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Pharmacological models and approaches for pathophysiological conditions associated with hypoxia and oxidative stress

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ABSTRACT

Hypoxia is the failure of oxygenation at the tissue level, where the reduced oxygen delivered is not enough to satisfy tissue demands. Metabolic depression is the physiological adaptation associated with reduced oxygen consumption, which evidently does not cause any harm to organs that are exposed to acute and short hypoxic insults. Oxidative stress (OS) refers to the imbalance between the generation of reactive oxygen species (ROS) and the ability of endogenous antioxidant systems to scavenge ROS, where ROS overwhelms the antioxidant capacity. Oxidative stress plays a crucial role in the pathogenesis of diseases related to hypoxia during intrauterine development and postnatal life. Thus, excessive ROS are implicated in the irreversible damage to cell membranes, DNA, and other cellular structures by oxidizing lipids, proteins, and nucleic acids. Here, we describe several pathophysiological conditions and *in vivo* and *ex vivo* models developed for the study of hypoxic and oxidative stress injury. We reviewed existing literature on the responses to hypoxia and oxidative stress of the cardiovascular, renal, reproductive, and central nervous systems, and discussed paradigms of chronic and intermittent hypobaric hypoxia. This systematic review is a critical analysis of the advantages in the application of some experimental strategies and their contributions leading to novel pharmacological therapies.

Contents

Abbreviations: BH2, dehydrobiopterin; BH4, tetrahydrobiopterin; CAO, cerebral artery occlusion; CAT, catalase; CKD, chronic kidney disease; COX, cycloxygenase; CuZnSOD, superoxide dismutase; eNOS, endothelial nitric oxide synthase; ET-1, endothelin; GSH, reduced glutathione; GSH-Px, glutathion peroxidase; GSSG, glutathione oxidized; HIF, hypoxia-inducible factor; IH, intermittent hypoxia; IP, prostacyclin receptor; IR, ischemia reperfusion; NADPH, nicotinamide adenine dinucleotide phosphate; ONOO-, peroxynitrite; OS, oxidative stress; PGI_2, prostacyclin; PO_2, oxygen partial pressure; ROS, reactive oxygen species; XO, xanthine oxidase; VEGF, vascular endothelial growth factor; TBARS, thiobarbituric acid-reactive substances; PA, perinatal asphyxia.

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1. Introduction

Disorders characterized by hypoxia, such as myocardial infarction, stroke, peripheral vascular disease, and renal ischemia, are among the most frequent causes of morbidity and mortality (Foltynie & Kahan, 2013). Moreover, during development, chronic hypoxia may cause intrauterine growth restriction (IUGR) and markedly affect the functions of the newly developed organs (Fowden et al., 2006). Hypoxia is defined as the threshold where the oxygen concentration is a limiting factor for normal cellular processes since oxygen is an essential component for metabolism, including ATP synthesis. Furthermore, the integration of local responses defines hypoxia as a paradigm of reactions affecting the whole organism (Kwasiborski et al., 2012). Subsequently, an oxygen gradient arises between affected and non-affected tissues, stimulating the migration and proliferation of endothelial cells and fibroblasts, which intend to reconstitute normal oxygen supply by increasing perfusion (Nauta et al., 2014). If this process fails, a prolonged inadequate vascular supply of oxygen leads to chronic hypoxia and can cause chronic diseases. Further, intrauterine chronic hypoxia may increase the risk of developing cardiovascular disease later in life, with cardiovascular impairment and endothelial dysfunction (Giussani & Davidge, 2013).

Several difficulties exist in translating basic science findings into clinical practice. For instance, patients usually have marked differences in hypoxia or ischemia duration, concomitant disease, age diversity, co-morbidities, and the medications used. Therefore, accurately representing the clinical situation when establishing an animal model, is a challenge. Moreover, animal models need to account for the differences in response and species-specific reactions in relation to hypoxia (Garcia-Dorado et al., 2009). Despite these limitations, results from animal studies have allowed us to gain considerable insight into the mechanisms of specific phenomena, aiding the design of clinical trials using new pharmacological approaches. The major advantages of hypoxic animal models include highly reliable mechanistic experimental data and the conservation of responses among mammalians.

The findings from animal-based research can establish a cause–effect relationship between a hypoxic protocol and endpoints, such as, reduction in cell death, function improvement, and tissue structure maintenance. This review describes some models for mechanistic studies in pathophysiological states, where the main means of damage are the induction of hypoxia and oxidative stress (OS).

2. Hypoxia and oxidative stress

2.1. General concepts

There is a balance between the production of ROS and the antioxidant system in healthy individuals. When this balance is slightly tipped in favor of ROS, there is continuous low-level oxidative damage in the biological system. This redox imbalance also plays a major pathophysiological role in several clinical conditions associated with hypoxia, such as cardiovascular and neurological dysfunction (Rodrigo et al., 2013).

