Chapter 1

Introduction
INTRODUCTION

Bronchopulmonary dysplasia (BPD) continues to be a significant cause of mortality and morbidity in the neonatal intensive care unit (NICU) and affects an estimated 500 very preterm infants (often with gestational ages <28 weeks and birth weights <1,000 g) in the Netherlands each year. BPD is a chronic lung disease typical for preterm infants born when lung development is far from complete. Soon after birth these infants develop respiratory problems (respiratory distress syndrome, RDS). They may need surfactant replacement to open up their alveoli and assisted ventilation with extra oxygen to guarantee optimal gas exchange during lung development. These acute respiratory problems affect both alveolarization and vascular development of the immature lung and ultimately lead to chronic lung disease, i.e. BPD. Until recently, preterm infants with BPD were weaned from the ventilator using glucocorticoids, such as dexamethasone. Glucocorticoids accelerate lung development, but their main drawback in pediatric pulmonology is their negative effect on alveolarization by inhibiting secondary septation, ultimately leading to a permanent simplification of the airspaces with enlarged saccular-like alveoli. This results in a permanent reduction of the gas-exchange surface area and lung function. In addition, the realization that early postnatal administration of glucocorticoids may affect neurodevelopment, has led to the advice by the American Academy of Pediatrics not to use postnatal glucocorticoids any longer. This advice has created a therapeutic vacuum in the treatment of preterm infants with BPD in the NICU. This thesis investigates the pathophysiology of hyperoxia-induced neonatal lung injury in the rat as a model for very premature infants suffering from RDS with a high risk of developing BPD and explores the therapeutic potential of the methylxanthine pentoxifylline and inhaled nitric oxide in the treatment and/or prevention of BPD.
PERINATAL LUNG DEVELOPMENT

For a better understanding of the pathogenesis of BPD knowledge of normal lung development and surfactant metabolism and function is necessary.

**Organogenesis**

Organogenesis of the lung can be divided into 5 distinct phases: embryonic, pseudoglandular, acinar or canalicular, saccular, and alveolar (reviewed in 15, 24). During the *embryonic phase* (26 to 52 days of gestation) an endodermal outgrowth derived from the primitive foregut divides and branches dichotomously to form the early tracheobronchial tree. In the *pseudoglandular phase* (52 days to 16 weeks of gestation) the primitive airway epithelium starts to differentiate and neuroendocrine, ciliated, and goblet cells appear, whereas cartilage and smooth muscle cells emerge from the mesenchyme. The airway branching pattern is completed in the *canalicular or acinar phase* (16 to 24-26 weeks of gestation) and the prospective gas-exchange region starts to develop: respiratory bronchioli emerge, interstitial tissue decreases, vascularization of peripheral mesenchyme increases, and distal cuboidal epithelium differentiates into alveolar type I and II cells signaling the start of surfactant production. Development of the distal pulmonary circulation by vasculogenesis with capillaries is present at 20 weeks. The *saccular phase* (24-26 to 36 weeks of gestation) is characterized by maturation of the surfactant system, growth of the pulmonary parenchyma and thinning of the connective tissue or interstitium. The capillary network comes in close contact with the developing airway epithelium in the primitive alveoli, enabling gas-exchange between blood and the environment in case of a premature birth from 24 weeks of gestation onward. The *alveolar phase* extends from 36 weeks of gestation to at least 18 postnatal months in which true alveoli, with increased acinar complexity and increased gas-exchange surface area, are formed. Alveoli are formed due to secondary septation from the present airspaces of smooth-walled transitory ducts and saccules with primitive thick septa. In addition, microvascular maturation takes place with fusion of the double capillary layer into a single medial layer facing both alveolar lumens of the septum. Thus, the process of alveolarization and terminal microvascular development begins in the late fetal period and proceeds after birth in humans.

**Pulmonary surfactant**

Surfactant synthesis (reviewed in 42, 100) starts in alveolar type II cells at 24-28 weeks of gestation. After secretion into the alveoli, surfactant lowers surface tension in the alveoli and distal bronchioli which promotes
lung expansion during inspiration and prevents alveolar collapse at expiration, both mandatory for gas-exchange and oxygenation. Surfactant deficiency in immature lungs triggers a cascade of alveolar instability and collapse, capillary leakage, and hyaline membrane formation leading to decreased gas-exchange, atelectasis, increase of the functional right-to-left shunt, pulmonary hypertension, respiratory acidosis and pulmonary edema with further inactivation of surfactant by plasma contents. Besides surface tension reduction, surfactant also plays a critical role in innate host defense and inflammation in the lung.

