muscle is triggered by an action potential-induced membrane depolarization. Direct coupling between RyR1 and the plasma membrane voltage sensor, DHPRα_1s during membrane depolarization causes a configuration change that influences opening of the RyR1 channel thus releasing Ca^{2+} in to the cytosol [9, 10].

RyRs have been shown to function as redox sensors [11], presumably due to the presence of free thiol groups of cysteine residues that are sentitive to redox reagents [12, 13]. About 100 cysteine residues per RyR1 subunit and 89 per RyR2 subunit have been identified and approximately a third of these from each isoform have free thiols [14, 15].

Modification of RyR channel protein by ROS has both physiological and pathological implications. The degree of either may be determined by the concentration of the ROS, length of exposure of the cell to ROS and the redox state of the intracellular milieux. Low concentrations of ROS, particularly H_2O_2 and HO^- [16, 17] and to a lesser extent, $O_2^{\cdot\cdot}$ [18] have been shown to trigger Ca^{2+} release through the skeletal and cardiac RyRs by increasing their sensitivity to Ca^{2+} as well as the channel open probability.

The mechanism by which ROS exert their effect has been demonstrated to involve the oxidation of RyR-SH group. $O_2^{\cdot\cdot}$ generated in skeletal muscles by mitochondrial complex I & III and by NADPH oxidase, in diaphragm muscles by Xanthine oxidase, [19, 20] is largely converted to the more diffusible H_2O_2 by SOD. H_2O_2 easily diffuses through the membrane into the cytosol where it oxidizes free thiols in the RyR and other ion channel proteins (Figure 1). Oxidation of the free reactive thiol (SH) groups induces formation of disulphide bonds between subunits within the RyR complex leading to conformation changes that alter channel activity and sensitivity to Ca^{2+} [21, 22]. This effect is reversed by reagents including glutathione (GSH), which reduces disulphide bonds to thiols [23, 24].

Some major sarcoplasmic reticulum (SR) protein components like calcium-ATPase and the Na^+-Ca^{2+} pump involved in Ca^{2+} regulatory mechanisms are also targets of ROS-mediated pathologies. The sarco(endo)plasmic reticulum calcium-ATPase and the Na^+-Ca^{2+} exchanger play major roles in skeletal and cardiac muscle calcium homeostasis. SERCA promotes Ca^{2+} uptake into the SR, which is directly coupled to ATP hydrolysis. The combined effect of SERCA and Na^+-Ca^{2+} exchanger is accompanied with diastolic relaxation of the heart muscles.

Association of ROS with pathological conditions has been suggested in the heart, including post ischemic injuries sustained during reperfusion [97, 98, and 99]. Most often, reperfusion of the heart following ischemia is performed in order to restore oxygen to the ischemic heart, thereby reversing the situation and preventing what would otherwise lead to a condition known as myocardial infarction. However, though reperfusion can be a lifesaver, cardiovascular injuries and myocardial stunning sustained in the heart by ROS [30] during or after reperfusion represent a severe pathological condition as well. Cardiac SERCA contains 25 cysteine residues, 2 of these have free thiol groups, which can be targeted for oxidation [25] and have been shown to be very sensitive to H_2O_2 [26]. ROS accumulation during reperfusion of the heart following ischemia [27] leads to calcium overload due to altered redox modulation of ion channels and pumps [28, 39]. This involves oxidation of the SH group of SERCA, thus inhibiting or inactivating SERCA activity [26]. Similarly, intracellular calcium overload can positively feedback and elicit a signaling cascade of events leading to formation of more ROS. This signaling event involves increased expression levels of xanthine oxidase and subsequent formation of $O_2^{\cdot\cdot}$ radical. The latter can then be converted to H_2O_2 and subsequently, the highly reactive; tissue damaging $^{\cdot}OH$ radical. It is important to note that mechanisms leading to calcium overload and reactive oxygen formation discussed in this review are not the sole features responsible for post ischemic heart

