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Hyperoxia-derived lung damage in preterm infants

Vineet Bhandari*

Division of Perinatal Medicine, Yale University School of Medicine, Department of Pediatrics, 333 Cedar Street, LCI 401B, New Haven, CT 06520-8064, USA

Summary

Hyperoxia-induced lung injury is characterized by an influx of inflammatory cells, increased pulmonary permeability, endothelial and epithelial cell death. This review highlights the mechanistic aspects of inflammation, vascular leak and cell death. The focus will be on agents that contribute to hyperoxia-induced lung injury in developmentally appropriate animal models, and those that have been detected in human premature neonates.

Keywords

Cell death; Cytokines; Oxidants; Oxygen; Pulmonary

Introduction

Exposure to high concentrations of oxygen is known to cause significant damage to the developing lung. Acute pulmonary injury secondary to hyperoxia is characterized by an inflammatory response with destruction of the alveolar–capillary barrier, followed by cell death.¹

Pathology

Morphologic studies in animal models have demonstrated that toxic concentrations of oxygen initially induce focal endothelial cell injury and, with continued exposure, necrosis of epithelial cells.^{2,3} After acute oxygen exposure, pulmonary microvascular endothelial cells rapidly die, leaving areas of denuded capillary basement membrane. Disruption of the alveolar–capillary membrane leads to flooding of the alveoli, causing significant perturbations in pulmonary mechanics and impairment of gas exchange.⁴ Investigators have confirmed the similarities in the stages and morphologic patterns of pulmonary oxygen toxicity in many animal species as well as man.⁵

Subsequently, pulmonary edema and the accompanying inflammatory processes decrease, even with continued exposure to hyperoxia.⁶ Despite the initial apparent histological improvement, chronic pulmonary inflammation ensues in the following few weeks.⁶ Such

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* Tel.: +1 (203) 785-2613; fax: +1 (203) 785-6974. vineet.bhandari@yale.edu.

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Conflict of interest statement

None declared.

long term consequences may depend on the lung's acute response to hyperoxia.⁶⁻⁸ There are significant differences in the response of the newborn from that of the adult, highlighting the developmental regulation of this process.^{9,10} Newborn animals of several species survive twice as long as adults in hyperoxia and have a significantly later onset of inflammation.^{9,10} Neonatal responses are unique, probably because injury occurs during the period of alveolar development.¹ Some aspects of the pathology of hyperoxia-induced acute lung injury in neonatal mice are illustrated in Fig. 1. Studies of hyperoxic chronic injury in newborn animals have shown morphologic changes similar to those seen in human bronchopulmonary dysplasia (BPD).¹¹

Inflammatory cells

The inflammatory cell influx is orchestrated and amplified by chemotactic factors.¹² Monocytes/macrophages and lymphocytes are not the only source of these chemotactic agents, as stromal, epithelial and endothelial cells can generate significant chemokine levels.¹² In such a scenario, alveolar or interstitial macrophages can respond to the exposure to hyperoxia with the expression of the early-response cytokines. These cytokines can then activate resident lung endothelial cells, epithelial cells, and fibroblasts, resulting in the production of chemokines.¹² This, in turn, would attract inflammatory cells, for example, neutrophils, to the lung.

Cell death

It has been postulated that tissue injury on exposure to hyperoxia occurs as a result of reactive oxygen species (ROS). Lung cells poison themselves by producing an excess of ROS.¹³ Inflammatory cells are also a potent source of ROS.¹⁴ Thus, inflammation and lung injury are frequently juxtaposed in animal models of hyperoxia-induced lung injury. This has led to studies investigating the mechanisms of hyperoxia-induced inflammation and the relationship between injury and inflammation in this disorder.¹⁵⁻¹⁷ Inhibitors of the migration of inflammatory cells into the lung have been found to be protective.¹² By contrast, hyperoxia can induce lung injury in animal models that lack leukocytes.^{18,19}