Pulmonary surfactant consists of about 90% lipids and 10% proteins and its composition is similar across species including man and rodents. Phospholipids make up 80-90% of the surfactant lipids, of which 70-80% accounts for phosphatidylcholine (PC). PC is the most important component to lower surface tension. Approximately 50 to 60% of PC is disaturated and largely consists of dipalmitoyl phosphatidylcholine (DPPC). Of the surfactant proteins two are large and hydrophilic (surfactant protein A [SP-A] and D [SP-D]), the other two are small and hydrophobic (SP-B and SP-C).

The functions of the surfactant proteins are summarized in Table 1. SP-A is the most abundant surfactant protein in the alveoli, and constitutes approximately 50% of all pulmonary surfactant proteins. Human SP-A, encoded by two distinct genes (SFTPA-1 and -2) and located on chromosome 10 (43), is a 26-36-kDA (monomer) hydrophilic collagen/lectin hybrid. The collagen-like domain interacts with phospholipids and the C-terminal domain resembles lectins, which play a role in host defense mechanisms. SP-A is expressed and synthesized by alveolar type II cells and to a lesser extent by non-ciliated Clara cells. SP-A has multiple functions, but knock-out studies have shown that SP-A is not pivotal for lung function, but critical for the recognition, binding, opsonization, and killing of various bacterial, viral, and fungal pathogens in the lung (36, 51). SP-D is a 42-kDA collagenous glycoprotein that is encoded by a single human SFTPD gene on chromosome 10. Like SP-A, SP-D is predominately synthesized by alveolar type II cells and non-ciliated Clara cells, but unlike SP-A, SP-D is not associated with surfactant lipids. SP-D deficient mice are susceptible to pulmonary infection, indicating the important role of SP-D in innate host defense (59, 60).

The small hydrophobic surfactant proteins, SP-B and SP-C, play a pivotal enhancing role in the adsorption of phospholipids at the air-liquid interface in the alveoli that is critical for maintaining the stability and morphological integrity of the alveolus. Together they account for only 1-2% of the surfactant weight. SP-B and SP-C both require specialized intracellular processing events to produce their mature forms. SP-B is a polypeptide with a molecular mass of 8.7 kDa, which is expressed in alveolar type II cells and Clara cells. Human SP-B is encoded by a single gene on chromosome 2 (SFTPB). SP-B is the only surfactant protein essential for life (69). Mutations in the SP-B gene result in lethal SP-B deficiency. SP-B is a member of the saposin-like family of peptides and is always associated with surfactant phospholipids. SP-B influences the processing and secretion of SP-C, because SP-B deficient mice also lack mature SP-C.
Surfactant deficiency is a common problem in very preterm infants and typically presents as RDS with nonspecific tachypnea, expiratory grunting, nasal flaring, cyanosis, and substernal and intercostal retractions. The previously used term hyaline membrane disease (HMD) is synonymous with RDS, and refers to the pathologic finding of membranes that stain like hyaline cartilage. These hyaline membranes consist of necrotic alveolar cells, plasma transudate, aspirated squamae, and fibrin, and line terminal bronchioles and alveolar ducts. Surfactant replacement therapy was introduced in the early 1990s and has greatly improved perinatal lung function and reduced mortality and morbidity of very preterm infants.

