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The effects of nitric oxide in acute lung injury

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Abstract

Acute lung injury (ALI) is a common clinical problem associated with significant morbidity and mortality. Ongoing clinical and basic research and a greater understanding of the pathophysiology of ALI have not been translated into new anti-inflammatory therapeutic options for patients with ALI, or into a significant improvement in the outcome of ALI. In both animal models and humans with ALI, there is increased endogenous production of nitric oxide (NO) due to enhanced expression and activity of inducible NO synthase (iNOS). This increased presence of iNOS and NO in ALI contributes importantly to the pathophysiology of ALI. However, inhibition of total NO production or selective inhibition of iNOS has not been effective in the treatment of ALI. We have recently suggested that there may be differential effects of NO derived from different cell populations in ALI. This concept of cell-source-specific effects of NO in ALI has potential therapeutic relevance, as targeted iNOS inhibition specifically to key individual cells may be an effective therapeutic approach in patients with ALI. In this paper, we will explore the potential role for endogenous iNOS-derived NO in ALI. We will review the evidence for increased iNOS expression and NO production, the effects of non-selective NOS inhibition, the effects of selective inhibition or deficiency of iNOS, and this concept of cell-source-specific effects of iNOS in both animal models and human ALI.

Keywords: Acute lung injury; Nitric oxide; Endothelial cell; Neutrophil; Macrophage; Permeability

1. Introduction

Acute lung injury (ALI) is a major clinical problem, contributing to the mortality of up to 100,000 critically ill patients in the United States annually. Intensive clinical and basic science investigation has led to a greater understanding of the pathophysiology of ALI. However, this knowledge has yet to be translated into new anti-inflammatory therapeutic options for patients with ALI, or into a significant improvement in the outcome of ALI.

ALI is characterized by up-regulation of a host of inflammatory mediators, including an increase in the expression and activity of inducible nitric oxide (NO) synthase (iNOS), resulting in increased NO production. Although the increased presence of iNOS and NO in ALI are thought to contribute importantly to the pathophysiology of ALI, this remains controversial. Indeed, non-selective inhibition of total NO production or selective inhibition of iNOS has not been consistently effective in the treatment of ALI. We have recently proposed the concept of cell-source-specific effects of NO in ALI. This idea has potential therapeutic relevance: rather than inhibition of NO production in all cells, targeted iNOS inhibition specifically to key individual cells may be a more effective therapeutic approach in patients with ALI.

In this paper, we will explore the potential role for endogenous iNOS-derived NO in ALI. We will review the evidence for increased iNOS expression and NO production, the effects of non-selective NOS inhibition, the effects of selective inhibition or deficiency of iNOS, and the concept of cell-source-specific effects of iNOS in both animal models and human ALI.

2. Acute lung injury

ALI is an important clinical problem which is characterized by high-protein pulmonary edema and severe hypoxemia and

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CLP, cecal ligation and perforation; cNOS, calcium-dependent NO synthase; EC, endothelial cell; iNOS, calcium-independent NO synthase; LPS, lipopolysaccharide; MPO, myeloperoxidase; 3-NT, 3-nitrotyrosine; NO, nitric oxide; NOS, NO synthase; SP-A, surfactant association protein-A.

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which is associated with significant morbidity and mortality. Indeed, ALI and its most severe form, the acute respiratory distress syndrome (ARDS) have case-fatality rates of approximately 40%, contributing to the mortality of up to 100,000 critically ill patients in the United States annually (Ware and Matthay, 2000; Rubenfeld, 2003). Despite great advances in our understanding of the inflammatory basis of the pathophysiology of ALI, this inflammation is not addressed by current ALI therapy (Abraham, 1999; Glauser, 2000; Wheeler and Bernard, 1999). Treatment of patients with ALI remains limited to supportive care, e.g. supplemental oxygen and mechanical ventilation, and thus, mortality has not improved significantly.

Many clinical disorders can result in ALI, either through a direct pulmonary insult, e.g. gastric acid aspiration, or an indirect pulmonary insult, e.g. sepsis. The most common cause of ALI remains systemic sepsis, which occurs in 1% of hospitalized patients (Bone et al., 1992; Angus et al., 2001). Regardless of the initiating cause, the pathophysiology of ALI is characterized by activation of many cells, including endothelial cells, neutrophils, and macrophages, and increased synthesis and release of many soluble mediators, such as TNFα and NO (Bone et al., 1992; Bone, 1994; Neumann et al., 1999). Neutrophils are central to the microvascular and tissue injury of ALI (Heflin and Brigham, 1981; Tate and Repine, 1983; Worthen et al., 1987; Downey et al., 1995; Wagner and Roth, 1999; Doerschuk, 2001). Pulmonary neutrophil infiltration is the result of a cascade of steps including reduced neutrophil deformability, pulmonary vascular sequestration (McCormack et al., 2000), neutrophil–EC adhesion, and *trans*-EC migration (Wagner and Roth, 1999; Doerschuk, 2001). Neutrophil infiltration is facilitated by endothelial cell (EC) barrier dysfunction (Furie et al., 1987; Garcia et al., 1998; Dull and Garcia, 2002).