At sites of tissue injury, cells can die via necrosis or apoptosis. Traditionally, these processes have been considered operationally and mechanistically distinct cell-death responses.²⁰ This distinction may not be as clear-cut as previously thought.¹⁴ Studies have shown that apoptosis-like DNA laddering and positive terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) can be seen in cells undergoing necrosis, that known inducers of apoptosis can cause cells to die via necrosis, that apoptosis and necrosis can be induced by the same agent in the same cell population, and that apoptosis and necrosis can be present simultaneously in tissues experiencing the same injury.²¹ It is possible that both pathways of cell death coexist or that another mechanism of cell death distinct from apoptosis or necrosis might be induced in hyperoxia.¹⁴ Furthermore, different cell types in the lung may undergo damage/death in response to hyperoxia via distinct or overlapping mechanisms depending on the concentration and duration of exposure to hyperoxia.

Activation of key caspases and components of the extrinsic/death receptor and intrinsic/mitochondrial cell death pathways appear to underlie the molecular mechanisms of hyperoxia-induced lung injury and cell death. The membrane (extrinsic) pathway triggers surface 'death receptors' such as Fas, which binds Fas ligand, and tumor necrosis factor (TNF) receptor 1, which binds TNF and lymphotoxin, which activate caspase-8. Other key regulators include the many members of the Bcl-2 gene family (which can be divided into three groups: antiapoptotic Bcl-2 and Bcl-x_L, proapoptotic Bax-type proteins, and proapoptotic BH3-domain-only family members), protein kinase B/Akt, and the redox-sensitive transcription factor NF- κ B.¹⁴ In the intrinsic response, mitochondrial dysfunction

signals cell death. In this response, BH3 domain-only family members such as Bid are activated and interact with Bax-type proteins (Bax, Bak, Bok) to form or interact with mitochondrial pores, release cytochrome *c*, activate caspase-9 and induce cell death.²² The mitochondrial-dependent pathway is probably more relevant in hyperoxia-induced lung injury, as mitochondria maintain the cellular levels of adenosine triphosphate (ATP) and are able to release death-promoting factors, such as cytochrome *c*.¹⁴

Recent studies have added to this pathogenic paradigm by demonstrating that ROS mediate their effects, in part, by inducing an endothelial and epithelial cell death response with features of apoptosis and necrosis^{13,16,23,24} and that a variety of exogenously administered regulators inhibit these toxic events by regulating local cell death responses.¹² In addition, although structural cell apoptosis (such as that seen secondary to hyperoxia) can stimulate tissue inflammation,^{25,26} hyperoxia-induced inflammation cannot be attributed solely to the nearby cell death response. A proposed model delineating some of the pathways to hyperoxia-induced lung injury is shown in Fig. 2.

Putative mediators of hyperoxia-induced lung injury in premature infants

Investigators have mostly adopted two approaches to identify mediators that may be involved in the process of hyperoxia-induced lung injury in premature neonates. The bench approach is to utilize in-vitro/in-vivo modeling systems and ascertain their responses to hyperoxia exposure. The bedside approach has been to access various compartments (tracheal, blood, urine, postmortem lung tissue) in premature human neonates and measure a variety of analytes, and report their association with long-term consequences of hyperoxia-derived lung injury.^{27,28} The former approach is scientifically sounder in that it isolates the injurious element (i.e. hyperoxia) and systematically evaluates the responses in specific cells or neonatal animals. While the latter approach addresses the clinical problem, it is difficult to tease out the specific contribution of hyperoxia to the overall pathology of pulmonary injury.

A combination of the above two approaches would ideally identify specific mediators that have evidence of biological validation in the process of hyperoxia-derived pulmonary injury. This, in turn, would allow such compounds to be targeted as potential biomarkers and/or therapeutic agents. Hence, for this review, the focus is on agents that have been reported to contribute to hyperoxia-induced lung injury in developmentally appropriate animal models and which have been detected in human premature neonates.