**Table 1.** Functions of the four surfactant proteins (Adapted and modified from reference 100).
BRONCHOPULMONARY DYSPLASIA

Clinical presentation

‘Classic’ BPD was first reported by Northway and colleagues in 1967 as a severe lung disease resulting from mechanical ventilation and oxygen exposure in preterm infants with RDS or HMD (70). BPD was defined as the presence of persistent respiratory symptoms and need for supplemental oxygen and an abnormal chest radiograph at 28 days after birth (70). Chest radiographs demonstrated a pattern of heterogeneous aeration of severe hyperexpansion with a combination of cystic emphysema, volume loss and fibrosis, the so called ‘bubbly lungs’ (85). Pathologic findings included necrotizing bronchiolitis, vascular smooth muscle hypertrophy and pulmonary hypertension, inflammation, pulmonary edema, and alveolar changes of overinflation and atelectasis with pulmonary fibrosis. Long-term follow-up studies showed that many BPD infants have recurrent (especially respiratory syncytial virus [RSV]) respiratory infections during infancy and reactive airways disease, upper airway obstruction, pulmonary hypertension and exercise intolerance in childhood and adolescence (57).

Due to pharmacological, nutritional and technical advances the survival of younger and smaller preterm infants with BPD has changed the clinical picture and definition of BPD over the past 40 years. The last decade the overall incidence of BPD is still the same, but the clinical course and pathology of BPD developing in infants after premature birth has changed after the introduction of surfactant therapy (16, 39, 40, 76). Of the original series published in 1967, surviving infants with BPD were born at 34-weeks gestation, weighed 2,200 g and the mortality was 67% (70). Today, 75% of the affected newborns weigh less than 1000 g at birth (39, 76). The risk of BPD rises with decreasing birthweight, with an incidence reported as high as 85% in neonates between 500-699 g versus 5% in neonates with birthweights over 1500 g (39, 76). Chronic oxygen dependency may even develop in premature newborns without severe RDS (39). Survival rates have dramatically increased from less than 10% to presently over 50% in extremely preterm infants of 24-26 weeks’ gestation (39). Also the histology features of classic progressive fibroproliferation are now generally less striking. In 1999 the term ‘New’ BPD was introduced by Jobe (40). ‘New’ BPD is a milder chronic disease in very preterm infants treated with less or no ventilatory support and lower inspired oxygen concentrations during the first postnatal days than in the days of ‘classic’ BPD. Their lung disease is more uniform and lung injury is milder with less inflammation and fibrosis, but histological studies show severely disturbed alveolar and vascular growth. This new BPD is fundamentally based on an inhibition of acinar and vascular growth during a vulnerable stage of lung development, whereas classic BPD was attributed primarily to the combination of oxygen injury and mechanical ventilation in prematurity. The differences in histologic findings of ‘classic’ BPD and ‘new’ BPD are shown in Table 2 (Adapted and modified from references 21 and 46). The chest radiographs of infants with BPD now
often appear hazy or dense and progress to a relatively uniform pattern of fine coarse reticular interstitial opacities with more uniform and smaller cystic lucencies (1). As a result of the shift in clinical and radiographic pulmonary changes new diagnostic criteria of BPD were developed based on time of clinical assessment and clinical severity (Table 3) (39).

<table>
<thead>
<tr>
<th>Classic BPD</th>
<th>New BPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternating atelectasis and hyperinflation</td>
<td>Less regional heterogeneity of lung disease</td>
</tr>
<tr>
<td>Severe airway epithelial lesions (e.g. hyperplasia, squamous metaplasia)</td>
<td>Rare airway epithelial lesions</td>
</tr>
<tr>
<td>Marked airway smooth muscle hyperplasia</td>
<td>Mild airway smooth muscle thickening</td>
</tr>
<tr>
<td>Extensive, diffuse fibroproliferation</td>
<td>Rare fibroproliferative changes</td>
</tr>
<tr>
<td>Hypertensive remodelling of pulmonary arteries, including endothelial edema, medial thickening, elastin deposition in normally non-muscularized pulmonary arterioles, and consequent right ventricular hypertrophy</td>
<td>Fewer arteries but ‘dysmorphic’; Less severe arterial/arteriolar vascular lesions</td>
</tr>
<tr>
<td>Decreased alveolarisation and surface area</td>
<td>Fewer, larger and simplified alveoli (alveolar hypoplasia, decreased acinar complexity)</td>
</tr>
</tbody>
</table>

**Table 2.** Differences in pathological features of ‘classic’ and ‘new’ BPD (Adapted and modified from references 21 and 46).