injuries. The development and formation of edema, acidosis and NO accumulation in the heart following reperfusion all contribute to inflict severe injuries to the heart muscle cells. In addition, a number of animal and in vitro studies have shown ROS to be involved in heart failure as well [31-34]. ROS involvement in the onset and progression of a significant number of coronary artery diseases [35, 36] is a cause of concern as they are thought to contribute to atherosclerotic lesions on the walls of blood vessels, formation of plaque in the vessels and finally rupture of the vessels leading to coronary thrombosis [37, 38] due to their oxidative action on low density lipoproteins (LDL).

ROS signaling in skeletal muscle adaptation, muscle wasting and protein loss:

ROS produced in skeletal muscle during muscular activity [101] or inactivity play important roles in the regulation of signaling pathways required to promote skeletal muscle adaptation and protection against stress [102] During physical exercise, production of antioxidants as well as scavenging enzymes increases in the mitochondria [103] and this may help keep down the concentration of ROS thereby protecting the cell against oxidative stress. Nevertheless, at elevated concentrations, ROS are said to regulate processes leading to muscle wasting and loss of important protein components involved in contraction and mobility by activating pathways involving protein degradation and apoptosis [46]. Skeletal muscle wasting and protein loss have been observed in a variety of diseases including cancer, AIDS, rheumatoid arthritis [39 40], severe burn injuries [41-44] and sepsis [45]. The most studied pathways involve the mitogen activated protein kinase (MAPK) pathway and the nuclear factor κB (NF-κB) pathway.

MAPK activation by ROS leads to actin-myosin degradation and/or protein degradation in skeletal muscles. MAPK's role in cellular signal transduction involves phosphorylation of important regulatory proteins involved in transcription [47]. The most studied MAPK subfamilies include c-Jun N-terminal kinase (JNK), p^{38} MAPK, and extracellular signal-regulated kinase (ERK) [48]. All three kinases can be activated by accumulation of ROS during oxidative stress. The mechanism of activation involves phosphorylation of p^{38} and JNK by an apoptosis-stimulating kinase 1 and is regulated by ROS and by endotoxins that also induce ROS production. Increased production of ROS during oxidative stress in skeletal muscle mitochondria stimulates activation of the p^{38} and JNK apoptotic pathways. The activation occurs through phosphorylation of tumor suppressor protein, p^{53} and NF-κB that induce expression of pro-apoptosis proteins via caspase-3 activation [49], or through ubiquitin-26S proteasome proteolytic pathways. Either pathway leads to actin-myosin degradation and/or protein degradation in skeletal muscles.

TNF-α/NF-κB signaling pathway is stimulated by increased level of $O_2^{\cdot\cdot}$ and H_2O_2 production in the mitochondria [50, 51]. Activation of NF-κB by TNF-α in turn stimulates ubiquitin conjugation and subsequent 26S proteasome-mediated I-κBα degradation. Studies conducted on transgenic animals [52] and on mouse skeletal muscle primary cultures [53] revealed a significant role played by TNF-α in stimulating muscle loss. In one of these studies, treatment of differentiated myotubes with TNF-α, even at low concentrations, resulted in a reduction in total protein content and loss of adult myosin heavy chain content [53].

ROS produced at sites of burn injuries may, in addition to wound healing, also contribute to modification of muscle constituent at burn site and sites distant from the wound. Proteolytic

pathways activated during burn injuries include the ubiquitin dependent pathways in the skeletal muscle [54, 55] mediated by ROS. A study conducted on rats with burn injuries showed a significant loss of body weight, reflected in a decrease in their protein content, compared to control rats which instead increased in weight [44]. A similar pattern was observed in the weight of the extensor digitorium longus (fast twitch muscles) and soleus from rats with burn injuries versus control animals [44].