Bombesin-like peptide (BLP)

BLP has been shown to impact on alveolar development in neonatal animals.²⁹ Using the baboon model, postnatal administration of anti-BLP antibody attenuated clinical and pathological features of hyperoxia-induced lung injury.³⁰ Urine BLP levels have correlated with the development of BPD in both the baboon model³⁰ and humans.³¹

Hepatocyte growth factor (HGF)

Exposure of neonatal rats to 60% O₂ led to an upregulation of lung HGF.³² However, anti-HGF treatment led to a simplified alveolar structure in neonatal rat pups in room air.³² By contrast, other investigators reported that rHGF partially protected against the inhibition of alveolarization and improved functional abnormality in a hyperoxia-induced neonatal mice model of BPD.³³ In preterm humans, lower tracheal aspirate levels of HGF were associated with more severe lung disease.³⁴

[A]Interleukins (IL)

[B]IL-1

The three known constituents of the IL-1 family, IL-1 α , IL-1 β and IL-1 receptor antagonist (IL-1RA), are structurally related to one another and bind with similar affinities to IL-1 receptors (IL-1R) on cells.¹² IL-1 α and IL-1 β are potent agonists that elicit broad-ranging biological responses in various cells, which are blocked by IL-1RA. Nearly all cell types that produce IL-1 α and IL-1 β also produce IL-1RA.¹² Both isoforms of IL-1 recruit cells to sites of inflammation and stimulate the production of proinflammatory mediators.¹² Once expressed, the members of the IL-1 gene family appear to work in concert to control crucial inflammatory and host defense responses.¹² The levels of IL-1RA detected at sites of inflammation do not appear to be high enough to completely block IL-1R signaling; thus, physiologically, IL-1RA may serve to downregulate the response to IL-1 rather than inhibit it.¹²

In neonatal mice exposed to >95% O₂, there was no change in lung mRNA for IL-1 α over a period of 10 days.⁹ By contrast, IL-1 α mRNA was increased in the lungs of newborn mice after 3 days of hyperoxia (85% O₂) and maintained an increase of 50–80% over 5–14 days of exposure compared with room air-exposed animals.¹ Increased expression of IL-1 α preceded the neutrophil influx (demonstrated in histological sections and bronchoalveolar lavage (BAL) fluid after 2 weeks of oxygen exposure) and thus may have a role in the inflammatory response.¹ Possible reasons for this discrepancy could lie with the different strains of mice used and exposure to different O₂ concentrations in the above two studies.

On the other hand, there was a 5-fold increase in lung mRNA for IL-1 β at 7 days of exposure.⁹ Acute alveolitis and slight edema were detected, but lethality was not observed till day 10.⁹ In newborn rabbits exposed to hyperoxia, IL-1 β was detected by 2–4 days of hyperoxia, being maximal at 6–10 days and decreasing thereafter.³⁵ This pattern of changes in IL-1 β was paralleled by the evidence of a rise, and then fall, of histologic inflammation.³⁵ In the immature baboon model of BPD, there were no significant differences between IL-1 β levels in the tracheal aspirates at any of the study times.¹¹ Using a transgenic system, IL-1 β overexpression in lung epithelial cells led to a BPD phenotype in neonatal mice.³⁶

[B]IL-6

Most, if not all, nucleated cells have been shown to express and synthesize IL-6 in vitro. The most prominent source appears to be stimulated monocyte/macrophages, fibroblasts, epithelial and endothelial cells.¹² There is evidence that IL-6 acts as an autocrine, paracrine, and exocrine inflammatory hormone.¹² The characteristic cytokine cascade response is well illustrated by the fact that mice treated with IL-1 showed a subsequent IL-6 response.¹² Interestingly, IL-6 has also been shown to have anti-inflammatory effects by inhibiting neutrophil influx in a model of acute lung inflammation¹² and by inducing IL-1RA (and soluble TNF receptor);¹² the latter two mediators would diminish macrophage-mediated inflammatory responses.¹² It is obvious that the timing and intensity of the effects of IL-6 are carefully controlled to elicit the appropriate effect needed in the inflammatory response.¹²