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>&lt; 32 weeks</th>
<th>&gt; 32 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time point of Assessment</td>
<td>36 weeks post-menstrual age or discharge*</td>
<td>&gt;28 days but &lt;56 days postnatal age or discharge*</td>
</tr>
<tr>
<td>Treatment with oxygen</td>
<td>&gt;21% for at least 28 days</td>
<td>&gt;21% for at least 28 days</td>
</tr>
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**Bronchopulmonary Dysplasia**

<table>
<thead>
<tr>
<th>Mild</th>
<th>Breathing room air at 36 weeks post-menstrual age, or discharge*</th>
<th>Breathing room air by 56 days postnatal age, or discharge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>Need for &lt;30% O₂ at 36 weeks post-menstrual age, or discharge*</td>
<td>Need for &lt;30% O₂ at 56 days postnatal age, or discharge*</td>
</tr>
<tr>
<td>Severe</td>
<td>Need for &gt;30% O₂, with or without positive pressure ventilation or continuous positive pressure at 36 weeks post-menstrual age, or discharge*</td>
<td>Need for &gt;30% O₂ with or without positive pressure ventilation or continuous positive pressure at 56 days postnatal age, or discharge*</td>
</tr>
</tbody>
</table>

**Table 3.** NIH diagnostic criteria for bronchopulmonary dysplasia. [*Whichever comes first] (Adapted from reference 39).
Experimental Bronchopulmonary dysplasia

Animal models are critical to further unravel the pathophysiology of BPD and to test potential treatment options for BPD. Hyperoxia exposure of premature baboons \((20, 22)\), neonatal mice \((13, 96)\), and rats \((17, 33, 75)\) induces a progressive chronic lung disease (experimental BPD), that closely resembles BPD in premature newborns. Nowadays in the postsurfactant era, premature infants at greatest risk for BPD are born at 24-28 weeks gestation during the late canalicular or saccular stage of lung development. The unique advantage of a rodent animal model is that lung injury is also induced during the saccular stage of lung development. In 2001 the National Institute of Child Health and Human Development (NICHD), the National Hearth, Lung and Blood Institute (NHLBI) and the Office of Rare Diseases (ORD) BPD workshop proposed to develop experimental BPD animal models for better characterization of the pathophysiology and new treatment strategies for BPD \((39)\).

Pathophysiology: general

The three key factors in the pathogenesis of BPD are lung immaturity, lung injury and inadequate repair. BPD represents the response of the lung to injury during the critical period of lung growth at the end of the canalicular and beginning of the saccular stage in which airspace septation and the vascular system are developing. After premature birth, multiple stimuli including oxidative stress, barotrauma, surfactant deficiency, inflammation, alveolar fibrin deposition, vascular maldevelopment, fluid management, patent ductus arteriosus (PDA), nutrition, and genetic background \((39, 40)\), act at the susceptible lung in a critical stage of development. Mechanical injury and oxygen toxicity were the two main factors invoked in classic BPD, whereas new or current BPD is associated with immaturity, perinatal infection and inflammation, PDA and disrupted alveolar and capillary development. The (im)balance between initiating factors and host characteristics probably determines whether BPD will occur in a preterm infant.

Supplemental oxygen given to preterm infants with respiratory failure challenges them to oxidative stress. Oxygen treatment induces the production of cytotoxic reactive oxygen species (ROS), that regulate signal transduction pathways and transcription factors, and aggravate inflammation. Preterm infants are highly susceptible to oxidative stress, because the antioxidant defense system is underdeveloped. Higher oxygen levels are related to worsening of BPD in humans. Resuscitation of depressed infants with 21% or 100% oxygen results in a decreased defense of the anti-oxidant glutathione and increased mortality after brief exposure to 100% oxygen \((77, 93)\).

