Animal models of bronchopulmonary dysplasia. II: the term rat models

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Abstract

Bronchopulmonary dysplasia (BPD) is the chronic lung disease of prematurity that affects very preterm infants. Although advances in perinatal care have enabled the survival of infants born as early as 23-24 weeks of gestation, the challenge of promoting lung growth while protecting the ever more immature lung from injury is now bigger. Consequently, BPD remains one of the most common complications of extreme prematurity and still lacks specific treatments. Progress in our understanding of BPD and the potential of developing therapeutic strategies have arisen from large (baboons, sheep and pigs) and small (rabbits, rats and mice) animal models. This review focuses specifically on the use of the rat to model BPD, and summarizes how the model is used in various research studies, the advantages and limitations of this particular model, and highlights recent therapeutic advances in BPD using this rat model.


Introduction

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease of very preterm infants. BPD interrupts lung development and has serious long-term respiratory complications that reach beyond childhood and into adult life (8, 25, 26, 41, 53). The multi-factorial etiology of BPD has prompted research to investigate the many factors contributing to the pathogenesis of BPD (reviewed in (38)), with the ultimate aim of developing effective therapies to prevent long-term pulmonary sequelae. In order to investigate the effectiveness of various therapeutic strategies on the development, structure, and function of the lungs, it is important to have animal models that reliably reproduce some of the features observed in very preterm infants developing BPD. In order to achieve this, known contributing factors of BPD, such as perinatal inflammation, growth restriction, hyperoxia and mechanical ventilation, have been used in both large and small animals to mimic the BPD-like lung injury. In particular, exposure of neonatal rats to hyperoxia is extensively utilized as a small animal model of experimental BPD.

Characterization of the rat model of experimental BPD

Exposing the immature rat lung to hyperoxic gas through neonatal life closely reproduces the histopathology observed in human infants with BPD. Studies have shown that exposure of the developing rat lung to hyperoxic gas can have detrimental effects, particularly on the structure of the gas-exchanging region (14, 30, 34, 46, 62, 66). The main overall finding, which is common to each study, is that exposure of the immature lung to hyperoxic gas impairs alveolarization, resulting in fewer and enlarged alveolar airspaces. Pulmonary hypertension,
disrupted vascular growth, vascular leakage, accumulation of plasma proteins, extravascular fibrin deposition, increased lung collagen content, increased inflammatory cell influx, and disorganized elastin deposition are also predominant characteristics observed in the parenchyma of lungs exposed to neonatal hyperoxia (14, 66, 67, 72). These alterations in the lung are hallmark features of BPD. Table 1 shows a non-exhaustive overview of various studies that have utilized the hyperoxic neonatal rat model in their investigations.

Advantages of the neonatal hyperoxia-induced rat model of experimental BPD

Owing to its many advantages, not only has this animal model been extensively utilized in studies that have investigated the independent effects of hyperoxia exposure on the developing lung, but it has also provided a platform for assessing various therapeutic strategies aimed at preventing or repairing lung injury in prematurely born babies. Detailed below are the advantages of this animal model.

Rats are born at a lung developmental stage equivalent to that of an extreme premature infant

Development of the lung is typically described to occur over five stages, which comprise the embryonic, pseudoglandular, canalicular, saccular, and alveolar stages (Table 2). In humans, term birth coincides with the alveolar stage of lung development. However, preterm birth precedes the final maturational steps and
can expose the lung to the external environment as early as the canalicular stage; the subsequent lung development stages must then occur *ex-utero*. Therefore, animal models of BPD ideally contain an element of lung immaturity. In this regard, the use of rats are beneficial because they commence alveolarization *ex-utero* and are born at a similar stage of lung development to that of a very preterm human infant (i.e. saccular stage). Although rats born at term have structurally immature lungs, they are functionally mature and this eliminates the requirement for various interventions (i.e. resuscitation, intubation, ventilation, synthetic surfactant, altered nutrition) normally needed to maintain a prematurely born infant or large animal model, such as sheep and baboons. Therefore, researchers can target the saccular stage of lung development in rats by commencing hyperoxia exposure from birth, which is equivalent to approximately 26-28 weeks gestation in the human (Figure 1). The transition to the alveolar stage occurs at postnatal day 5 in rats, with complete lung development occurring post-weaning at approximately 30 days postnatal age. Being born at an earlier stage of lung development without the need for induced preterm birth is probably the most advantageous aspect of this hyperoxia rat model of BPD. It is important to note however, that the ability for rats to be born at term with anatomically immature lungs is largely due to the production of surfactant at birth. In contrast to human lungs, it has been shown that all four surfactant proteins (SP-A, -B, -C, and -D) can be detected in the type II pneumocytes in newborn rat lungs (49). In humans, the pulmonary surfactant system matures between 29-32 weeks of gestation, which coincides with the
saccular stage of lung development (52). Therefore, infants born prior to 29 weeks of gestation often require administration of synthetic surfactant to prevent alveolar collapse. It is through the adaptation of the rat’s surfactant system to respiration in the *ex-utero* environment, in spite of a structurally immature lung, that enables neonatal transition without the need for respiratory support.

