Respiratory Physiology & Neurobiology 209 (2015) 106–114

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Effects of hyperoxic exposure on signal transduction pathways in the lung☆

Andrea Porzionato[a], Maria Martina Sfriso[a], Andrea Mazzatenta[b], Veronica Macchi[a], Raffaele De Caro[a], Camillo Di Giulio[b,*]

[a] Department of Molecular Medicine, University of Padova, Padova, Italy
[b] Department of Neurosciences and Imaging, University of Chieti, Chieti, Italy

ARTICLE INFO

Article history:
Accepted 1 December 2014
Available online 6 December 2014

Keywords:
Reactive oxygen species
Bronchopulmonary dysplasia
Oxidative damage
ERK
JNK
p38

ABSTRACT

Exposure to supraphysiological concentrations of oxygen is often applied in clinical practice to enhance oxygenation in acute or chronic lung injury. However, hyperoxic exposure is associated with increased reactive oxygen species production, which can be toxic to pulmonary endothelial and alveolar epithelial cells. Oxidative stress activates the pathways of the mitogen-activated protein kinases family: extracellular signal-regulated kinase (ERK1/2), C-Jun-terminal protein kinase (JNK1/2), and p38 kinase. Several studies have suggested that ERK activation in lung cells has a protective effect in response to hyperoxia, through stimulation of DNA repair and antioxidant mechanisms, and prolonged cell survival. Conversely, JNK1/2 and p38 kinase have been most frequently reported to have roles in induction of apoptotic responses. Moreover, exogenous factors, such as ATP, retinoic acid, substance P, thioredoxin, inosine and laminin, can have cytoprotective effects against hyperoxia-induced cell damage, through promotion of ERK activation and/or limiting JNK and p38 involvement.

Published by Elsevier B.V.

1. Introduction

Mechanical ventilation with supraphysiological concentrations of oxygen is often necessary to treat newborns, older children, and adults with respiratory distress due to hypoxemia, acute respiratory distress syndrome, or chronic obstructive pulmonary disease. However, in lung cells, hyperoxia can increase reactive oxygen species (ROS), which are extremely toxic and can cause cell injury and death (Xu et al., 2006). Hyperoxic lung damage is characterized by an extensive inflammatory response and damage to the alveolar–capillarity barrier, which can lead to impaired gas exchange and pulmonary edema. Such pathological changes in hyperoxic lungs are accompanied by injury and apoptotic or necrotic death of pulmonary cells (Mantell and Lee, 2000; Petrache et al., 1999). However, prolonged exposure to hyperoxia results in a scenario of both acute and chronic lung diseases, such as an acute inflammatory lung injury and bronchopulmonary dysplasia (BPD), respectively. In the acute inflammatory lung injury, NADPH oxidase activation generates ROS (e.g., superoxide anions, hydrogen peroxide, hydroxyl radicals, hypochlorous acid), which can directly injure pulmonary cells via lipid peroxidation, protein sulfhydryl oxidation, enzyme inactivation, DNA damage, and depletion of cellular reducing agents (Cacciuttolo et al., 1993; Zhang et al., 2003). The final effects in mammalian endothelial and epithelial cells are stress responses, and modulation of cell growth, inflammation, and/or death (Lee and Choi, 2003).

BPD is the most common chronic lung disease of prematurity, and it results in impaired alveolar growth and a dysmorphic vascular architecture (Thebaud and Abman, 2007). In its classic form, BPD is strongly correlated to oxygen toxicity and mechanical injury. Moreover, most animal models of BPD involve hyperoxic exposure, such as with premature baboons, or newborn rats or mice in the early postnatal period. From a histopathological point of view, hyperoxia disrupts postnatal alveolar development, which leads to smaller numbers of enlarged and simplified alveoli, thicker septa, and an increase in alveolar macrophages (e.g., Dauger et al., 2003; Balasubramaniam et al., 2007; Porzionato et al., 2012a, 2013a; Grisafi et al., 2012, 2013). Experimental hyperoxic models of BPD also result in changes in

☆ This paper is part of a special issue entitled "Molecular basis of ventilatory disorders" guest-edited by Dr. Mietek Pokorski.

* Corresponding author. Tel.: +39 0871 3554044; fax: +39 0871 3554045.
E-mail address: digiulio@unich.it (C. Di Giulio).

http://dx.doi.org/10.1016/j.resp.2014.12.002
1569-9048/Published by Elsevier B.V.

microvascular development and thickening of the medial muscle layer of arteries, with pulmonary hypertension (e.g., Jones et al., 1984; Koppel et al., 1994; Porzionato et al., 2012a, 2013a; Grisafi et al., 2012, 2013). Moreover, hyperoxic exposure has also been reported to increase the number of lung mast cells, which preferentially accumulate around vessels (Brock and Di Giulio, 2006).

Hyperoxic exposure and oxidative stress are known to activate many different cascades of intracellular signaling pathways. In particular, protein kinases, such as mitogen-activated protein kinases (MAPKs), might have roles in the production of the histopathological and functional changes that characterize hyperoxic lung damage. A thorough understanding into the regulation of the main signal transduction pathways in hyperoxia that can lead to alveolar epithelial cell injury and cell death might also provide the basis for effective therapeutic interventions. This review focuses on pulmonary cell responses to hyperoxia, with particular reference to the role of the main MAPKs.