In the premature baboon model of BPD, tracheal aspirate IL-6 levels on days 9–10 and 16–44 were significantly increased when compared with those at 48–72 h.¹¹ Neonatal mice exposed to hyperoxia had an 8-fold increase in lung mRNA for IL-6 after 7 days of exposure.⁹ Newborn mice with increased lung IL-6 levels had increased mortality on hyperoxia exposure.³⁷ Newborn rats exposed to 100% O₂ (for 9 days) had significant pulmonary edema and increased cellularity on days 1 and 3, which resolved by days 6 and 9.⁶ IL-6, primarily of non-macrophage origin, was detected in the BAL on days 6 and 9, but

not earlier.⁶ Placement of these animals in room air 4 days after hyperoxia (day 13) resulted in non-detectable (control) levels of IL-6.⁶ Newborn rats exposed to 48 h of hyperoxia (95% O₂) had increased levels of IL-6 mRNA; dexamethasone treatment reduced these levels.³⁸

[B]IL-8/cytokine-induced neutrophil chemoattractant-1 (CINC-1)

Neutrophil chemokines such as IL-8, which acts through the C-X-C chemokine receptor-2 (CXCR2), regulate chemoattraction. CINC-1, the rat analogue of IL-8, belongs to the C-X-C chemokine family and is produced by an array of different immune and non-immune cells.¹²

Exposure to prolonged hyperoxia (10 days) led to an upregulation of CINC-1 in premature rat lungs³⁹ and newborn rabbits.³⁵ IL-8 protein levels were significantly increased and highly correlated with the neutrophil presence in the BAL fluid.³⁵ In the premature baboon model of BPD, tracheal aspirate IL-8 levels on days 6–8 and 9–10 were significantly increased when compared with other time points.¹¹ Increased IL-8 levels in tracheal aspirates obtained during the clinical course appeared to correlate when lung infection was suspected clinically in several of these animals.¹¹

In newborn rats exposed to hyperoxia (95% O₂), CINC-1 levels increased about 10-fold by day 8 versus controls.⁴⁰ There was no statistically significant difference in survival between newborn rats exposed to hyperoxia in those treated with anti-CINC-1 or control-IgG (on days 3 and 4 of hyperoxia exposure) or no treatment. However, anti-CINC-1-treated newborn rats had significantly lower neutrophil numbers in the BAL and tissue myeloperoxidase activity.⁴⁰ On histology, there was decreased cellular influx in alveolar spaces, and septal thickening with no differences apparent in elastin staining in the lungs of anti-CINC-1-treated newborn rats.⁴⁰ Furthermore, there was preservation of alveolar volume and surface area in anti-CINC-1-treated newborn rat lungs compared with hyperoxia-exposed controls.⁴¹ The above data are consistent with a role for IL-8 in the influx of neutrophils in response to hyperoxia,³⁵ though the influence of infection cannot be excluded.¹¹

[B]IL-10

In murine systems, IL-10 production often correlates with production of other Th2 cytokines (such as IL-4); but, in humans, IL-10 is produced by both Th1 and Th2 type T-cell clones.¹² IL-10 suppresses activation of macrophages and dendritic cells, inhibiting their abilities to secrete cytokines and function as accessory cells for T-cell and NK-cell stimulation.¹² Consistent with its inhibitory effects on a number of in-vitro assays of cell-mediated immunity, IL-10 has been shown to be a potent inhibitor of inflammatory responses in vivo.¹²

Neonatal mice exposed to hyperoxia did not demonstrate any changes in the lung mRNA of IL-10 at any time point.⁹ No significant differences were observed in the IL-10 levels from the tracheal aspirate specimens obtained from the baboon model of BPD.¹¹

IL-1 β and -6 are particularly active in the acute phase response to injury, while IL-8 is a potent chemotactic agent for recruitment of neutrophils. Typically viewed as proinflammatory, these cytokines have been shown to be elevated very early in the respiratory course of the human preterm population that ultimately develops BPD, and in those with BPD at the time their tracheal aspirates are assessed. These findings have been replicated in multiple studies.²⁷ Studies have found that serum and tracheal aspirate IL-10 levels were decreased in those infants who developed BPD.²⁷

Keratinocyte growth factor (KGF)

In neonatal rats exposed to hyperoxia, KGF treatment was protective against lethality, but did not impact on the impaired alveolarization (the pathologic hallmark of BPD).^{42,43} Tracheal aspirate KGF concentrations were higher in survivors without BPD, compared to those with BPD.⁴⁴

Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is one of the ligands for CCR2.¹² It is a chemoattractant for monocytes, lymphocytes and basophils. It is produced by a variety of cells in response to inflammatory stimuli.¹²

On gene expression profiling with confirmation by real-time reverse transcriptase–polymerase chain reaction (RT–PCR), premature rat lungs exposed to prolonged hyperoxia (10 days) had an upregulation of MCP-1.³⁹ Newborn rats were exposed at birth to hyperoxia or room air and given anti-MCP-1 or IgG control injections on days 3–5.¹⁸ At 1 week, anti-MCP-1-treated pups had reduced leukocyte numbers, both macrophages and neutrophils, in the BAL versus controls. CINC-1 and tissue carbonyls (a measure of protein oxidation) were reduced in the lung of anti-MCP-1-treated pups.¹⁸ MCP-1 levels were increased in tracheal aspirates of very low birth weight infants who developed BPD.⁴⁵

Matrix metalloproteinase 9 (MMP9)

MMP9 has been found to be increased in neonatal rat lungs exposed to hyperoxia in one study,⁴⁶ whereas another reported a decrease.⁴⁷ Consistent with the former, MMP9 was found increased in the baboon model of BPD.⁴⁸ By contrast, MMP9 null mutant neonatal mice were protected from hyperoxia-induced lung injury,⁴⁹ whereas in the IL-1 β transgenic model of BPD, absence of MMP9 worsened the phenotype.⁵⁰ Most of the preterm human studies support the contention that hyperoxia leads to increased MMP9 levels in the lung, and is associated with BPD.^{51,52}

Transforming growth factor beta (TGF β)

TGF β and/or constituents of its signaling pathways have been noted to be upregulated on exposure to hyperoxia in neonatal rats⁵³ and mice.^{54,55} Lung overexpression models of TGF β in neonatal rodents also have features consistent with BPD.^{56,57} Preterm infants who developed BPD had significantly increased levels of TGF β 1 in tracheal aspirates, when compared with controls.²⁷

Tumor necrosis factor alpha (TNF α)

TNF α was increased in the lungs of neonatal rat⁶ and mice⁹ pups exposed to hyperoxia. In human neonates, tracheal aspirate levels of TNF α were increased in neonates who subsequently developed BPD.²⁷

Vascular endothelial growth factor (VEGF)

VEGF is a widely expressed dimeric glycoprotein, but the highest level of expression in normal tissues is in the lung.¹² The biological activity of VEGF is dependent upon its interaction with specific transmembrane receptor tyrosine kinase (RTK) receptors; the two well-defined ones are VEGFR1/Flt-1 and KDR/Flk-1/VEGFR2.¹² A wide variety of cells express VEGF receptors, including activated macrophages, neutrophils, vascular endothelial cells, and Type II cells.⁴ VEGF promotes endothelial cell growth and remodeling. In the pulmonary system it appears to be essential for the appropriate development of alveolar tissue; in rats its antagonism has been shown to result in markedly impaired alveolarization,

especially earlier in the course of lung development.¹² In heavily vascularized tissues, such as the lung, VEGF exists in high concentrations. During hyperoxic episodes when damage to the microvasculature occurs, VEGF plays a role in the remodeling process, and its levels are increased, sometimes disproportionately.

On gene expression profiling with confirmation by real-time RT-PCR, premature rat lungs exposed to prolonged hyperoxia (10 days) underwent a downregulation of VEGFR2.³⁹ In newborn rabbits, the amount of VEGF in the BAL fluid increased 2-fold on exposure to 95% O₂, dropped to barely detectable levels at the 50% lethal dose time point, and increased 8-fold compared with control levels during the first 5 days of recovery (in 60% O₂).¹² By 2 weeks of recovery, VEGF levels had reached normal values.¹² In the premature fetal baboon delivered at 125 days (term is 140 days), and treated with oxygen and mechanical ventilation as needed for 14 days, overall expression of VEGF mRNA and protein was markedly decreased; expression of VEGFR1 was decreased by 30–40% while VEGFR2 mRNA expression was unchanged.¹² Exposure of neonatal rats to hyperoxia for 12 days impaired alveolarization and vessel density that persisted despite recovery in room air at day 22.¹² rhVEGF treatment, started at day 14 during the recovery period, enhanced vessel growth and alveolarization in infant rats.¹²