*Rats have a short estrous cycle and gestation, and a large litter and pup size*

Another advantage of using the rat, or any rodent for that matter, is the relatively short duration of the estrous cycle; approximately 4-5 days in rats (68). This very short estrous cycle provides many opportunities for the female to become pregnant compared to larger animal models such as the sheep and baboon, which have estrous/menstrual cycles of approximately 17 and 33 days, respectively (6, 54). Furthermore, the short estrous cycle enables timed-pregnancies to be easily planned and achieved. The duration of gestation is also relatively short in rats, approximately 21-22 days. This short duration enables the production of multiple litters in a tight timeframe. The large litter size of the rat (average of 11 pups per litter) provides a considerable number of subjects per hyperoxia exposure set-up. Furthermore, in comparison to the mouse, rat pups are relatively larger (4.5-6g at birth vs. 1-1.5g mouse birth weight) and this enables ease of use at a younger age. For example, surgical manipulations may be achieved with more ease, and the amount of tissue obtained at harvest is greater.
Short lifespan enables timely long-term follow-up

Recent evidence shows that BPD has long-term respiratory complications, which reach beyond childhood and into adult life, with follow-up studies demonstrating increased risk of respiratory symptoms (i.e. cough, wheeze), poor lung function, and low exercise capacity (8, 18, 25, 26, 41, 53, 69). Therefore, long-term studies in experimental models are now gaining greater interest. The average lifespan of the rat ranges from 24-36 months, thus making long-term research studies extremely feasible over a shorter timeframe, especially when compared to larger animal models such as sheep and baboons. An earlier study using the rat model clearly demonstrated the persistence of neonatal hyperoxia-induced lung injury into adult life, with analysis occurring at 60 days of age, equivalent to approximately late-adolescence in the human (50). Likewise, at postnatal day 51, rats presented with persistent alveolar enlargement, medial wall thickness and right ventricular hypertrophy (22). The realization of these advantages has lead to interesting therapeutic studies exploring the optimal timing of cell-based therapies in rats up to 70 days (1) and even 6 months of age (~mid-adulthood) (45).

Commercial availability of analysis products

Not only is it important to utilize an animal model that best suits the experiment, but also it is advantageous to have the tools required for reliable analysis of the collected data. The readily available analysis products for use with the rat are yet another benefit of using the rat model of BPD. Products such as antibodies for
use in immunohistochemistry, Western blotting, and flow cytometry, ELISA kits, primers for use in PCR, and specific cell lines are all commercially available. Therefore, the type of analysis in this model is not a limiting factor in a study.

**Flexible, “multi-hit” model**

Another attraction of the rat model of BPD is its flexibility, as demonstrated by the possibility to add secondary insults to the existent experimental model. Since BPD has a multi-factorial etiology, it is advantageous to be able to investigate the effects of various contributing factors in experimental models. Although hyperoxia is used to induce the BPD-like lung injury, additional factors such as inflammation due to chorioamnionitis, growth restriction, and prenatal/postnatal dexamethasone can also be introduced into the model, either combined during the hyperoxia-exposure period or separately. Earlier studies on the effects of prenatal dexamethasone have been investigated in the hyperoxia-induced rat model of BPD, in both the prematurely delivered rat as well as the more commonly utilized term-born rat model (15, 28). The addition of inflammation to the rat model of BPD, achieved by intra-amniotic or postnatal administration of LPS, has also been extensively researched (16, 17, 37). This double-hit model, incorporating the added effect of antenatal inflammation to mimic chorioamnionitis, showed that the administration of LPS exacerbated the already damaging effects of hyperoxia on the developing alveolar structure. Since severe BPD is associated with adverse neuro-development, several investigators have
now taken further advantage of this model by exploring the effects of perinatal
events on the developing brain (7).

Although the rat BPD model has many advantages, like all animal models it also
has its limitations.