If the initial increase of ROS is relatively small and occurs in a short period of time, the antioxidant response may be adequate to manage the excess ROS and restore the original redox balance. This physiological response involves a slight increase and/or temporary shift in the intracellular thiol/disulfide redox status toward oxidative conditions (Droge, 2002). However, when the elevated ROS levels are persistent (chronic OS), pathophysiological conditions may arise. Noteworthy, the redox reaction rates and production of ROS is segmented in biological systems by subcellular compartmentation. Thus, as a biological defense, organelles that exhibit high redox reaction rates provide membrane-limited compartments (e.g., mitochondria has electrochemical mechanisms aimed at ATP production, peroxisomes are enriched in H_2O_2 linked metabolism), and both reductants and oxidants have different distributions among compartments. The thioredoxin redox system and reduced glutathione (GSH)/ glutathione oxidized (GSSG) system are "redox buffers" that protect proteins from oxidation and to maintain the redox balance within the cell. These systems are extremely redox dynamic exhibiting different redox potentials depending on the compartmentation (e.g., mitochondria, cysotol, nucleus, endoplasmic reticulum, Golgi, lysosome, peroxisome, or extracellular space), as well as on the physiological/pathological state of the cell. Therefore, it is critical to define the key point of divergence between an organism that can reset the redox status to its original balance and an organism that cannot restore the original healthy condition.

In essence, oxidative stress is established when ROS generation overwhelms an organism's antioxidant capacity. Furthermore, OS has been defined as a disturbance in the pro-oxidant/antioxidant balance toward the former, leading to potential damage (Sies, 1991).

Therefore, oxidative stress can result from the following scenarios:

i. *Diminished levels of antioxidants*, for instance, mutations affecting the activities of antioxidant defense enzymes, such as superoxide dismutase (CuZnSOD), catalase (CAT), or glutathione peroxidase (GSH-Px), or toxins that deplete antioxidant defenses. Many xenobiotics are metabolized by conjugation with reduced glutathione (GSH); high doses of these substances can deplete GSH and cause oxidative stress, even if the xenobiotic is not itself a generator of reactive species. Deficiencies in dietary minerals (e.g., Zn^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, and Se), which act as cofactors for several antioxidant enzymes, can also cause oxidative stress.
ii. Increased production of reactive oxygen species. ROS are molecules, which are produced as a result of oxygen metabolism caused by mitochondrial function, nicotinamide adenine dinucleotide phosphate (NADPH), cyclooxygenase (COX), and xanthine oxidase (XO), among others. Since the rate of mitochondrial electron transfer is orders of magnitude greater than thiol-related oxidative reactions, this organelle produces high amounts of ROS. The occurrence of particularly high-reduced state of thioredoxin system in mitochondria (−360 mV, compared with −290 mV in nucleus and −270 mV in cytosol) preserves the "physiological insulation" of the cell from high-flux electron transfer systems. Cellular environmental alterations such as hypoxia and hyperoxia alter these enzymatic functions and lead to excessive ROS formation (Clanton, 1985). In addition, exposure of cells or organisms to decreased or elevated levels of radical superoxide ($O_2^{\cdot-}$), exposure to other toxins that are oxidant species (e.g., NO_2) or are metabolized to generate reactive species (e.g., paraquat), or the excessive activation of "natural" systems that produce such species (e.g., the inappropriate activation of phagocytic cells in chronic inflammatory diseases) (Clanton, 1985; Halliwell & Whiteman, 2004). In addition, the supraphysiological activation of pro-oxidant enzymes such as NADPH oxidase and XO (through increasing oxidative conversion of xanthine dehydrogenase) or an increase in the bioavailability of transition metals in their redox active state (e.g., Cu^{+} and Fe^{2+}) can lead to a general oxidant environment.

2.2. Alterations induced by hypoxia

The deprivation of oxygen and nutrients results in a series of abrupt biochemical and metabolic changes in various tissues (Zepeda et al., 2013). The absence of oxygen halts oxidative phosphorylation, leading to mitochondrial membrane depolarization, ATP depletion, and inhibition of cellular transportation. During the absence of oxygen, cellular metabolism switches to anaerobic glycolysis, resulting in the accumulation of lactate, which reduces intracellular pH. Consequently, the intracellular accumulation of protons activates the Na^{+}-H^{+} ion exchanger, which pumps protons out from the cell in exchange for Na^{+} entry. Diminishing ATP during hypoxia impairs the function of the Na^{+}/K^{+}-ATPase, thereby exacerbating the intracellular Na^{+} overload. In response, the reverse activation of the Na^{+}-Ca^{2+} ion exchanger results in overloading intracellular Ca^{2+} as the cell tries to expel Na^{+}