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Mitochondrial reactive oxygen species: A double edged sword in ischemia/reperfusion vs preconditioning

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ABSTRACT

Reductions in the blood supply produce considerable injury if the duration of ischemia is prolonged. Paradoxically, restoration of perfusion to ischemic organs can exacerbate tissue damage and extend the size of an evolving infarct. Being highly metabolic organs, the heart and brain are particularly vulnerable to the deleterious effects of ischemia/reperfusion (I/R). While the pathogenetic mechanisms contributing to I/R-induced tissue injury and infarction are multifactorial, the relative importance of each contributing factor remains unclear. However, an emerging body of evidence indicates that the generation of reactive oxygen species (ROS) by mitochondria plays a critical role in damaging cellular components and initiating cell death. In this review, we summarize our current understanding of the mechanisms whereby mitochondrial ROS generation occurs in I/R and contributes to myocardial infarction and stroke. In addition, mitochondrial ROS have been shown to participate in preconditioning by several pharmacologic agents that target potassium channels (e.g., ATP-sensitive potassium (mKATP) channels or large conductance, calcium-activated potassium (mBKCa) channels) to activate cell survival programs that render tissues and organs more resistant to the deleterious effects of I/R. Finally, we review novel therapeutic approaches that selectively target mROS production to reduce postischemic tissue injury, which may prove efficacious in limiting myocardial dysfunction and infarction and abrogating neurocognitive deficits and neuronal cell death in stroke.

Introduction

Acute coronary artery disease and stroke are the number one and third leading causes, respectively, of death and disability among Americans and in most westernized cultures. Ischemia caused by vascular obstruction in the cerebral circulation is the most common cause of stroke, although increased microvascular permeability and intracerebral hemorrhage can also result in decreased perfusion. If diagnosed in a timely manner, significant I/R injury can be avoided by prompt treatment with thrombolytic agents or by physical removal of the obstruction using angioplasty approaches. Neurons or myocytes that are supplied by vessels downstream from the occlusion die from prolonged ischemia and comprise the infarcted region of the tissue that is termed the ischemic core. The cells in this region never regain function and are dead prior to therapeutic intervention in the brain or progress irreversibly to death in the case of severe cardiac ischemia. Of greater clinical interest are cells that die in a delayed manner after reperfusion is initiated. This population of neural or myocyte cells surrounds the ischemic core and is referred to as the penumbra in stroke and area-at-risk in myocardial I/R. These jeopardized cells are not fully reliant on blood flow from the occluded artery, with modest perfusion maintained by collateral blood vessels that provides resistance to ischemic damage. Although penumbral neurons or at-risk myocytes do not succumb to the initial ischemia-induced cell death, they progress to death during reperfusion in a delayed manner that resembles apoptosis. The delayed onset of death provides a window of opportunity for therapeutic intervention. Recognition of this initial penumbral resistance to cell death led to the concept that treatments targeting these cells should be initiated prior to or in the first hours after recanalization of the obstructed vessel.

While significant progress has been made with regard to identifying ROS as key mediators of both detrimental and protective responses in I/R, therapeutic antioxidant management of I/R syndromes such as myocardial infarction, stroke, and circulatory arrest has proven disappointingly ineffective [1–4]. This is most likely due to a number of factors including the fact that untargeted application of antioxidants may not differentiate between detrimental vs beneficial ROS generation. However, recent breakthroughs regarding use of targeted antioxidant therapies to enhance therapeutic efficacy of treatments to ameliorate oxidative stress in I/R injury have rekindled interest in use of agents that modify oxidative stress in I/R.

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Particularly promising developments have arisen with regard to targeted delivery of therapeutic agents to the mitochondria, as a means to reduce ROS-dependent I/R injury. The aim of this review is to summarize evidence supporting a role for mitochondrially-derived ROS in the pathogenesis of I/R injury and for their participation in the beneficial protective actions of preconditioning. We begin with a generalized review of the multifactorial pathogenetic mechanisms of I/R injury, followed by a brief description of mitochondrial sources of ROS, before moving on to a review of the evidence supporting a pivotal role for mitochondrial oxidants in heart and brain injury induced by I/R. We conclude this review with a brief discussion regarding the roles for mitochondrial ROS as redox signaling molecules that underlie metabolic and flow-dependent vasodilation and finish with a summary of work implicating mitochondrial oxidant generation as an essential trigger for activation of cell survival programs that enhance tolerance to I/R, which again emphasizes the Jekyll and Hyde nature of mitochondrial ROS production. From this discussion, it will be apparent that mitochondrial ROS can exert detrimental or beneficial effects, a double-edged sword that is probably explained by the type of oxidant, the amount of ROS produced, and the subcellular site of their production in these two vary different situations (preconditioning vs I/R).

General Concepts of I/R Injury

In tissue subjected to ischemia followed by reperfusion (I/R), pathologic mechanisms are elicited that produce reversible cell injury and dysfunction, which can progress to irreversible damage if the nature and extent of ischemia is prolonged or if the pathologic sequelae to reperfusion are of sufficient magnitude (reviewed in [5]). This damage is referred to as I/R injury and can be divided into three phases (Fig. 1). During ischemia (the first phase of injury), interruption of the blood supply to an organ causes a reduction in oxygen and nutrient delivery to the affected tissues. This disrupts ATP generation via oxidative phosphorylation, causing cells to alter their metabolism and impairs energy-dependent cellular function. Reduced ATP availability limits ion pumps in cell membranes, resulting in calcium overload, structural disorganization, and apoptotic, necroptotic, and necrotic cell death. In addition, ischemia induces conformational changes in enzymes such as xanthine oxidase and elicits the formation of proinflammatory mediators and expression of adhesion molecules that promote leukocyte/endothelial cell adhesive interactions. These latter processes do not directly contribute to injury during the ischemic phase, but rather set the stage for the second stage of I/R injury (ie, that due to reperfusion), wherein tissue injury is exacerbated when the blood supply is re-established (Fig. 1). Paradoxically, the lack of oxygen during ischemia and the replenishment of oxygen during reperfusion both contribute to the total injury sustained by tissues subjected to I/R. The clinical outcome is also determined by a third phase of ROS production that occurs during post-reperfusion repair that is characterized by tissue remodeling and adaptation (Fig. 1).