**Limitations of the rat model of experimental BPD**

*Postnatal growth restriction*

Several studies have documented the occurrence of postnatal growth restriction
following neonatal exposure to hyperoxic gas. Indeed, this can be
disadvantageous if the primary outcome of the study is to investigate the
independent effects of hyperoxia alone on lung development. It is well
documented that both *in-utero* and postnatal growth restriction can negatively
influence the development of the lung. In rats, poor nutrition after birth, coinciding
with the saccular and alveolar stages of lung development, has been shown to
result in enlarged alveoli, thicker septa and reduced elastin deposition in the lung
parenchyma (19). Furthermore, postnatal undernutrition in rats can affect the
bronchiolar epithelium, leading to altered proportions of Clara and ciliated cells
(40). Postnatal growth restriction, however, can be avoided in some cases
through the use of dam rotation during the hyperoxia-exposure period. This
technique is a necessary step when using hyperoxia-exposures greater than
80% oxygen as it prevents oxidative toxicity and death in the adult dams. It is
important to note, however, that in studies utilizing 100% oxygen, postnatal
growth restriction has been shown to be unavoidable even when employing the
dam rotation technique (64, 65). In studies utilizing lower oxygen concentrations,
oxidative toxicity and death is not an issue and the dam rotation technique is not
necessarily used. This, however, can lead to maternal weight loss during the
hyperoxia-exposure period, which impacts offspring nutrition and postnatal
growth. Therefore, it is important to document body weights throughout the
experiment, as this may be a confounding factor.

Clinical relevance of oxygen concentrations and antioxidant defense systems
Another disadvantage of the hyperoxia-exposure rat BPD model is that the levels
of oxygen utilized far exceed what is currently used in the NICU. Although current
clinical practice uses lower oxygen concentrations than were used in the past,
few experimental studies have investigated the effects of breathing hyperoxic gas
containing less than 60% oxygen on lung development. However, when using
this model as a platform for assessing therapies, it is understandable that
investigators use a high oxygen concentration to induce a greater degree of lung
injury. Low oxygen concentrations in clinical practice are used to minimize the
amount of lung injury in preterm infants. Therefore, similar oxygen levels in a rat
model may not produce such obvious lung injury and may generate difficulty in
assessing therapeutic potential. Another limitation of the rat model of BPD is their
increased tolerance to hyperoxia-exposure. It has been shown that the neonatal
rat has a mature antioxidant enzyme activity at the time of birth and in early
neonatal life (55, 71). Conversely, preterm infants have been reported to exhibit significantly higher oxidative stress levels combined with low levels of antioxidant enzyme activity over the first 100 days of life compared to term-born infants (42). This makes the neonatal rat potentially more resistant to oxidative stress compared to the preterm infant at the same stage of lung maturation.

Strain-dependent variations

Various strains of the rat are commonly used in research, for example Sprague-Dawley, Long-Evans, Fischer, Wistar, and Lewis. However, this can pose a limitation to the use of the rat in experimental models of BPD. Recently, it has been shown that strain-dependent differences exist in oxygen-induced retinopathy rat models (24, 63). However, it is unknown as to whether this strain related difference might also extend to the effects on the developing lung. Although the majority of studies use the Sprague-Dawley strain in their hyperoxia-induced rat models of BPD, others have shown similar BPD-like outcomes in the Wistar rat strain (22, 65).

Variations in the rat model of experimental BPD

With variations in research come variations in the animal models used. While many studies have investigated the effects of hyperoxia-exposure alone on the developing lung, others have used it as a platform to test new therapies. The variations to the model will depend on the primary aim of the study. Studies that investigate therapeutic potential are likely to use the most injurious model; this
likely incorporates a high oxygen concentration combined with early exposure. Other studies that investigate the effects of hyperoxia are likely to use different combinations of time and/or oxygen concentration to determine the impact on lung development, structure, and function.

**Duration of hyperoxia-exposure**

Hyperoxia-exposure in the rat model of BPD is generally commenced within the first few days of life while the neonatal rat is still in the saccular stage of lung development (Figure 1). Breathing hyperoxic gas from birth is achieved by placing the pregnant rat dam in the chamber on the expected day of delivery; this ensures that the pup's first breaths are of hyperoxic gas. Other commonly used practices include placing the pups in the hyperoxic chamber within the first 24 hours, or even by postnatal day 4. The duration of the hyperoxia exposure can vary, however most studies keep the pups in hyperoxia for up to 14 days postnatal age (see Table 1).

**Oxygen concentration**

Studies that use the rat BPD model as a platform to test therapeutic potential generally expose the rat pups to very high oxygen concentrations, above 90%, as this ensures a severe oxygen-induced lung injury model. Other studies have also investigated the effect of lower oxygen concentrations, with exposures of 60% oxygen (30, 34, 72). Use of 60% oxygen has been described to produce heterogeneous changes in rat lung morphology, with patchy areas of
parenchymal thickening and small airspaces interspersed with areas of enlarged airspaces (30, 72). Although still moderately high, these oxygen concentrations are closer to the levels that are clinically used in preterm infants.

