Oxidative stress and ischemia

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Abstract

In the mid 1950s, a small number of scientists first postulated the role of oxidative stress and oxygen-derived free radicals in the pathophysiological mechanisms underlying various types of human disease, including ischemic disease. However, before 1975, because of the technical difficulty of measuring the amounts of free radicals and quantitating oxidative damage, it was very difficult to prove that free radicals could contribute to cell pathology. For that reason, the importance of oxidative stress in biological systems was not definitely recognized until the early 1980s, when measurement of short-lived oxygen-derived reactive species and their detection was made possible by the advent of sophisticated techniques such as electron paramagnetic resonance (EPR) spectroscopy or fluorescent probes. These enabled both the study of free radical biochemistry and the acquisition of useful information about the nature and consequences of free-radical-induced protein and lipid oxidation, both in vitro and in vivo.
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Introduction

Under anaerobic conditions, the mammalian heart cannot produce sufficient energy to maintain essential cellular processes, because it is an obligate aerobic organ. Thus cardiac function and viability need a constant and adequate supply of oxygen (from 8–15 mL O_2/min per 100 g tissue at rest, to more than 70 mL O_2/min per 100 g during exercise). However, the role of oxygen in the heart goes well beyond its role in energy production and remains complex. Indeed, if some oxygen-derived compounds, such as reactive oxygen species (ROS) and oxygen-derived free radicals (ODFR) can participate as helpful molecules in cell signaling process, they can also exert deleterious effects, contributing to the pathogenesis of cardiac dysfunction and inducing irreversible myocardial tissue damage or cell death [1].

Oxygen is thus both vital and deleterious, and it is now widely accepted that molecular oxygen, although essential for maintaining cell viability in living organisms, may also act as one of the primary factors involved in the pathogenesis of several forms of tissue injury [1]. Nowadays, overproduction of ROS is considered to be a common feature of a broad range of diseases, and oxidative stress is generally believed to make a significant contribution to ischemic diseases, postischemic reperfusion injury, drug-induced cardiomyopathy, heart failure, hypertension, inflammatory diseases, cancer, adult respiratory distress syndrome, organ transplantation, neurologic diseases, smoking-related diseases, and acquired immunodeficiency syndrome, among many others [2]. Moreover, oxidative damage is considered to be a major factor in the progressive decline of tissue functions associated with aging [3].

Reactive oxygen species

The way in which oxygen may exert oxidative stress is through its reactive forms. Partial reduction of molecular oxygen can generate reactive oxygen species (ROS), including non radical ROS [hydrogen peroxide (H_2O_2) or singlet oxygen ($^1O_2$)], in addition

to the free radicals, superoxide radical ($^{\bullet}O_2^-$) and hydroxyl radical ($^{\bullet}OH$). Chemically, a free radical is any chemical species (atom, ion, or molecule) containing in its outer orbitals a single, unpaired electron that confers to the radical a very high reactivity.

As free radicals are highly unstable and extremely reactive, they can attack almost all cellular components. Thus oxygen radicals react with membrane lipids, especially those containing unsaturated double bonds, leading to the formation of lipid peroxides* or hydroperoxides*, and aldehydes*. Membrane proteins, especially those containing sulfhydryl groups, are also important targets for oxyradicals, leading to marked alterations in cellular ionic homeostasis. Finally, several enzymes are inactivated by ROS, including catalase*, glyceraldehyde-3-phosphate dehydrogenase*, glutathione peroxidase*, adenylate cyclase*, myofibrillar ATPase, and creatine kinase [2].

Counterbalancing of reactive oxygen species

As ROS are highly reactive, they are able to oxidize most of the biomolecules within the cell, leading to tissue injury and cell death. ROS formed in the cell are normally removed by endogenous antioxidants, which constitute a primary means of detoxifying ROS and preventing ROS-induced cellular damage [4].

Under normal conditions, several cellular antioxidant systems exist to prevent oxidative stress and maintain the redox balance of the cell. Thus ROS are cleared from the cell either by enzymatic systems, including superoxide dismutases (SODs), catalase, and glutathione peroxidase, or by non enzymatic antioxidants, including ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), β-carotene, glutathione, ubiquinone*, lipoic acid, and urate [1,2].

Under the catalyzing effect of SODs, superoxide anions can be first dismutated into hydrogen peroxide; catalase then catalyzes the decomposition of hydrogen peroxide into water and oxygen, leading to the removal of superoxide anion. Another enzyme, glutathione peroxidase, has an important role in protecting against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate.

Ascorbic acid scavenges free radicals and reduces them to hydrogen peroxide, which can be further catalyzed by catalase to form water and oxygen. Alpha-tocopherol* can transfer a hydrogen atom with a single electron to free radicals, thus removing the radicals before they can interact with cell membrane proteins or generate lipid peroxidation.