Role of ROS in cancer development and tumor progression:

ROS involvement in carcinogenesis and tumor progression [110] can be observed directly by modification of macromolecules [104] or indirectly by activating and stabilizing the transactivating factor, hypoxia inducible factor-1 (HIF-1α).

Macromolecules, including DNA, RNA, lipids, lipoproteins and cell membrane components are sensitive to damages mediated by ROS.

Damage caused to DNA by $^{\cdot}OH$, $O_2^{\cdot-}$ or H_2O_2 results from reaction with pyrimidine and purine bases as well as with chromatin proteins. These damages induce mutagenesis, DNA strand breaks and alter chromatin structure, leading to genomic instability [56, 57], which plays an important role in carcinogenesis [58]. The most common mutations caused by ROS are G → T transversions resulting from oxidized guanine, which easily mispairs with A [104]. In addition to mitochondria, two other important sites of ROS production in the fatty liver cells have been suggested: peroxisomes and microsomes [2]. Elevated concentrations of ROS in liver cells have been shown to stimulate lipid peroxidation [59]. Prolonged inflammation resulting from ROS-mediated oxidation of lipids and cholesterol in the liver cell membrane is a pathological event in cellular transformation and can lead to extensive scarring (liver fibrosis) [59]. Depending on the degree of liver injury caused by ROS, secondary infections like liver cirrhosis may develop as well as the onset of liver cancer. Transformed cells in many tissues lack cell cycle check points to control the overexpression of oncogenic growth factors and their kinase receptors. As a result of this, cell proliferation and tumor formation progress with ease [60]. Modification of protein phosphatases by H_2O_2 modulates processes that lead to apoptosis suppression, cell survival and proliferation [64]. ROS-mediated oxidation of the reactive cysteine residues in protein tyrosine phosphatases inhibits the action of this enzyme, thus enhancing activation of tyrosine kinase signaling through activator proteine-1 (AP-1) [61, 62]. AP-1 is a transcription factor consisting of jun-jun-homodimer or jun-Fos heterodimer, and activation of AP-1 is required for tumor progression [63].

Hypoxia inducible factor (HIF-1) is a transcription factor composed of HIF-1α and HIF-1β subunits [65]. The expression and stabilization of HIF-1α depends on the oxygen condition of the cellular environment [71, 73]. Under normal oxygen tension (normoxia), HIF-1α is unstable, has a very short half-life and is targeted for proteasome degradation via the prolyle 4-hydoxylase (PHD) pathway. Hydroxylation of proline residues 402 or 564 in the human HIF-1α is required for the binding of Von Hippel Lindau tumor suppressor protein (VHL) [66, 67], a complex with an ubiquitin ligase (E3) activity. Ubiquitinated HIF-1α is then rapidly degraded by the 26S proteasome [68, 69]. Under hypoxic condition (less oxygen), $O_2^{\cdot-}$ produced in the mitochondria complex III of the electron transport chain is rapidly converted to H_2O_2 by superoxide dismustase (SOD). The resulting H_2O_2 diffuses in to the cytosol where it inhibits PHD activity through