The varied oxygen concentrations and durations used in different studies appear to have an effect on mortality in the rat model, as evidenced by different reported survival rates (Table 3). In studies that have reported survival rates of rat pups exposed to hyperoxia, there seems to be quite a large range in the percentage survival, from as low as 5% up to 100% survival rate. It is important to note, however, the differences in hyperoxia-exposure between models as this can influence survival rates. Rat pups that are prematurely delivered and exposed to 100% oxygen from birth until postnatal day 14 are affected the most, exhibiting a ≥95% mortality rate. Whereas rat pups born at term and exposed to 60% oxygen from postnatal day 1 to 14 display a 100% survival rate (29). Survival rate appears to be affected by the percentage of oxygen used, as demonstrated in a study that reported survival rates of 40%, 56%, and 81% in rat pups exposed to 95% oxygen from birth to postnatal day 14, 95% oxygen from birth to postnatal day 7 followed by 7 days in 60% oxygen, and 60% oxygen from birth to postnatal day 14, respectively (30). Not only can hyperoxia-exposure affect the survival rate of the rat pups, but it can also affect the body weight of the survivors; many studies using the rat BPD model have reported reduced body weight (refer to Table 3).
The mouse model is addressed in a partner review that is published in this issue (insert reference of mouse model). However, it is useful to briefly outline the overlap between the rat and mouse models as well as the advantages of one over the other. In terms of similarities between the rat and mouse models of hyperoxia-induced lung injury, both rodents have the advantages of a short estrus cycle, short gestation, large litter size, and short lifespan. As discussed above, these characteristics are beneficial to researchers because timed-pregnancies can be easily organized, they are provided with a short timeframe for the production of offspring, they have many offspring to use for experiments, and need not wait many years to investigate long-term outcomes of events that occurred in fetal or neonatal life. One aspect that makes rats beneficial over mice is their larger size, which makes handling, surgical manipulations, and tissue harvesting able to be achieved with greater ease. The main beneficial aspect of the rodent model of hyperoxia-induced experimental BPD is that they are born at term at a lung developmental stage that is equivalent to that of an extreme premature infant, which eliminates the need for induced preterm birth in order to investigate an immature lung. This element of lung immaturity in both the rat and mouse makes them attractive models for experimental BPD. One beneficial aspect of the rat, however, is the possibility of further accentuating the element of lung immaturity by inducing premature birth approximately 0.5-1 day early (term is 21-21.5 days; see Table 3). This has not been described in mice. Conversely, there is larger availability of reagents for mice. More importantly the access to
transgenic mice has substantially advanced our understanding of normal and impaired lung development (39). However, in 2004 the genomic sequencing of the Brown Norway rat was completed, and in 2009 the first targeted knockout rats were generated using zinc-finger nuclease technology, a technique that was already well established in mice (reviewed in (23, 31)). Furthermore, in 2010 the first gene knockout rats were generated using homologous recombination in embryonic stem cells (23, 31). Further advances in genetic manipulation will enable easier generation of transgenic rats.

**Contribution of the rat BPD model to our knowledge on lung development and its use in investigating therapeutic strategies for lung repair**

Use of the neonatal hyperoxia-induced rat model of BPD has contributed greatly to our current understanding of normal lung development. In particular, knowledge about various angiogenic pathways and growth factors involved in lung development have stemmed from studies using this model. Furthermore, the use of the rat BPD model as a pre-clinical model to test various therapeutic strategies has proven to be a very effective tool (see Table 1).

The normal progress of alveolarization throughout lung development is highly dependent on angiogenesis. The importance of vascular endothelial growth factor (VEGF) in normal lung development and its impairment in BPD has been demonstrated through the use of the rat BPD model (35, 58). Pharmacological inhibition of VEGF during normal postnatal rat lung development was shown to
significantly restrict body and organ growth, reduce the number of lung capillaries, and impair alveolar development, resulting in lung structure reminiscent of BPD (58). Furthermore, lung mRNA levels of VEGF in the hyperoxia-induced rat model of BPD were significantly reduced, highlighting the critical role of VEGF in lung development (58). Administration of VEGF into the rat BPD model, either via intratracheal adenovirus gene transfer or intramuscular administration of recombinant human VEGF, proved beneficial in ameliorating the BPD-like lung injury induced by hyperoxia (35, 58). VEGF-induced angiogenesis is mediated, in part, by nitric oxide (NO). Activation of NO stimulates the production of guanosine 3',5'-cyclic monophosphate (cGMP), which is inactivated by phosphodiesterase enzymes (PDE). Increased intracellular cGMP levels can also be achieved pharmacologically with inhaled NO or inhibitors of PDE. The beneficial effects of inhaled NO were demonstrated in a study using the rat model of BPD (57). Administration of inhaled NO reduced pulmonary fibrin deposition and improved alveolar development, evident through reduced septal thickness; anti-inflammatory effects were also observed with the reduction in leukocyte influx (57). Sildenafil protects the activity of cGMP and increases NO through its specific inhibition of PDE5. The beneficial effects of sildenafil treatment in hyperoxia-induced lung injury have been demonstrated using the rat BPD model; preservation of alveolar growth and lung angiogenesis and a reduction in the pulmonary inflammatory response was observed, as well as reduction of pulmonary hypertension and restoration of right ventricular hypertrophy (20, 36). Pharmacological inhibition of another PDE, PDE4,
Piclamilast treatment has also been investigated in the rat model of BPD (22). Piclamilast treatment reduced pulmonary fibrin deposition and septum thickness, but did not restore alveolarization or angiogenesis; however, partial reversal of pulmonary hypertension was observed (22). L-citrulline is another factor that is involved in the production of NO, which is produced during NO synthesis from L-arginine. In the hyperoxia-exposure model of rat BPD, reduced plasma levels of L-citrulline have been demonstrated (61). Treatment with L-citrulline increased the plasma concentrations of L-citrulline and contributed to the preserved alveolar and vascular growth, as well as the reduction in pulmonary arterial medial wall thickness and right ventricular hypertrophy (29, 61). Pharmacological inhibition of endothelin receptor type A using ambrisentan treatment has also been investigated (64). Ambrisentan reduced lung fibrin and collagen III deposition, but did not restore the impairment in alveolarization and angiogenesis, nor reduce the influx of macrophages and neutrophils; however, it did improve arterial medial wall thickness and right ventricular hypertrophy (64). Hyperoxia-induced lung injury in this rat BPD model has also been successfully treated by prophylactic administration of apelin, a vasodilator and angiogenic factor (21). Apelin treatment was shown to improve alveolarization and angiogenesis, reduce pulmonary fibrin deposition, inflammation, and septum thickness; apelin also reduced arteriolar wall thickness and right ventricular hypertrophy. Furthermore, treatment with adrenomedullin, another vasodilator that promotes angiogenesis, was shown to attenuate arrested lung angiogenesis and alveolar development, as well as prevent pulmonary hypertension in the rat model of BPD (59). A
different angiogenic factor that has been investigated in this rat model of BPD is
treatment with recombinant human erythropoietin (rhEPO) (44). Administration of
rhEPO was shown to improve the alveolar structure, enhance vascularity, and
also decrease fibrosis, but it did not have an effect on the increase in pulmonary
smooth muscle content (44).