Mechanical ventilation leads to barotrauma and volutrauma in the preterm lung. Barotrauma and volutrauma overstretch or overdistend the underdeveloped lung parenchyma and lead to tissue disruption that induces
a reparative mechanism including cellular influx, inflammation, elastosis, and distorted acinar and vascular growth (87). Excessive and disordered elastin is a key feature in the fibroproliferative changes in the histology of BPD. Increased elastin has been found in infants who died of BPD (87). Mechanical ventilation in newborn mice with room air results in expression of elastin-related genes. Moreover, mechanical ventilation with 40% oxygen not only leads to an upregulation of genes related to elastin, but also to reductions in lung abundance of proteins that affect the formation of alveoli and lung capillaries (12). Pulmonary complications from the treatment of RDS with ventilatory and oxygen support include pulmonary air leaks (pulmonary interstitial emphysema, pneumo-mediastinum, and pneumothorax), pulmonary edema, and concurrent pulmonary infection. Pulmonary complications due to mechanical ventilation and oxygen supplementation will lead to prolonged ventilatory and oxygen support, further increasing the risk of developing BPD. Chronic inflammation and edema as a complication of barotrauma also suppresses surfactant function. As surfactant reduces surface tension and minimizes alveolar collapse, surfactant deficiency or inactivation requires even more aggressive ventilation leading to more lung tissue damage.

Lung inflammation is defined by an increased amount of inflammatory cells in the airspaces and lung tissue producing pro-inflammatory mediators. The inflammatory response can be triggered by infectious factors and a number of non-infectious factors, including oxygen, free radicals, positive-pressure ventilation, ventilation with an excessive tidal volume and increased pulmonary blood flow caused by a PDA. Both intra-uterine (chorioamnionitis or antenatal infection) and extra-uterine inflammation (nosocomial infection) contribute to the development and severity of BPD. Chorioamnionitis, a common clinical problem associated with preterm delivery, or antenatal exposure to pro-inflammatory cytokines may prime the fetal developing lung for minimal postnatal injury, resulting in abnormal alveolarization and pulmonary vascular development (83).

An arrest in both the formation of the alveolar and vascular system of the lung is the key characteric of BPD. The abnormal growth of the developing pulmonary microcirculation results in elevation of the pulmonary artery pressure. In normal lung morphogenesis development of the distal epithelial and capillary networks are very closely related. Inhibition of vascular development in fetal mouse lung explants resulted in abrogation of epithelial branching morphogenesis (92). Treatment with three different anti-angiogenic agents, including fumagillin and thalidomide, attenuated both vascular growth and alveolarization in the lungs of newborn rats (38). These data suggest a close interaction between epithelial and endothelial cells in the developing lung. Lung epithelium probably stimulates capillary morphogenesis through elaboration of angiogenic factors, and the vascular system likely has a regulatory role on the epithelium. A major factor in lung angiogenesis is vascular endothelial growth factor (VEGF), and its two receptors Flk-1 (also known as VEGF receptor-2) and Flt-1 (also known as VEGF receptor-1). VEGF is produced by both alveolar type 2 and bronchiolar cells, and stimulates vascularization at the leading edge of branching
airways. Lung VEGF expression is reduced in hyperoxia induced BPD in animal models (62, 63). Recombinant human VEGF treatment improves alveolarization and vascular growth in hyperoxia-exposed newborn rats (55). Transgenic mice overexpressing IL-13 stimulate pulmonary VEGF expression and improved survival in hyperoxia (25). In conclusion, vascular maldevelopment results in pulmonary hypertension and contributes to the development of BPD. Primary injury to either the airspace or lung circulation may have profound secondary effects on the other.

Pulmonary edema is an important factor in preterm infants with BPD. Interstitial and intra-alveolar protein rich pulmonary edema is the result of increased permeability of the alveolar-capillary membrane in the preterm lung. Pulmonary edema is mainly due to immaturity and probably aggravated by barotrauma (41), activated plasma proteins, including fibrin, and inflammatory cells (14). The plasma proteins in intra-alveolar edema contribute to the formation of hyaline membranes. As mentioned previously, pulmonary edema inactivates surfactant. Pulmonary edema may also be the result of a PDA or excessive fluid administration, which both negatively influence pulmonary function, and are associated with BPD (72). PDA induces systemic-to-pulmonary shunting, thereby increasing the pulmonary circulation leading to pulmonary edema and endothelial injury (7). Increased inflammatory parameters have been measured in preterm infants with a PDA (30). The incidence of BPD decreased after the implementation of indomethacin therapy for ductal closure (19).

Inadequate nutrition and certain genetic factors also predispose to development of BPD. Adequate nutrition is essential for normal lung development and repair. Nutritional deficits in animal models lead to impaired alveolarization and thickened septa for age (64). Variants of polymorphisms for surfactant proteins are related to BPD development (31).