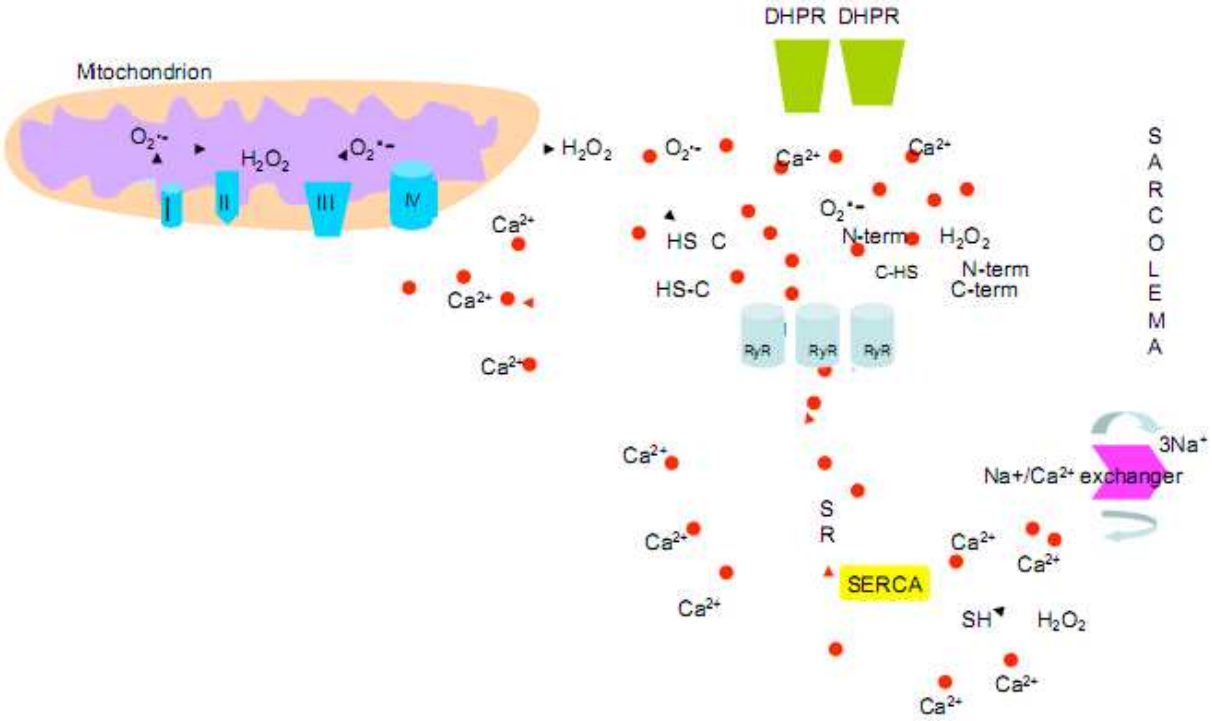
oxidation of Fe^{2+} in the catalytic domain of PHD [70]. This stabilizes HIF-1α and results in its accumulation. It then translocates into the nucleus where it complexes with HIF-1β and binds to HIF-responsive elements (HREs) and modulates the expression of genes, among which are those that favor cancer survival and growth of tumors [106]. A number of studies have shown that acute hypoxia results in increased ROS level in the mitochondria [70, 71] and are required for the inhibition of PHD thus activating HIF-1α signaling pathway in hypoxic cells [70, 72-75].

Effects of ROS on the bioavailability of NO:

Nitric oxide (NO) is a biologically active gaseous molecule produced in the endothelium [76], neurons and immune cells including monocytes, neutrophils and macrophages [105]. It is generated from its precursor, L-arginine by nitric oxide synthases in the presence of oxygen and by the reduction of inorganic phosphates.