The roles of various growth factors have also been researched with the use of
the hyperoxia-induced rat model of BPD. Intraperitoneal injection of recombinant
human KGF prevented neutrophil influx in broncholaveolar lavage and the
reduction of whole lung DNA content and cell proliferation rate in hyperoxia-
exposed rat pups (27). However, KGF did not appear to improve alveolarization,
indicating that its beneficial effects are largely anti-inflammatory. Using the rat
model of BPD, the critical role for platelet-derived growth factor (PDGF) in
secondary septation during normal postnatal lung development has also been
demonstrated, and how its expression is depressed and delayed in hyperoxia
exposure (9). Furthermore, insulin-like growth factor (IGF) gene expression
changes in normal lung development and in the rat model of BPD have been
documented, with altered IGF expression patterns correlating with the patchy
areas of parenchymal thickening induced by hyperoxia exposure (30). More
recently, the effect of connective tissue growth factor (CTGF), which plays a role
in tissue development and remodeling, has been assessed. Hyperoxia-exposure
up-regulates the expression of CTGF, therefore administration of a CTGF-
neutralizing antibody was able to protect alveolarization and vascular
development, as well as pulmonary hypertension in the rat BPD model (2). More recently other experimental techniques in the rat BPD model, such as the use of siRNA to inhibit expression of important lung development factors, have helped identify possible targets for lung repair in BPD (51, 60, 70).

Several studies have also focused on the inflammation and oxidative stress factors associated with the hyperoxia-induced rat model of BPD. Pharmacological inhibition of glycogen synthase kinase (GSK)-3β, a key regulator of NF-κB activity and inflammatory response, has been shown to reduce hyperoxia-induced lung inflammation, improve alveolarization and angiogenesis, and decrease pulmonary vascular remodeling and pulmonary hypertension (33). Treatment with pentoxifylline (PTX) has also shown beneficial effects in the rat model of BPD: reduced lung edema, decreased macrophage infiltration, and increases in the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase were reported (3). Also, PTX treatment increased both gene and protein expression of VEGF and improved pulmonary vascularization, but it did not shown beneficial effects in terms of improving alveolarization or attenuating pulmonary fibrosis (3). Administration of curcumin, a potent anti-inflammatory and antioxidant agent, in the rat model of BPD has been shown to inhibit oxidative stress and also effectively blocked activation of transforming growth factor beta (TGF-β) and subsequent hyperoxia-induced lung injury (47, 48). More recently the effects of resveratrol, which exhibit anti-inflammatory and antioxidant activities, has been assessed in the rat BPD model (43). Administration of resveratrol was shown to reduce pulmonary smooth
muscle content, decrease NO and tumor necrosis factor alpha levels, as well as increase the levels of the antioxidant enzymes superoxide dismutase and glutathione. Treatment with gadolinium chloride, which acts on reducing the lung macrophage content, has been shown to prevent right ventricular hypertrophy and pulmonary vessel smooth muscle hyperplasia in response to hyperoxia-induced lung injury; however, treatment did not have any beneficial effects on the morphological changes within the lung parenchyma (34). Interestingly, in another study that inhibited the hyperoxia-induced lung neutrophil influx with a CXC chemokine receptor-2 antagonist, increased alveolar formation was observed (72).