Pathophysiology: inflammation and coagulation

The inflammatory response and an imbalance of the coagulation and fibrinolytic cascades, leading to pulmonary fibrin deposition, play pivotal roles in the pathophysiology of BPD. The contribution of inflammation and coagulation seems to be of crucial importance in the arrest in alveolarization and vascular development. Inflammation and coagulation are closely related processes, but the exact mechanism of their relationship in BPD is not clear.

Activation of the inflammatory response has been detected in both preterm infants and animal models with (experimental) BPD. Persistence of neutrophils in bronchoalveolar lavage fluid (BALF) correlated with the development of BPD (3, 71). Increased levels of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β and mediators reflecting neutrophil recruitment and activation, including soluble intercellular adhesion molecule (s-ICAM), chemokines IL-8 and MCP-1, and neutrophil elastase have been observed in tracheal aspirates of infants developing BPD (52-54). Moreover, in premature baboons (22), mice (96) and rats (10) with experimental BPD pro-inflammatory cytokines and inflammatory cells are elevated in BALF or
lungs and lung tissue. Antichemokine treatment with anti-CINC-1 (4, 27), anti-MCP-1 (94) and anti-MIP-2 (27) attenuates neutrophil and/or alveolar macrophage accumulation in BALF and preserves alveolar development of hyperoxia-exposed newborn rats. These studies indicate the impact of activation of the inflammatory response on alveolar enlargement, one of the key findings of BPD.

Pro-inflammatory cytokines create a procoagulant and antifibrinolytic state that may lead to fibrin deposition in the airspaces and microvasculature of the lungs. IL-6 is an important intermediate factor in coagulation activation in endotoxemia (91) and anti-IL-6 infusion in low grade endotoxemia in chimpanzees results in attenuation of coagulation. The procoagulant state caused by pro-inflammatory cytokines is frequently accompanied by inhibition of the fibrinolytic system or natural anticoagulants. Fibrinolysis decreases after TNF-α infusion (89, 90). During inflammation the fibrinolytic system is stimulated by increasing levels of plasminogen activators, which are released from the endothelium. TNF-α and IL-1 are able to reduce the levels of tissue-type plasminogen activators. Moreover, both pro-inflammatory cytokines have an additional antifibrinolytic effect by increasing PAI-1 release (11, 78). The natural anticoagulant APC system is impaired by the pro-inflammatory cytokines TNF-α and IL-1β through downregulation of the key mediator thrombomodulin on the endothelial cell surface (67, 68).

The inflammatory response can activate the coagulation cascade, and, in turn, coagulation may influence inflammation (Figure 1). Tissue Factor (TF), a transmembrane bound protein, is the physiologic initiator of the extrinsic coagulation pathway with fibrin deposition as ultimate result. Fibrin is degraded into fibrin degradation products after the conversion of plasminogen into plasmin by plasminogen activators, i.e. tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) bound to its receptor uPAR. The generation of plasmin is inhibited by plasminogen activator inhibitors (PAI-1,-2 and -3), of which PAI-1 is believed to be the most powerful regulator. Fibrinolysis is also regulated by two other antifibrinolytic factors, α2-antiplasmin and thrombin-activatable fibrinolysis inhibitor (TAFI). α2-antiplasmin inactivates free plasmin not bound to the fibrin network, whereas TAFI inhibits the binding of plasmin on the fibrin network by removing the carboxyterminal lysine and arginine residues.