Increased generation of reactive oxygen species (ROS) has been suggested as a major contributor to the pathogenetic mechanisms underlying ischemia, reperfusion, and the later post-reperfusion phase of I/R injury (Fig. 1). As a second paradox, ROS appear to exert both detrimental and beneficial effects in I/R, causing damage that leads to neurocognitive defects in stroke and contribute to the expansion of infarct size as a result of their production during ischemia and reperfusion while subserving a signaling function to promote fibrosis, angiogenesis, and vascular remodeling during the repair phase (Fig. 1). Moreover, ROS signaling promotes the activation of cell survival programs when the heart or brain (or other organs) are exposed to preconditioning stimuli (such as short bouts of ischemia, moderate ethanol ingestion, or a wide variety of pharmacologic agents) prior to the onset of prolonged I/R. The double-edged sword effects of I/R-induced ROS generation may be related to species of ROS produced,

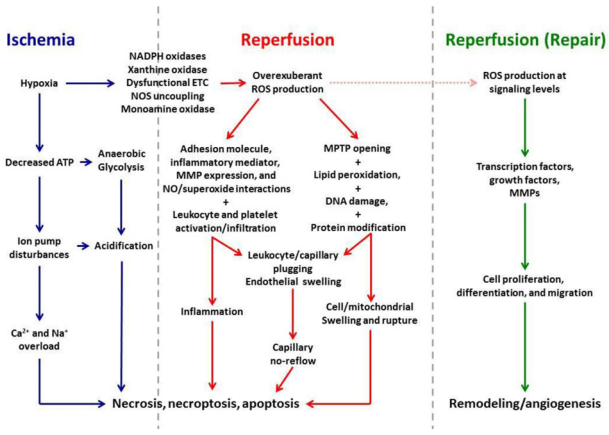

Fig. 1. Mechanisms contributing to tissue injury in ischemia/reperfusion (I/R). Cellular hypoxia secondary to ischemia results in decreased ATP production, which in turn, disrupts ion pump function, leading to accumulation of Na^+, Ca^{2+}, and H^+, with cellular acidification further promoted by a shift to anaerobic glycolysis for energy production. Activation and upregulated expression of enzymes capable of producing reactive oxygen species (ROS) and electron transport chain (ETC) dysfunction are also initiated during ischemia. These events set the stage for a burst of ROS generation when molecular oxygen is reintroduced to ischemic tissues when the blood supply is re-established. ROS-dependent expression of proinflammatory stimuli and expression of adhesion molecules by endothelial cells and leukocytes precipitates the infiltration and activation of neutrophils, T cells and monocytes. Phagocytic Nox2 activation results the respiratory burst of superoxide production that further magnifies the massive oxidative stress that directly damages virtually every biomolecule found in cells and induces the programmed cell death responses, apoptosis and necroptosis. Postischemic ROS generation also activates matrix metalloproteinases (MMPs) and other proteases that act to cleave proteins and receptors, thereby impairing their function. The net impact of these ROS-dependent events is opening of mitochondrial permeability transition pores (MPTPs), which contributes to swelling and lysis of cells. Increases in leukocyte stiffness induced by hypoxia and acidosis during ischemia lead to impaction of these cells in capillaries, an effect that is exacerbated by ROS-dependent endothelial cell swelling which in turn reduces their diameter when the blood supply is re-established. Thus, a nutritive perfusion impairment becomes prominent during reperfusion, despite repair of the precipitating ischemic event. In direct contrast to these catastrophic effects of ROS generation secondary to events occurring in ischemia and early reperfusion, oxidant production also occurs at later stages of reperfusion as tissue repair is initiated. However, ROS production occurs at lower levels that allow oxidant species to serve as signaling molecules that participate in transcriptional activation of growth factors and promote cell proliferation, differentiation and migration. The net effect of these processes is tissue and vascular remodeling, including angiogenesis. While some of these repair processes help restore organ function, others such as tissue fibrosis contribute over time to eventual organ failure. The mechanisms depicted in this figure emphasize the concept that ROS generation play key roles in all three phases of ischemia/reperfusion injury and cell death.

the amount of oxidants generated, and the subcellular location and cellular source of their production under a given set of conditions, as well as at what time during the three phases of responses to I/R they are formed.

Reperfusion represents the second phase of I/R injury and precipitates the generation of ROS that is fueled by the reintroduction of molecular oxygen to the tissues (Fig. 1). Xanthine oxidase- and phagocyte NADPH oxidase-derived oxidants can damage virtually every biomolecule found in cells and tissues. Although essential for cell survival, re-establishing the blood supply to ischemic tissues also delivers blood-borne formed elements (platelets and leukocytes), which become activated and establish adhesive interactions with the walls of postcapillary venules (Fig. 1). Upon transmigration into the tissues, these activated leukocytes release their cytotoxic arsenal of ROS and hydrolytic enzymes to exacerbate parenchymal cell injury. ROS induce tissue dysfunction by directly damaging cells via a number of mechanisms including peroxidation of cell membrane and organelle lipids, oxidizing DNA, activation of matrix metalloproteinases and calpains, producing osmotic cell lysis, induction of no-reflow, and causing opening of the mitochondrial permeability transition pore (Fig. 1). ROS may also induce cell dysfunction and death by indirect