Excessive production of ROS, overwhelming these mechanisms of defense, is referred to as ''oxidative stress''. Uncontrolled oxidative stress may lead to cellular damage with marked biochemical, metabolic, and functional abnormalities resulting from ROS-induced alterations of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA.

Oxidative stress may result from three different mechanisms: (i) an increase in the production of pro-oxidant agents (ROS); (ii) a decrease in antioxidant defense mechanisms; (iii) a failure to repair oxidative damage.

Oxidative stress and myocardial ischemia

In the heart, ROS can be formed by a variety of mechanisms, including generation during oxidative phosphorylation in the mitochondria as a byproduct of normal cellular aerobic metabolism. They can also be produced by several enzymatic reactions, such as those catalyzed by xanthine oxidase*, NAD(P)H oxidases, and cytochrome P450, or by auto-oxidation of catecholamines or uncoupling of nitric oxide synthase*. In the heart, formation of ROS can also be induced by the action of cytokines and growth factors.

In clinical conditions of myocardial ischemia, early reperfusion is essential to prevent irreversible cardiac damage. However, reperfusion is often associated with some specific deleterious effects brought about by oxidative stress upon reflow, such as life-threatening ventricular arrhythmias and persistent contractile dysfunction (myocardial stunning) [5].

More than 30 years ago, the pioneering work of David Hearse and colleagues [6] clearly demonstrated that the reintroduction of oxygen after a period of severe hypoxia in an isolated, buffer-perfused heart preparation is associated with the occurrence of ''oxygen paradox''. Oxygen paradox is characterized by the development of a sudden alteration in the integrity of cardiac cells and a definitive loss of myocardial contractile function, associated with severe and irreversible ultrastructural damage. The demonstration that molecular oxygen is involved in the development of such reoxygenation injury was provided by the observation that, if reperfusion of the anoxic heart with an oxygenated buffer enhanced cellular injury, longer periods of anoxic perfusion did not produce any extent of tissue injury [5].

Therefore, it is important to emphasize the fact that ROS play an important part in ischemia-induced tissue damage and reperfusion injury. Because of their clinical implications, the deleterious effects of the oxidative stress that occurs in conditions of ischemia-reperfusion as the result of both an impairment of the antioxidant defense system and increased production of ROS have been under intense investigation [5,7,8]. Over more than 20 years, many

experimental studies have clearly shown that, according to several indices of cellular oxidation (oxidation of glutathione, NADPH, ascorbic acid, and tocopherol, and the appearance of protein carbonyls*), the ischemic heart is under oxidative stress, and that this stress is exacerbated by reperfusion. In these conditions, oxidative stress has been shown to correlate with cardiac dysfunction, increased lipid peroxidation, increased membrane permeability, alterations in the function of ionic channels, ionic pumps, and ion exchangers, connexin-43 remodeling, inflammatory processes, and apoptosis signaling [9–12].

Antioxidant therapies and cardioprotection

Numerous animal studies have reported beneficial effects of antioxidant therapies aimed at preventing alterations in the activity of cardiac antioxidant defense mechanisms or at scavenging excessive ROS production. For instance, in in-vivo conditions as well as in isolated heart models, superoxide dismutase mimics, such as organic salen manganese complexes, have been reported to improve postischemic recovery and to limit infarct size [13]. Similarly, in rats receiving a high-selenium diet, cardiac glutathione peroxidase activity was significantly increased, whereas remodeling of connexin-43 was prevented and reperfusion arrhythmias significantly reduced [12]. Selenium was also able to reduce the vulnerability of senescent rat heart to ischemia [14]. In fact, there is now much experimental evidence that nutritional factors may contribute to improving cardiac antioxidant defense mechanisms and to increasing the cardiac resistance to ischemia.

Conclusion

During the past two decades, the terms ''free radicals'', ''oxidative stress'', and ''antioxidants'' have become commonly used in discussions of the cellular mechanisms of an increasing number of diseases [15], even though there are still many gaps in our understanding of the role of ROS and oxyradicals in the pathogenesis of such diseases. Available evidence from animal studies indicates that antioxidant reserve might well be an important factor in promoting tissue protection against injury that is mediated by oxidative stress. Antioxidant enzyme mimics or dietary factors implied in catalytic antioxidation hold much promise for treating conditions in which the damaging oxidant molecule is continuously overproduced after an insult such as myocardial ischemia followed by reperfusion.

However, although antioxidant therapy may be of some interest for limiting the damage caused by ROS, an important question remains regarding the physiological role of antioxidants in biological signaling. ■

* See glossary for definition of these terms.

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