Excess procoagulant and decreased fibrinolytic activity in the lung lead to fibrin deposition in alveoli, interstitium and capillaries (8, 34, 35). In PAI-1 deficient mice exposed to hyperoxia fibrin deposition is decreased, leading to a less severe phenotype and increased survival (8). In baboons with sepsis-induced acute lung injury TF blockade reduces intra-alveolar fibrin deposition and early collagen formation (98). Fibrin is not only the result of coagulation activation initiated by TF, but also has profibrotic (34, 35) and pro-inflammatory properties via activation of NF-κB and AP-1 (82). In addition, fibrin deposition can hamper gas-exchange by inactivating lung surfactant (80) and thus favoring alveolar collapse. Therefore, intra-alveolar fibrin deposition may function as a key marker for the severity of BPD with respect to coagulation activation and impaired fibrinolysis.
Figure 1. Schematic representation of the coagulation and fibrinolytic cascades. (A) Taken from Wagenaar et al., Free Radic Biol Med; 2004. Tissue damage results in the local expression of the physiological activator of the coagulation cascade tissue factor (TF). TF binds to factor VII/VIIa. This complex activates factors IX and X. Factor Xa activates prothrombin (factor II), resulting in thrombin (factor IIa) generation. Also, generation of factor Xa results in an inhibition of the extrinsic pathway by tissue factor pathway inhibitor (TFPI). At low concentration thrombin acts as an anticoagulant. After binding to its cofactor thrombomodulin (TM), thrombin activates protein C. Activated protein C (APC) inhibits the coagulation cascade by inactivation of factors VIIIa and Va, which act as cofactors of factors IXa and Xa, respectively. High concentrations of thrombin are
procoagulant. It results in even higher thrombin concentrations via the factor XIa feedback loop. Proteolytic cleavage of fibrinogen (Fg) results in fibrin formation. Hyperoxia results in a local upregulation of TF and fibrinogen expression and a downregulation of TM expression, resulting in a procoagulant environment. High concentrations of thrombin are antifibrinolytic via the activation of thrombin-activatable fibrinolysis inhibitor (TAFI), bound to its cofactor TM. (B) Fibrinolysis is the process by which fibrin degradation takes place. Fibrin is degraded by plasmin after proteolytic cleavage of plasminogen by plasminogen activators, i.e., tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) bound to its receptor uPAR. Plasmin formation is regulated by plasminogen activator inhibitors (PAI-1, -2, and -3) of which PAI-1 is believed to be the most important PAI in fibrinolysis. Plasmin bound to the fibrin network is prevented from inactivation by α2-antiplasmin. Binding of plasmin to the fibrin network is prevented by TAFI, which removes the carboxyterminal lysines from the fibrin network that serve as binding sites for plasmin. Hyperoxia results in a moderate upregulation of the profibrinolytic factor uPA and its receptor uPAR, but not of tPA expression, and a tremendous upregulation of the inhibitor of fibrinolysis PAI-1. This will probably result in an antifibrinolytic environment. Hyperoxia results in a local procoagulant and antifibrinolytic environment, ultimately resulting in fibrin deposition in the developing neonatal lung. Solid lines indicate activation and dotted lines indicate inhibition. Factors in bold are upregulated and factors in italic are downregulated in our experiments.

INTERVENTION

The importance of extravascular fibrin deposition through an imbalance in the interrelated processes of activation of the inflammatory response and coagulation and/or fibrinolytic cascades in BPD suggests a potential role for compounds with both anti-inflammatory and anticoagulant properties.

Pentoxifylline

The methylxantine derivative pentoxifylline (PTX) is a nonspecific phosphodiesterase inhibitor and acts as an immunomodulation agent. PTX has been used clinically in the treatment of peripheral arterial disease because it enhances the deformability of red blood cells and thereby improves the microcirculatory blood flow (73, 95). PTX increases intracellular cAMP levels and decreases the TNF-α production with beneficial effects on the inflammatory response. PTX attenuates neutrophil sequestration, prevents pulmonary vascular permeability to protein to the alveolar lumen (97), and inhibits the production of free oxygen radicals (88). The anti-inflammatory and anticoagulant properties of PTX have been shown in baboons suffering from lipopolysaccharide (LPS)-induced endotoxemia (58). In severe acute alcoholic hepatitis PTX improves the short-term survival (2). Positive effects of PTX have also already been reported in septic preterm infants, in whom administration of PTX reduces the treatment requirements after the first month of life (56). In a recent LPS-induced endotoxemia rat model, PTX administration resulted in a significant decreased production of IL-8, MMP-2 and MMP-9 in combination with a reduction of NF-κB and ICAM-1 activity in lung tissue leading to less severe lung injury (23).
Inhaled nitric oxide

