Oxidative stress in experimental bronchopulmonary dysplasia

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Chapter

6

General discussion

GENERAL DISCUSSION

An animal model to study bronchopulmonary dysplasia

The aim of this study was to generate and characterize animal models for BPD to develop new and less harmful treatment strategies for premature infants who develop bronchopulmonary dysplasia (BPD) after suffering from respiratory distress syndrome (RDS). We started out with the characterization of a premature rat model in which continuous neonatal exposure to 100% oxygen leads to the development of lung injury, which closely resembles that of premature infants who develop BPD (28). This animal model formed a perfect basis to investigate the pathophysiology of BPD and the potential therapeutic strategies. Nowadays in the postsurfactant era, premature infants at greatest risk for BPD are born at 24-28 weeks of gestation during the late canalicular or saccular stage of lung development (12, 13). So the unique advantage of this animal model is that lung injury is induced during the saccular stage of lung development and mimics the key finding of BPD in premature infants, i.e. leads to an arrest in alveolarization and vascular growth of the immature lung (12, 13). We extensively characterized this model by studying gene expression profiles with DNA microarray analysis, confirmed by RT-PCR. These gene expression profiles will be especially important for researchers focusing on specific genes for target therapy. The characterization of this animal model was not only essential for our own studies, but also offers new opportunities to other investigators focusing on BPD in premature infants.

Investigation of new therapeutic strategies in premature infants with RDS, who are prone to develop BPD, is complicated by multiple morbidity and ethical constraints. Further advances in the clinical setting will benefit from preliminary studies in an animal model, but are limited by the intrinsic problem that an animal model can never fully replace human clinical research. Although the pathophysiologic parameters measured in premature rat pups exposed to hyperoxia closely resemble the clinical parameters of infants treated for BPD in the neonatal intensive care unit (NICU), the investigative methods continue to be different. However, the advantange of the use of an animal model is that parameters like survival, histopathology and analysis on lung homogenates can be extensively explored, which is never possible in a human model.

Surfactant deficiency in premature birth

Lack of surfactant is a pivotal problem in infants suffering from RDS and prone to develop BPD because of immaturity of their lungs. Intratracheal administration of exogenous surfactant has greatly improved pulmonary care, morbidity and mortality of premature infants with RDS (19, 21). The expression profiles of the four surfactant proteins in the experimental BPD

model demonstrate an important role for surfactant protein A (SP-A) and bronchial Clara cells in the defense against oxidative stress. Currently available synthetic surfactant preparations contain the hydrophobic surfactant proteins SP-B and SP-C (29) which are responsible for the surface activity of surfactant, but not the hydrophilic surfactant proteins SP-A and SP-D which have an important immunological function. Supplementation of SP-A in exogenous surfactant might improve the resistance of the immature lung against oxidative lung injury.

The development of new therapeutic modalities for BPD

New treatment options for BPD are extremely important as the current use of glucocorticoids to wean premature infants from the ventilator is under close scrutiny due to its association with long-term neurological side-effects. 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 BPD. Interruption of this vicious cycle can be achieved by either preventing or counterbalancing the activation of the coagulation cascade and inhibition of fibrinolysis triggered by pro-inflammatory cytokines, or preventing or counterbalancing the inflammatory response promoted by coagulation proteases. In this thesis we studied two therapeutic interventions in experimental BPD: subcutaneous administration of the methylxantine pentoxifylline, a weak non-selective phosphodiesterase (PDE) inhibitor and inhalation of the vasodilator nitric oxide (iNO).

Although the effect of pentoxifylline on lung injury is very promising, it is unlikely that pentoxifylline will be introduced in the NICU. However, other methylxanthines, including theophylline, aminophylline and caffeine, are widely used in the NICU to treat apnea of prematurity and weaning from the ventilator, because they increase respiratory drive leading to oxygen consumption (6, 8, 22). Until now, pentoxifylline has been used clinically to treat intermittent claudication, due to arterial obstruction in the limbs (23), and vascular dementia (24). Little is known about the multiple side effects of pentoxifylline and more research needs to be done to explore the exact working mechanism of pentoxifylline and to guarantee safe use in the clinical setting.

