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Review

Neuronal oxidative stress in acute ischemic stroke: Sources and contribution to cell injury

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ABSTRACT

Oxidative stress has emerged as a key deleterious factor in brain ischemia and reperfusion. Malfunction of the oxidative respiratory chain in mitochondria combines with the activation of cytoplasmic oxidases to generate a burst of reactive oxygen species that cannot be neutralised by the cell's antioxidant mechanisms. As a result, oxidative stress contributes directly to necrosis and apoptosis through a number of pathways in ischemic tissue. Pharmacological intervention with antioxidants or enhancers of endogenous antioxidant molecules is proving to be difficult due to the speed and scope of the oxidative impact. Additionally, the knowledge that neuronal fate in ischemic stroke is tightly linked to other brain cells like endothelial cells and astrocytes has shifted the focus of study from isolated neurons to the neurovascular unit. For this reason, recent efforts have been directed towards understanding the sources of oxidative stress in ischemic stroke and attempting to block the generation of oxygen radicals.

1. Introduction

A common controversy in the recent study of neurodegenerative disorders has been whether oxidative stress is the cause or consequence of changes that contribute to neurodegeneration (Andersen, 2004). This is not the case in ischemic stroke, as the primary cause of the injury is well understood. Ischemic stroke begins with the occlusion of a brain artery, with the consequent restriction in the delivery of basic nutrients to a cerebral region (Woodruff et al., 2011). The affected area of the brain receives insufficient oxygen and glucose to keep cells functioning and this alters cellular functions at the electrochemical and metabolic level as well as with regard to the release of toxic products (Woodruff et al., 2011). Under these conditions, a cell can lose function and yet survive even if tissue irrigation by collateral vessels is minimal (Moskowitz et al., 2010). However, the accumulation of toxic products such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) can tip the balance between life and death in the undesired direction (reviewed by Chen et al., 2011). This is thought to occur upon reperfusion, when the occlusion is removed (spontaneously or pharmacologically) and blood is restored to the area. At this stage, the delivery of oxygen and glucose necessary for the generation of ATP can occur, allowing the initiation of a cascade of adverse reactions, one of which is the generation of more ROS. The resulting oxidative stress causes further damage and may ultimately result in the initiation of pathways that lead to necrotic and apoptotic cell death.

The cell is equipped to combat oxidative stress at three levels: by regulating ROS generation (Groeger et al., 2009; Li et al., 2011), by eliminating ROS with the help of neutralising enzymes and scavenger molecules (Watson et al., 2012), and by repairing those proteins, lipids or DNA that have been affected by oxidative stress (Liu et al., 2011; Gao et al., 2012). However, once ischemia takes place, increased neutralising enzymes and scavengers are insufficient to cope with the oxidative load (Kaur et al., 2011; Sharpe et al., 1994). Additionally, repair mechanisms cannot withstand the amount of damage inflicted, and consequently the cell succumbs to the stress and undergoes apoptosis or necrosis. However, these three levels offer potential targets for therapeutical interference with oxidative stress. In this review we will cover current research aimed at understanding ischemic stroke-related oxidative stress, its molecular mechanisms, its effects on cell viability and the potential sites of intervention currently under research focus.

2. ROS generation in ischemia

Healthy mitochondria use oxygen to generate ATP by oxidative phosphorylation at the mitochondrial respiratory chain (MRC), and as a consequence small levels of the ROS superoxide ($O_2^{\cdot -}$) are produced in the mitochondrial matrix. $O_2^{\cdot -}$ gets converted to another ROS, H_2O_2, by the enzyme superoxide dismutase (SOD), and H_2O_2 leaves the mitochondria to act as an intracellular messenger (Rice, 2011). In ischemia (Fig. 1, top), however, oxygen becomes depleted

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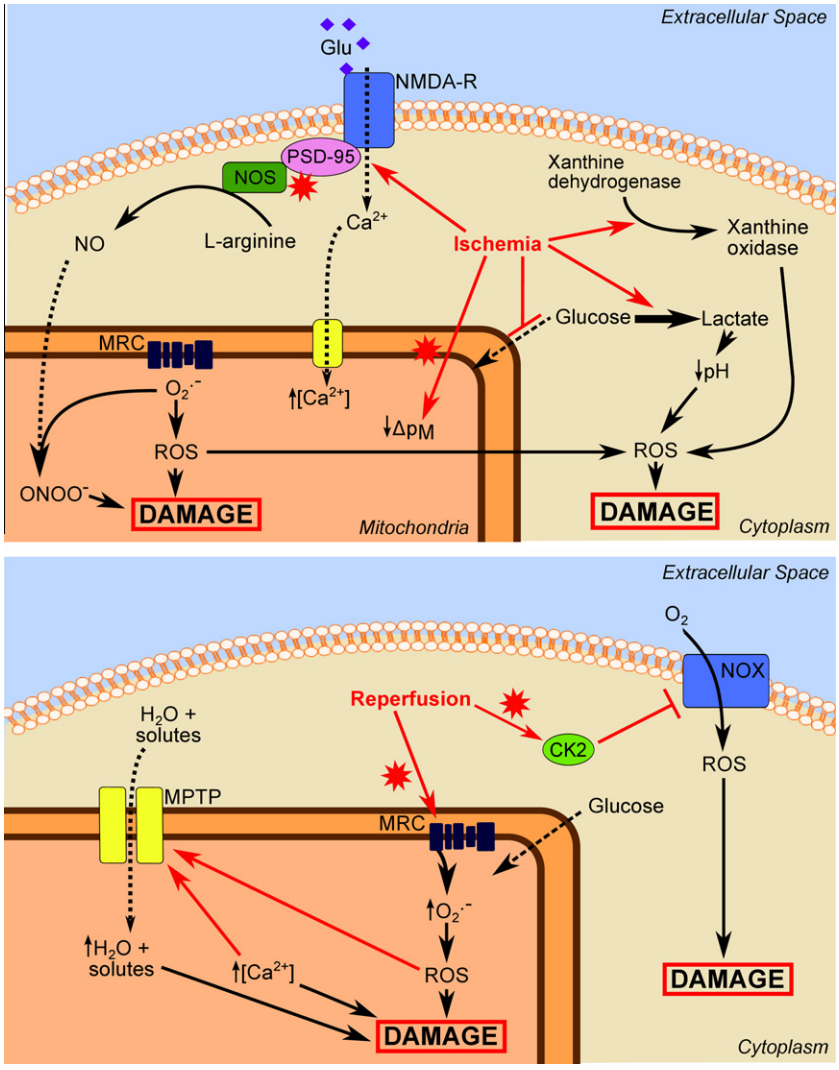