A novel therapeutic strategy for infants with respiratory failure is administration of inhaled nitric oxide (iNO). NO is an important mediator of biological processes in the pulmonary epithelium, such as neurotransmission, pulmonary vasodilatation, smooth muscle contraction, inflammatory mechanisms, ciliary motility, mucin secretion and plasma exudation (9, 29, 37, 74). It exerts its biological effects via the activation of guanylate cyclase resulting in the production of cyclic 5’-guanose monophosphate (cGMP) (66). Endogenous NO is synthesized from arginine and oxygen by three NO synthases (neuronal [nNOS], inducible [iNOS] and endothelial [eNOS]), that have been identified in the human and animal lung (29, 49, 81). NO is able to attenuate the procoagulant activity induced by acute lung inflammation in rats (44) and plays an important role in the regulation of the pulmonary vascular tone and lung liquid production (26). Moreover, NO seems to promote the formation of alveoli and branching morphogenesis in the developing lung (5, 61, 99). In animals with chronic lung disease, NO also reduces abnormal elastin deposition (65, 87), decreases lung neutrophil accumulation (47), and has a positive effect on early pulmonary function (47, 65). In the NICU iNO is used as a vasodilator to alleviate persistent pulmonary hypertension of the newborn, a complication in full-term infants with respiratory failure. These data indicate that iNO treatment of preterm infants with respiratory distress syndrome may be beneficial to improve oxygenation and reduce the survival rate and/or development of BPD.

Several clinical trials do support this hypothesis. Unfortunately, the results are controversial. After treatment with early low-dose iNO many clinical studies reported an improvement in oxygenation (32, 48, 50, 84), and a reduction of the need for extracorporeal membrane oxygenation (18) and a shorter stay at the NICU (86). The study of Schreiber et al. is the only clinical trial showing both a decrease in the incidence of neonatal chronic lung disease and death (79). Therefore, Field advised in 2005 that near-term and term infants with respiratory failure should enter a trial with iNO, but that preterm infants should be treated conventionally (28). Recently, two large multicenter, randomized and placebo-controlled iNO trials have been conducted in preterm infants (6, 45). In the study of Kinsella et al. preterm infants were treated with 5 particles per minute (ppm) iNO after birth for 21 days. The incidence of BPD or death was not reduced, but iNO did diminish the risk of brain injury (45). Ballard et al. treated preterm infants between day 7 and 21 of age with decreasing iNO concentrations beginning at 20 ppm, resulting in a better pulmonary outcome (6).
AIMS AND OUTLINE OF THIS THESIS

Due to a lack of patient materials and ethical reasons animal models of BPD are critical for characterization the pathophysiology of BPD and testing of potential treatment options. In chapter 2 of this thesis we characterize a rat model for experimental BPD, induced in neonatal pups by prolonged exposure to hyperoxia, by investigating histopathology and differential gene expression profiles in the lung and demonstrate its significance for studying BPD in premature infants. In chapter 3 we describe the spatial and temporal expression of surfactant proteins in this experimental BPD model.

Since inflammation and unbalanced coagulation and fibrinolysis, leading to extravascular fibrin deposition in the lung, are two interrelated processes that play a pivotal role in the pathophysiology of inflammatory lung disease, we investigated whether the pathophysiology of experimental BPD could be improved by interrupting the vicious cycle of inflammation and coagulation. Fibrin deposition can be prevented directly via inhibition of the coagulation cascade and/or stimulation of the fibrinolytic cascade or indirectly via inhibition of the inflammatory response, thereby preventing activated leucocytes to perform their procoagulant and antifibrinolytic activity. In chapters 4 and 5 intervention studies in experimental BPD are described which study the potential therapeutic effect of agents with anti-inflammatory and/or anticoagulant activity for premature infants who are at risk of developing BPD. The role of pentoxifylline, a methylxantine derivative and weak non-selective phosphodiesterase inhibitor with anti-inflammatory and anticoagulant properties, and with positive effects on capillary blood flow in experimental BPD is presented in chapter 4. The role of nitric oxide, a gas that is involved in multiple (patho)physiological processes in the injured lung, including pulmonary vasodilatation, inflammation and plasma exudation, is presented in chapter 5. In chapter 6 the presented studies of chapters 2-5 and the future perspectives are discussed. In chapter 7 a summary is given of this thesis.
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