Studies done in intubated premature neonates reveal an increase by at least 3–4-fold over the first 3–10 days after birth.⁵⁸ The discrepancy with the fetal baboon studies could be due to different concentrations of isoforms of VEGF being secreted in the BAL fluid, or other variables such as chorioamnionitis or corticosteroid administration in humans.⁵⁸ However, the subset of human infants that recover without BPD have a trend towards increasing VEGF levels over time, whereas babies who did develop BPD appeared to have decreased VEGF levels.⁵⁸ Others have reported that infants had significantly lower or no difference in tracheal aspirate VEGF levels between BPD and no BPD infants.²⁷ In a recent study, a phasic pattern of VEGF concentrations was noted in infants who go on to develop BPD. These infants had an initial spike over the first 12 h of postnatal life, followed by a decrease over the next few days and then a subsequent significant increase.⁵⁹ These data appear to conform to the neonatal animal studies enumerated above.

In summary, the data would suggest that exposure to hyperoxia leads to a biphasic VEGF response. Initially, there is an increased amount of VEGF release that could account for lung injury by causing vascular permeability alterations,⁶⁰ followed by a decrease in VEGF. If adequate recovery is to occur, there is a tremendous surge in VEGF levels, allowing for angiogenesis and alveolarization that underlie the process of lung healing and repair.

Conclusions

Inflammatory cells produce cytokines and chemoattractants for augmentation of the inflammatory response, in an attempt to curb the damage of the initial and ongoing insults secondary to hyperoxia. Subsequent disruption of the alveolar capillary unit with increased vascular permeability, and cell death result in decreased pulmonary tissue integrity. This injurious process, with a simultaneous attempt at repair, mediated via a variety of factors, results in lung pathology that culminates with characteristic features of BPD in preterm infants.

Practice points

- Hyperoxia-induced lung injury is characterized by inflammation, edema and cell death.

- There is significant developmental regulation of the response of the immature lung to hyperoxia.
- Mediators involved in hyperoxia-induced lung injury responses that have also been detected in preterm neonates include BLP, HGF, IL-1 β , IL-6, IL-8, IL-10, KGF, MCP1, MMP9, TGF β , TNF α and VEGF.

Research directions

- Biological validation of other pulmonary biomarkers detected in preterm infants (examples: angiopoietin 2, macrophage migration inhibiting factor, parathyroid hormone-related protein) in developmentally appropriate animal models of hyperoxia-induced lung injury and vice versa (example: cathepsin-S).
- Understanding the paracrine response of stem cells in hyperoxia-induced lung injury.

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Figure 1. Photomicrographs ($\times 110$, upper panel; $\times 20$ lower panel; hematoxylin and eosin stain) of neonatal lung injury noted in newborn mice at postnatal day 2, after 100% O_2 exposure since birth. Note the alveolar exudates and presence of inflammatory cells in the hyperoxia-exposed lungs, compared with litter-mate controls in room air. RA, room air; HYP, hyperoxia.

Figure 2.

A proposed model of hyperoxia-derived lung damage in neonates delineating some of the pathways. Hyperoxia exposure leads to release of certain mediators [exemplified by vascular endothelial growth factor (VEGF) and angiopoietin 2] that disrupt the alveolar–capillary membrane leading to pulmonary edema which contributes to lung injury. Other cytokines [examples of which are interleukin (IL)-1, IL-6, IL-8, transforming growth factor (TGF) β, tumor necrosis factor (TNF) α, VEGF] are also released from lung cells that attract inflammatory cells to the lung. These inflammatory cells as well as hyperoxia per se release reactive oxygen species, which can initiate the mitochondrial-dependent cell death pathway. The cytokines and cell death mediators contribute to pulmonary injury resulting in hyperoxia-derived lung damage. PKC, protein kinase C.