The use of the rat BPD model as a pre-clinical model to test various therapeutic strategies has proven to be a very effective tool. Recent studies using this model have highlighted its importance in investigations of cell-based therapies to prevent or repair lung injury in BPD (1, 5, 11-13, 45, 62, 67, 73, 74). Animal and human studies suggest that damage or depletion of stem/progenitor cells in the developing lung likely contributes to the pathogenesis of BPD. This formed the rationale for stem/progenitor cell supplementation for the prevention or repair of lung injury. Observations using the rat model of experimental BPD have shown significantly reduced numbers of circulating and resident mesenchymal stromal cells (MSCs) in the lung in response to hyperoxia-induced injury (62). Pre-clinical studies using the rat model provided proof of concept for the lung protective effect of bone marrow-derived and umbilical cord blood-derived MSCs.
Mechanistic studies in the rat model demonstrated the pleiotropic effects of MSCs, capable of attenuating various disease processes contributing to BPD including inflammation, fibrosis, and oxidative stress leading to improved alveolar and lung vascular growth and decreased pulmonary hypertension. Intraperitoneal administration of MSC-free conditioned media in this rat BPD model also demonstrated the paracrine mechanism of action of MSCs (11-13, 45, 62, 67). Further pre-clinical studies using this model have also evaluated the optimal route of administration, dose of MSCs, and the timing of the dose (11-13). The long-term safety of MSC administration has also been highlighted using the rat model of BPD; studies demonstrate no adverse effects at both 2.5 and 6 months of age in the rat (1, 45). This has allowed the design of a first-in-human pilot study (10). Based on the promising evidence from these pre-clinical studies in experimental rat BPD models, phase I and II clinical trials are currently underway in preterm infants.

The abovementioned studies highlight the usefulness of this small animal model in not only driving progress in our understanding of disease mechanisms, but also paving the way for new treatments into the clinic.

**Conclusions**

Animal models that reliably reproduce the BPD-like lung injury observed in preterm infants are imperative to improve the outcome of extreme prematurity and may also lead to benefits for pediatric and adult lung disease. Due to its
many advantages, the rat model of experimental BPD, which mainly employs hyperoxia-exposure during the saccular to alveolar stages of lung development, is one of the commonly used small-animal models. Not only has this model provided a further understanding of the effects of hyperoxia-exposure on the developing lung, but it has also aided in finding novel therapeutic opportunities for BPD. Technological progress will allow the generation of transgenic rats and the development of *in vitro* models mimicking alveolar and vascular growth (32). Used in combination, these novel tools will further accelerate knowledge on lung development, injury and repair that will ultimately benefit patients.
Table 1. Overview of studies utilizing the rat model of experimental BPD