Fig. 1. Overview of ROS production in brain ischemia and reperfusion. (A) ROS production in ischemia. Lack of oxygen promotes the interruption of oxidative phosphorylation at the mitochondrial respiratory chain (MRC). Mitochondrial depolarisation ($\downarrow p_M$) together with intracellular acidification ($\downarrow$pH) caused by an accumulation of lactate as an anaerobic product of glycolysis, result in increased levels of superoxide ($O_2^{\cdot -}$) conversion to other ROS. Activation of the NMDA receptor by glutamate increases Ca^{2+} influx as well as the activation (via the adaptor protein postsynaptic density (PSD)-95) of neuronal nitric oxide synthase (nNOS), which generates nitric oxide (NO) from L-arginine. Xanthine oxidase, which results from the conversion of xanthine dehydrogenase under these conditions, contributes to the production of ROS. NO reacts with $^{\cdot -}$ to generate the harmful radical peroxynitrite ($ONOO^-$). All of these ROS have potential damaging roles in mitochondria and in the cytoplasm. B. ROS production in reperfusion. In addition to the pathways initiated during ischemia, oxygen availability after ischemia resets the MRC resulting in a large increase in $^{\cdot -}$ mostly from complex I. Oxidative stress and increased Ca^{2+} cause opening of the mitochondrial permeability transition pore (MPTP). This allows entry of water and solutes from the cytoplasm resulting in mitochondrial swelling and damage. Generation of ROS also occurs in the cytoplasm through the action of NADPH-oxidase (NOX). Upon reperfusion, the inhibitory enzyme casein kinase 2 (CK2) is downregulated contributing to activation of NOX. The resulting oxidative stress outweighs antioxidant defences and leads to cell damage, necrosis or apoptosis. Asterisks indicate the pathways discussed in this review.

prior to glucose, as shown by the fact that interstitial oxygen pressure in the penumbra, the brain area partially irrigated by collateral vessels that surrounds the acutely injured core, decreases to 33% only 1 h after ischemia (Liu et al., 2004). This favours the glycolytic pathway as the means of anaerobic ATP production (Hertz, 2008). The consequence is an accumulation of lactic acid resulting in acidosis, which promotes pro-oxidant and detrimental changes in neurons such as the inactivation of antioxidant defences, release of oxidant iron from proteins (Ying et al., 1999), and increased glutamate toxicity (Lewerenz et al., 2010). Other contributors to ROS in ischemia are the enzyme xanthine oxidase and mitochondrial depolarisation (Abramov et al., 2007).

Recent studies aiming at preventing ROS generation due to mitochondrial depolarisation have focused on uncoupling factors. Uncoupling refers to the entry of protons to the mitochondrial matrix across the inner mitochondrial membrane (IMM) independently of ATP synthesis, resulting in the depolarisation of the IMM and decreased ROS generation (Mattiasson et al., 2003). One of these factors is uncoupling factor 2 (UCP2), a protein present in low levels in healthy neuronal mitochondria that becomes upregulated as a result of ischemia (Mattiasson et al., 2003). These authors showed that only 3 min of global brain ischemia were sufficient to significantly increase UCP2 mRNA upon reperfusion in the rat hippocampus. UCP2 overexpression protected neurons from cell death in an *in vitro* model of stroke characterised by 90 min of oxygen and glucose deprivation (OGD), and it effectively reduced infarct size after 50 min of middle cerebral artery occlusion (MCAO), the most common model of focal ischemia, and 24 h of reperfusion (Mattiasson et al., 2003). Besides manipulation of UCP2 and other uncoupling proteins, compounds with mild uncoupling properties, like 2,4-dinitrophenol (DNP), have proved effective in limiting ischemia and reperfusion (I/R) injury. In fact, DNP, when applied at the onset of

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