The prolonged survival, the reduction of alveolar fibrin deposition, the attenuation of the inflammatory response and the improved alveolarization presented in this thesis with iNO in experimental BPD underscore the promising findings of iNO in BPD. However, the positive effects of 5 ppm of iNO in clinical trials among premature infants are still hotly debated (5, 14-16). Higher doses of iNO led to more sustainable improvements in lung function in our experimental model and therefore future clinical trials should consider delivering higher doses of iNO to establish a more uniform result in the affected premature infants. Although expensive, the use of iNO is easy to realize in the clinical setting and various clinical trials have already demonstrated the absence of worrisome side-effects of iNO. Testing higher

doses of iNO will undoubtedly lead to a consensus on the use of iNO in premature infants suffering from RDS and prone to develop BPD.

The promising results of pentoxifylline and iNO in experimental hyperoxia-induced BPD warrant further exploration of other specific phosphodiesterase inhibitors. Pentoxifylline is a weak non-specific phosphodiesterase inhibitor with anti-inflammatory and anticoagulant properties and with positive effects on capillary blood flow. Recently, our laboratory investigated the role of specific PDE4 and -5 inhibition in experimental BPD. PDEs belong to an enzyme family with 11 different members, iso-enzymes PDE1-11, which exert their biological function by inactivating the intracellular messengers cAMP and/or cGMP by hydrolysis (7, 11, 20). PDE4 is a cAMP-specific phosphodiesterase that consists of 4 sub-families (PDEA-D). The anti-inflammatory properties of rolipram, which is the specific prototypic PDE4 inhibitor and piclamilast, which is a second generation PDE4 inhibitor, was studied in experimental BPD. PDE4 inhibitor therapy in our rat BPD model prolonged the median survival up to 7 days, reduced alveolar fibrin deposition, lung inflammation, and vascular leakage and improved mRNA expression of genes involved in inflammation, fibrin deposition and alveolarization, whereby piclamilast outperformed rolipram (27).

The biological effects of NO can be mediated via the cGMP pathway after activation of guanylate cyclase or S-nitrosylation: the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine (reviewed in 9). Signaling via S-nitrosylation or cGMP may lead to a similar biological response in experimental BPD, resulting in reduced inflammation and pulmonary hypertension and improved alveolarization after iNO or ethyl nitrite therapy (2, 10). The role of cGMP signaling in experimental BPD can be studied in more detail by specific activation of guanylate cyclase or specific inhibition of cGMP degradation by PDE5 (1; Figure 1).