<table>
<thead>
<tr>
<th>Experimental hyperoxia-exposure model</th>
<th>Dam rotation used?</th>
<th>Investigations and Outcomes</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 95% O₂: birth to 14d, 95% O₂: birth to 7d, followed by 60% O₂: 7-14d, or 60% O₂: birth to 14d | Yes, every 24h | Reduced survival  
Inhibited lung DNA synthesis  
Patchy areas of parenchymal thickening with increased immunoreactive IGF-1 and type 1 receptor | (30) |
| 60% O₂: birth to 14d | Yes, every 24h | Increased mRNA for PDGF-B, -βR and –αR  
Reduced and delayed PDGF-A, -B, and –αR immunoreactivities | (9) |
| 60% O₂: birth to 14d | Yes, every 24h | Alveolar simplification  
Increased pulmonary hypertension  
Increased whole lung content of the neutrophil chemoattractant CINC-1 up to 7d and increased lung neutrophil content up to 4d  
Accumulation of pulmonary macrophages  
Treatment with a CXC chemokine receptor-2 antagonist inhibited lung neutrophil influx and improved lung histology  
Treatment with a 21-aminosteroid antioxidant attenuated macrophage accumulation and pulmonary hypertension  
Treatment with gadolinium chloride reduced lung macrophages, prevented pulmonary hypertension and pulmonary vessel smooth muscle hyperplasia but did not improve lung histology | (34, 72) |
| 95% O₂: birth to 14d | Yes, every 48h | Reduced survival and impaired exercise capacity  
Alveolar simplification: larger and fewer alveoli  
Increased lung fibrosis and inflammation  
Reduced number of circulating and resident MSCs  
Decreased lung capillary density  
Increased pulmonary hypertension  
Decreased lung VEGF and VEGFR-2 expression  
Decreased plasma concentrations of NO precursors  
Decreased expression of activated lung Akt  
Adenovirus-mediated VEGF gene therapy improved survival and | (4, 12, 13, 36, 45, 58, 59, 61, 62, 67) |
| 75% O₂: 2-14d, followed by room air to 22d | Not reported | Lung capillary formation, preserved alveolar development  
Sildenafil treatment preserved alveolar growth and lung angiogenesis, decreased pulmonary hypertension  
Administration of MSCs, PCs, or cell-free conditioned media improved survival and exercise capacity, preserved alveolar and vascular development, attenuated lung fibrosis and inflammation, and reduced pulmonary hypertension  
Supplementation with L-citrulline improved plasma concentrations of NO precursors and prevented alveolar simplification, lung vascular growth and pulmonary hypertension  
Adenovirus-mediated Akt gene therapy increased lung Akt concentrations, preserved alveolar growth, attenuated lung apoptosis, and reduced pulmonary hypertension  
Adrenomedullin administration attenuated arrested lung vascular and alveolar development, decreased right ventricular hypertrophy and pulmonary artery medial wall thickness |
| --- | --- | --- |
| 100% O₂: birth to 10d, or 100% O₂: birth to 9d, followed by room air to 18d | Yes, 24h in O₂ followed by 48h in room air | Alveolar simplification: increased mean linear intercept and septal thickness  
Increased pulmonary fibrin and collagen III deposition and pulmonary arteriolar medial wall thickness, lung edema, reduced pulmonary vessel density, inflammatory cell influx, and right ventricular hypertrophy  
Sildenafil treatment improved alveolarization and angiogenesis, attenuated increased fibrin deposition, reduced influx of inflammatory cells, improved right ventricular hypertrophy  
Picolamilast treatment reduced fibrin deposition, decreased septal thickness, decreased right ventricular hypertrophy, reduced arteriolar wall thickness, improved right ventricular function  
Ambrisentan treatment improved survival, reduced lung fibrin and collagen III deposition, arterial medial wall thickness and right ventricular hypertrophy |
<table>
<thead>
<tr>
<th>Study Condition</th>
<th>Experimental Details</th>
<th>Outcomes</th>
</tr>
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<tbody>
<tr>
<td>100% O₂: preterm birth (at ~E21.5d) to 10d, or 100% O₂: preterm birth (at ~E21.5d) to death (survival experiments)</td>
<td>Yes, 24h in O₂ followed by 48h in room air</td>
<td>Apelin treatment improved alveolarization and angiogenesis, reduced pulmonary fibrin deposition, decreased inflammation, septal thickness, arteriolar wall thickness, and right ventricular hypertrophy.</td>
</tr>
</tbody>
</table>
| >95% O₂: birth to 10d | Yes, every 24h | Alveolar simplification: increased septal thickness, increased mean linear intercept and reduced alveolar surface area. Increased pulmonary fibrin deposition and macrophage influx. Increased pulmonary inflammation. Upregulation of CINC-1, MCP-1, amphiregulin, PAI-1, SLPI, MMP12, p21, metallothionein, and heme oxygenase. Downregulation of FGFR4, and VEGFR-2. Increased protein in BAL fluid. Pentoxifylline treatment reduced fibrin deposition and expression of MCP-1, reduced protein in BAL fluid. Inhaled NO therapy improved survival, improved alveolarization, reduced fibrin deposition, reduced influx of leukocytes, induced down-regulation of pro-inflammatory genes. 
(56, 57, 66) |
<p>| 90% O₂: birth to 14d | Not reported | Alveolar simplification: increased mean linear intercept, reduced secondary septal count. Reduced vascular density, activation of β-catenin signaling, pulmonary hypertension. Increased GSK-3β phosphorylation. Increased inflammation, pulmonary vascular remodeling, and pulmonary hypertension. Administration of CTGF neutralizing antibody prevented β-catenin signaling activation, improved alveolarization and vascular development, attenuated pulmonary hypertension. Pharmacological inhibition of GSK-3β decreased its phosphorylation, decreased MCP-1 expression and lung damage. (2, 33) |</p>
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<th>O2 Concentration</th>
<th>Time</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% O2: 1-14d</td>
<td>Not reported</td>
<td>Alveolar simplification: increased alveolar size, reduced mean linear intercept, increased septal thickness, reduced secondary crests. Reduced VEGF gene expression and lung vessel density.</td>
<td>(29)</td>
</tr>
<tr>
<td>&gt;95% O2: within 12h of birth to 7d, followed by 60% O2: 7-28d</td>
<td>Yes, every 24h</td>
<td>Growth restriction up to 21d. Alveolar simplification: large airspaces, fewer secondary septa, thick interstitium. Increased fibrosis and alveolar cell apoptosis. Increased inflammatory cell recruitment. Increased CTGF mRNA expression and protein up to 14d. CTGF localized to fibroblasts. Increased lung collagen levels from 21-28d.</td>
<td>(14)</td>
</tr>
</tbody>
</table>