2. Mitogen-activated protein kinases

MAPKs are important intermediates in signal transduction pathways, and they have been conserved through evolution. To date, four different mammalian MAPK cascades have been described: extracellular signal-regulated kinase 1 and 2 (ERK 1/2); c-Jun N-terminal kinase (the JNK family); p38; and ERK5 (Plotnikov et al., 2011). Each cascade involves the three core kinases MAP3K, MAPKK, and MAPK, and usually also include additional upstream (MAP4K) and downstream components. These cascades can be activated by various extracellular stimuli, such as hyperoxia, and they control a wide range of fundamental cellular processes, such as cell growth, proliferation, differentiation, motility, stress responses, survival, and apoptosis (Lee and Choi, 2003; Plotnikov et al., 2011; Son et al., 2011; Zaher et al., 2007).

Each MAPK carries out its function through dual specific phosphorylation. The signals transmitted need to be transported into the nucleus and to modulate specific intracellular and nuclear substrates that trigger the activities of transcription factors, transcription suppressors, and chromatin remodeling proteins in de novo gene expression (Plotnikov et al., 2011). Currently, some potential targets of the MAPK pathways include the family of pro-apoptotic caspases, cytokines (e.g., interleukin [IL]-6, IL-11 and IL-β), growth factors, and heme oxygenase (HO-1). The downstream effectors of hyperoxia-induced activation of MAPKs are also redox transcription factors, such as nuclear factor-kappa β (NF-κβ), AP-1 and NF-E2-related transcription factor 2 (Nrf2), which are involved in protective responses against oxidative damage (Zaher et al., 2007).

Dysregulation or incorrect functioning of the MAPK pathways are involved in the progression of various diseases, such as cancer, diabetes, autoimmune diseases, and developmental abnormalities (Plotnikov et al., 2011). One of the main damaging stimuli that can mediate or induce activation of MAPK pathways is the production of ROS, such as superoxide, hydroxyl radicals, and hydrogen peroxide, following hyperoxic exposure (Petrache et al., 1999). Furthermore, ROS can induce oxidative modifications of signaling proteins, by altering the structure and function of the proteins through modification of critical amino-acid residues. Indeed, mimicking oxidative stress through direct exposure to exogenous H_2O_2 leads to activation of MAPK signaling. MAPKs can also be activated by oxidative modifications to intracellular kinases; e.g., MAP3Ks that are involved in MAPK signaling cascades. Another potential mechanism for MAPK activation by ROS is the inactivation and degradation of MAPK phosphatases, which are tyrosine and serine/threonine phosphatases that are involved in negative regulation of MAPKs. Other factors that activate MAPK pathways are environmental stress and growth factors (Son et al., 2011).

2.1. ERK

The MAPK/ERK pathway is also known as the Ras–Raf–MEK–ERK pathway, and it is a kinase cascade that has a central role in the signaling of a wide variety of extracellular agents that operate through different receptors-tyrosine kinases (RTKs), such as the epidermal growth factor (EGF) receptors. Growth factor receptors are most commonly activated by ligand-induced dimerization or oligomerization, which phosphorylates RTKs. For example, insulin-like growth factor-1 (IGF-1) contributes to the activation of the ERK pathway via phosphorylation of EGF receptors (Son et al., 2011). In most cases, the activation of these receptors is transmitted to the small GTPase Ras by several mechanisms, and this in turn activates Raf, a MAP3K. Thereafter, the signal is transmitted to the MAPKKs MEK1 and MEK2, which, again in turn, transmit their signals to ERK1/2.

Ligand-independent clustering and activation of growth factor receptors can be induced directly by exposure to different oxidative-stress-inducing agents, such as osmotic stress and ultraviolet radiation (Meves et al., 2001). The first step is the activation of cell-surface receptors through chemical cross-linking or aggregation of receptors and membrane rafts, which leads to the production of ROS, as second messengers of this intracellular signal transduction. The second step involves chemical modifications to protein tyrosine kinases, to initiate the tyrosine-phosphorylation–dependent local switch for activation of the catalytic activity of the enzymes (Nakashima et al., 2005). Following this activation pathway, oxidative stress induces EGF receptor activation through RTK phosphorylation, and H_2O_2 has been proposed to be a mediator of ligand-independent phosphorylation of growth factor receptors in response to oxidative stress (Meves et al., 2001).

Phosphorylation of ERK1/2 supports the activation of hundreds of substrates in many cellular locations, including the cytoplasm, mitochondria, Golgi complex, endosomes, cellular membranes, and in particular, the nucleus. The final effect of activation of the ERK cascade is the induction of several processes, which include induction or suppression of transcription and chromatin remodeling, which mainly promotes cell proliferation and differentiation, but also cell apoptosis under some stress response conditions.

The ERK cascade is generally considered to be a survival mediator that is involved in the protective actions of growth factors against cell death. For its role under hyperoxic conditions, most studies have reported pro-survival effects (Ahmad et al., 2004; Buckley et al., 1999, 2005; Carnesecchi et al., 2009; Chen et al., 2010; Kannan et al., 2006; Jones and Agani, 2003; Lang et al., 2010; Li et al., 2006, 2011a; Monick et al., 2004; Papaiahgari et al., 2004; Parinandi et al., 2003; Truong et al., 2004; Xu et al., 2006; Waldow et al., 2008), although under some conditions, pro-apoptotic actions have also been reported (Petrache et al., 1999; Zhang et al., 2003).

Various studies have reported increased activation of ERK in response to hyperoxia, with this phenomenon frequently ascribing to cellular ROS generation (e.g., Kannan et al., 2006; Kim et al., 2012; Monick et al., 2004; Truong et al., 2004; Xu et al., 2006; Zhang et al., 2003). The role of the ERK pathway in hyperoxic exposure is illustrated in Fig. 1. Some of the mechanisms involved in ERK activation in response to hyperoxia and ROS generation will be discussed here.

Mitochondria are the major source of ROS production under normoxic and hyperoxic conditions in which the ERK pathway is involved. In the mouse lung epithelial cells (MLE-12), ROS generation due to hyperoxic exposure results in disruption of both