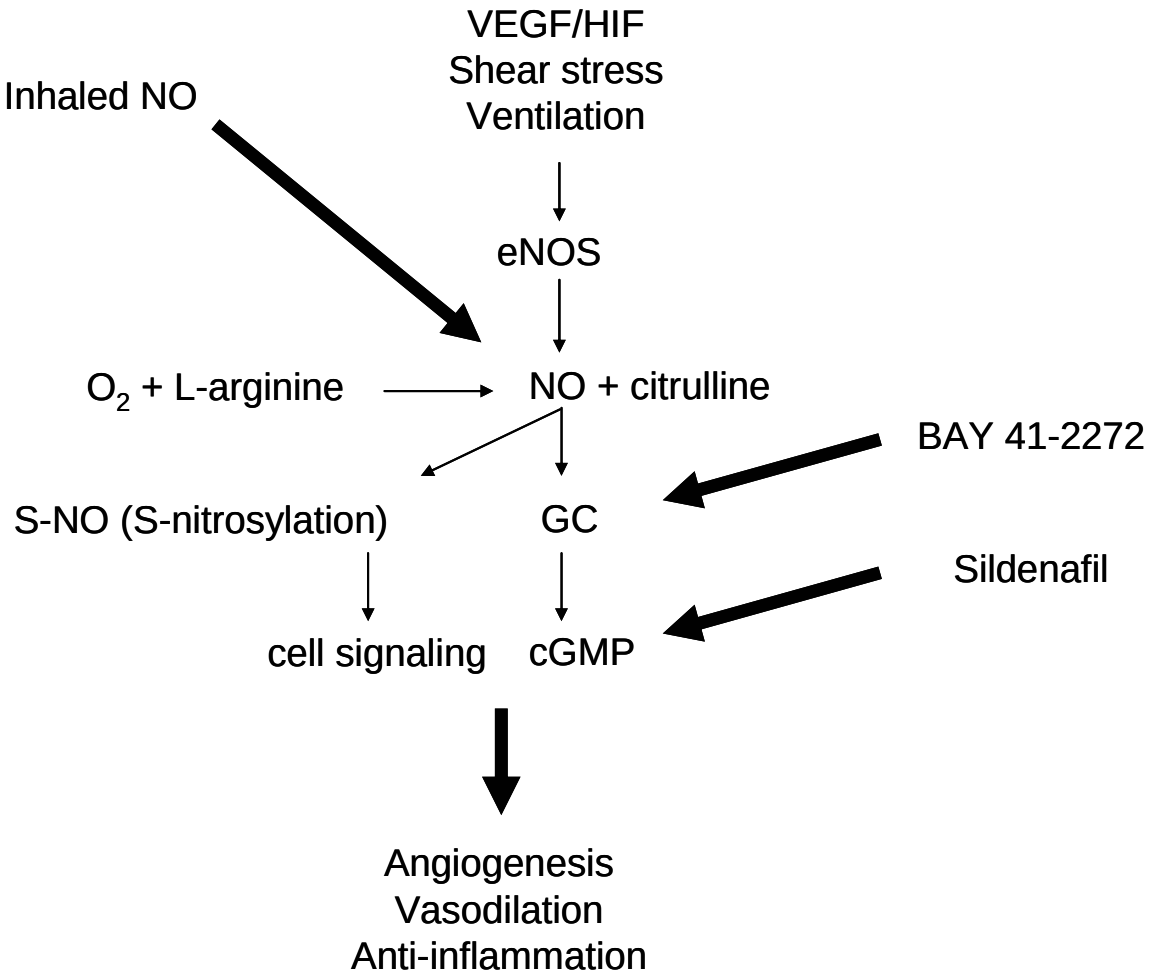

Figure 1. Schematic representation of the NO signaling pathways in experimental BPD. [VEGF: vascular endothelial growth factor; HIF: hyperoxia inducible factor; NO: nitric oxide; eNOS: endothelial nitric oxide synthase; S-NO: S-nitrosothiol; GC: guanylate cyclase; BAY 41-2272: soluble guanylate cyclase activator; Sildenafil: specific phosphodiesterase-5 inhibitor; cGMP: cyclic guanosine monophosphate]

Increased intracellular cGMP levels by iNO and PDE5 inhibition lead to vasodilatation and subsequent prevention of persistent pulmonary hypertension of the newborn, which is a common complication in full-term infants with respiratory failure.

In experimental BPD the effect of PDE5 inhibition was studied with sildenafil, a selective PDE5 inhibitor. PDE5 inhibitor therapy prolonged survival, reduced fibrin deposition, attenuated the inflammatory response, and improved lung histopathology by improving alveolar development (18, 26). Interestingly, sildenafil improved the mRNA expression of vascular endothelial growth factor receptor-2 (VEGFR2) and fibroblast growth factor receptor-4 (FGFR4). VEGFR2, one of the receptors of vascular endothelial growth factor (VEGF), and FGFR4 are two pivotal genes in the normal formation of the alveolar and/or vascular system, that are both affected in BPD. The angiogenic growth factor VEGF is an upstream effector of the NO-cGMP pathway and is absolutely critical for vascular development and

promotes vessel growth and remodeling. VEGF-induced lung angiogenesis is in part mediated by NO. Neonatal treatment with the VEGF inhibitor, SU5416, downregulates endothelial NO synthase protein and NO production (3, 4). Treatment with iNO improves vascular and alveolar growth in experimental BPD (3, 4) and recombinant human VEGF treatment improves alveolarization and vascular growth in hyperoxia-exposed newborn rats (17). Thébaud and Abman (25) recently hypothesized that disruption of angiogenesis impairs alveolarization, and that preservation of vascular growth and endothelial survival promotes growth and sustains the architecture of distal airspaces.

The data on (specific) PDE inhibitors and iNO suggest that the (VEGF)-NO-cGMP signaling pathway is involved in the pathogenesis of an arrest in alveolar and vascular development in BPD and indicates that further research in this signaling pathway may shed more light on the potential of PDEs and iNO to prevent the development of BPD in premature infants with BPD.

Summary for the future

The experimental BPD animal model presented in this thesis is a valuable tool to further unravel the key findings in BPD leading to an arrest in alveolarization and distorted vascular development, and more importantly to develop new treatment modalities for BPD. The experimental use of phosphodiesterase inhibitors and iNO has shown a clear potential as intervention therapy in BPD by interrupting the vicious cycle of inflammation and coagulation, and interacting in the development of alveolar and vascular growth. These findings open up new avenues to prevent or attenuate hyperoxic lung injury and ultimately may reduce pulmonary morbidity and mortality of premature infants with RDS and BPD.

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