NO is an important signaling gaseous molecule which participates in many physiological and pathological processes in human systems. NO stimulates relaxation of endothelial smooth muscles thus facilitating vasodilation and increasing blood flow. It also inhibits platelet aggregation and reduces monocyte and leukocyte adhesion to the endothelial walls [77-79]. An important signaling pathway through which NO exerts its effect involves activation of guanylyl cyclase which catalyzes cyclic guanosine monophosphate (cGMP) formation. The latter activates cGMP-dependent protein kinase 1 which in turn phosphorylates proteins involved in relaxation of vascular smooth muscles, inhibition of platelet aggregation and reduction of monocyte adhesion. Therefore mechanisms that alter or greatly reduce the bioactivity or bioavailability of NO in mammalian systems will ultimately affect the endothelial function, vascular contractility and permeability of muscular arteries [77, 80, 81], and also cause severe damage to biological systems that depend on NO to regulate their proper function. For example, the reaction of $O_2^{\cdot-}$ or $^{\cdot}OH$ with NO inactivates NO and reduces its bioavailability [82, 83], thereby leading to endothelial dysfunction [84]. Their reactions also generate the very highly reactive oxidant, peroxinitrite ($ONOO^{\square\square}$) [85, 86], in blood vessels, impairing vascular relaxation and causing tissue injuries through peroxidation of lipids and lipoproteins. Lipid peroxidation is one of the most deleterious effects caused by ROS which, if unabated can result in an irreversible destruction of cell membranes. Like the other ROS, peroxinitrite also inactivates enzymes through oxidation of critical cysteine residues of enzymes which participate in the cell energy generating process, like creatine kinase [89], glyceraldehyde-3-phosphate dehydrogenase [90], and the complexes I, II, and III of the mitochondrial respiratory chain [91, 92]. Proteins containing heme prosthetic groups hemoglobin, myoglobin and cytochrome [93, 94, 95] are also targets of peroxinitrite attack. Peroxinitrite modifies these proteins by oxidizing ferrous heme to the corresponding ferric heme. In their ferric state, hemoglobin and myoglobin are defective in binding and transporting oxygen. Similarly, oxidation of ferrous heme to the ferric heme in cytochromes will disrupt the sequential transfer of electrons along the respiratory chain. ROS-impaired NO bioavailability represents a central feature of endothelial dysfunction leading to atherosclerosis, diabetes, heart failure [87], and the development and maintenance of hypertension during oxidative stress build-up in the kidneys [88].

Figure 1. Redox regulation of ion channels and membrane proteins by ROS. ROS released from mitochondria diffuse into the cytosol. Here they come in contact with free thiol (SH) groups of cisteine residues of ion channel proteins and proteins involved in skeletal and cardiac muscle calcium homeostasis like RyR, SERCA and Na^+/Ca^{2+} exchanger. Oxidation of free reactive thiol in RyR induces formation of disulphide bonds within subunits of RYR complex. This leads to conformation change that alters channel activity and releases calcium from intracellular store in the sarcoplasmic reticulum (SR) to the cytosol. Oxidation of free reactive SH group of SERCA inhibits uptake of calcium from cytosol back into the (SR) and oxidation of the Na^+/Ca^{2+} exchanger affects the ionic equilibrium of sodium and calcium with more calcium in the cytosol



CONCLUSION

ROS production in cells leads to a variety of pathological conditions, including cellular damage, onset of cancer and tumor progression, cardiovascular diseases and neurodegenerative diseases. At the same time ROS play important physiological roles in signal transduction processes leading to muscle contraction and neuronal plasticity. Nevertheless, the beneficial role of ROS is far more overweighed by the numerous pathological conditions they have been found to associate with. The influence of ROS on these pathologies appears to be more complicated than that discussed in this review. Even more complicated is the mode of action and signaling pathways through which ROS operate. The severity of cellular damage caused by ROS may depend on the tissue or cell type, the level of production of the ROS and the length of time a cell or tissue is exposed to ROS before being removed by scavengers or antioxidants. Given the availability of these scavengers at the disposal of the cell, one would expect an efficient monitoring of the concentration and level of production of ROS in the cell. This, however, is not usually the case as many biological processes in the cell which generate ROS as by-products occur at a fast pace at the same time or different time scales.

Considering the potential and significant role played by ROS in severe pathological conditions leading to a variety of diseases one would be tempted to suggest that enhanced scavenging of the ROS may help prevent the diseases and or alleviate the condition. This strategy has been attempted in the case of cancer in the early transformation process, but only very few studies showed positive outcomes for patients with the advanced disease (96). Nevertheless, it would be of paramount importance to invest on extensive research on the development of new, and to improve upon existing, antioxidant species that would help protect the cell from damages caused by ROS and at the same time improve the beneficial effects the cells can derive from the ROS. How this can be achieved would be the subject of intense scientific investigation.

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