A key step in pulmonary neutrophil infiltration is neutrophil–EC adhesion, mediated through the specific interaction of surface adhesion molecules on each cell, including neutrophil β2-integrins (e.g. CD11b/CD18) and EC adhesions (e.g. ICAM-1) (Hynes, 1992; Adams and Shaw, 1994; Hynes, 1992). Following neutrophil–EC adhesion, neutrophils subsequently migrate through inter-EC gaps in response to chemotaxins (e.g. IL-8 in humans, MIP-2 in mice) (Del Maschio et al., 1996; Burns et al., 1997; Garcia et al., 1998; Haelens et al., 1996), putatively through the homotypic interaction of PECAM-1 on the surface of both neutrophils and EC (Muller et al., 1993; Schenkel et al., 2004). Neutrophils may contribute to EC barrier dysfunction and ALI through several mechanisms. Neutrophils release a variety of pro-inflammatory factors (e.g. oxidants, proteolytic enzymes) that may directly injure EC and contribute to pulmonary oxidant stress (Burg and Pillinger, 2001; Babior et al., 2002; Dull and Garcia, 2002; Allport et al., 2002). Alternatively, neutrophil–EC adhesion may activate intracellular signaling cascades in the EC which can result in barrier dysfunction (Del Maschio et al., 1996; Huang et al., 1993; Garcia et al., 1998).

The end-result of this complex, integrated inflammatory response involving cells and soluble mediators is injury and dysfunction of the alveolar capillary–epithelial permeability barrier. In direct pulmonary insults, the alveolar epithelial cell is the principal target, exhibiting features of injury and apoptosis. In contrast, in sepsis-induced ALI, the pulmonary microvascular EC is one of the key targets of the systemic inflammatory response (Curzen et al., 1994; Reinhart et al., 2002). The importance of EC injury in sepsis has recently been highlighted, as markers of EC injury correlate with survival in human sepsis and ALI (Ware et al., 2001).

In summary, injury and death of pulmonary microvascular EC and alveolar epithelial cells leads to barrier dysfunction and the leak of plasma into the pulmonary interstitium and alveolar spaces, resulting in high-protein pulmonary edema, as well as neutrophil infiltration into the lungs (Wang et al., 2002; Razavi et al., 2002; Granger and Kubes, 1994; Garcia et al., 1998; Wagner and Roth, 1999). We will consider the effects of NO on each of the key pathophysiologic features of ALI, including EC and alveolar epithelial cell injury and death, pulmonary oxidant stress, pulmonary protein leak, and pulmonary neutrophil infiltration.

3. Endogenous NO synthesis in ALI

NO in mammalian cells is produced by a family of NO synthases (NOS). NOS isoenzymes are classified as low-output, calcium-dependent (cNOS) or high-output, calcium-independent, cytokine-inducible NOS (iNOS) isoforms. In the lung, cNOS is constitutively expressed in EC (ecNOS) and in neurones (nNOS). cNOS is important in pulmonary homeostasis, including mediating direct and neurogenic pulmonary vasodilatation, bronchodilation, and immune modulation (Scott and McCormack, 1999a; Mehta and Drazen, 2000; Scott et al., 1996). However, pulmonary cNOS has a lesser role in sepsis and ALI, in which we and others have shown cNOS is down-regulated (Razavi et al., 2002; Scott et al., 2002; Ermert et al., 2002).

In contrast, iNOS expression is induced in the majority of mammalian cell types upon exposure to inflammatory stimuli, including cytokines, bacteria, and bacterial products (e.g. lipopolysaccharide [LPS]) (Hibbs et al., 1988; Lamas et al., 1991; Nakayama et al., 1992; Nathan, 1992; Kolls et al., 1994; Robbins et al., 1994; Ermert et al., 2002). It should be noted that there is evidence for constitutive expression of iNOS in some cells, such as bronchial epithelial cells in humans (Guo et al., 1995). With regard to ALI, all of the key cellular participants (e.g. neutrophils, macrophages, EC, epithelial cells) can express iNOS under inflammatory conditions (Robbins et al., 1993; Nathan and Hibbs, 1991). Neutrophils and macrophages express high levels of iNOS and are important sources of iNOS-derived NO. Moreover, NO mediates many of the actions of neutrophils and macrophages, e.g. microbial and tumor cell killing, cytotoxicity (Hibbs et al., 1988; McCall et al., 1989; Munoz-Fernandez et al., 1992; Amin et al., 1995; Bratt and Gyllenhammar, 1995; MacMicking et al., 1995; Evans et al., 1996; Tsukahara et al., 1998; Boyle et al., 2000). Of relevance to ALI in humans, enhanced iNOS expression and/or activity have been reported in stimulated human neutrophils and macrophages (Munoz-