Abbreviations: BAL, bronchoalveolar lavage; bFGF, basic fibroblast growth factor; CINC-1, cytokine-induced neutrophil chemoattractant-1; CTGF, connective tissue growth factor; d, days; E, embryonic; FGFR4, fibroblast growth factor receptor-4; GSK-3β, glycogen synthase kinase-3β; h, hours; IGF-1, insulin-like growth factor-1; KGF, keratinocyte growth factor; MCP-1, monocyte chemotactic protein-1; MMP12, matrix metalloproteinase-12; MSCs, mesenchymal stem cells; NO, nitric oxide; O2, oxygen; PAI-1, plasminogen activator inhibitor-1; PCs, perivascular cells; PDGF, platelet-derived growth factor; SLPI, secretory leukocyte protease inhibitor; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2.
<table>
<thead>
<tr>
<th>Stage of lung development</th>
<th>Rats (days)</th>
<th>Human (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation Length</td>
<td>Approx. 22 days</td>
<td>38-40 weeks</td>
</tr>
<tr>
<td>Embryonic</td>
<td>0 – 13</td>
<td>0 – 7</td>
</tr>
<tr>
<td>Pseudoglandular</td>
<td>13 – 18</td>
<td>5 – 16/17</td>
</tr>
<tr>
<td>Canalicular</td>
<td>18 – 20</td>
<td>16/17 – 24/26</td>
</tr>
<tr>
<td>Saccular</td>
<td>20 – 5d postnatal</td>
<td>24 – term</td>
</tr>
<tr>
<td>Alveolar</td>
<td>5 – 30d postnatal</td>
<td>36 – early childhood</td>
</tr>
<tr>
<td>Experimental hyperoxia-exposure model</td>
<td>Rat strain</td>
<td>Survival rate</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>60% O₂, 1-14d</td>
<td>Sprague Dawley</td>
<td>100% at 14d</td>
</tr>
<tr>
<td>&gt;95% O₂, birth to 8d followed by room air to 60d</td>
<td>Sprague Dawley</td>
<td>97% at 8d</td>
</tr>
<tr>
<td>95% O₂, 3-10d followed by room air 24d</td>
<td>Sprague Dawley</td>
<td>92% at 10d, 45% of survivors at 10d survived to 24d</td>
</tr>
<tr>
<td>85% O₂, birth to 7d followed by room air to 14d</td>
<td>Sprague Dawley</td>
<td>~90% at 7d, 58% at 14d</td>
</tr>
<tr>
<td>(a) 85% O₂, birth to 7d, or (b) 85% O₂, birth to 14d</td>
<td>Sprague Dawley</td>
<td>(a) 85% at 7d (b) 77% at 14d</td>
</tr>
<tr>
<td>90% O₂, birth to 14d followed by room air to 70d</td>
<td>Sprague Dawley</td>
<td>82% at 14d, 100% of survivors at 14d survived to 70d</td>
</tr>
<tr>
<td>100% O₂, preterm birth (at ~E21.5d) to 10d, or 100% O₂, preterm birth (at ~E21.5d) to death (survival experiments)</td>
<td>Wistar</td>
<td>77% at 10d 15% at 14d</td>
</tr>
<tr>
<td>(a) 100% O₂, birth to 10d, or (b) 100% O₂, birth to 9d followed by room air to either 18d or 51d</td>
<td>Wistar</td>
<td>(a) 77% at 10d (b) 73% at 9d, 80% of survivors at 9d survived to 18d, &gt;95% of survivors at 18d survived to 51d</td>
</tr>
<tr>
<td>100% O₂, preterm birth (at ~E21.5d) to 10d, or 100% O₂, preterm birth (at ~E21.5d) to death (survival experiments)</td>
<td>Wistar</td>
<td>75% at 10d 5% at 12d</td>
</tr>
<tr>
<td>100% O₂, preterm birth (at ~E21.5d) to 10d, or 100% O₂, preterm birth (at ~E21.5d) to death (survival experiments)</td>
<td>Wistar</td>
<td>75% at 10d 25% at 12d 5% at 14d</td>
</tr>
<tr>
<td>95% O₂, birth to 14d followed by room air to 21d</td>
<td>Sprague Dawley</td>
<td>75% at 14d, 100% of survivors at 14d survived to 21d</td>
</tr>
<tr>
<td>90% O₂, birth to 14d followed by 60% O₂, 14-21d</td>
<td>Sprague Dawley</td>
<td>71% at 21d</td>
</tr>
<tr>
<td>(a) 100% O₂, birth to 10d, or (b) 100% O₂, birth to 9d followed by room air to 18d</td>
<td>Wistar</td>
<td>(a) 70% at 10d (b) 70% at 9d, 80% of survivors at 9d survived to 18d</td>
</tr>
<tr>
<td>90% O₂, 3-13d</td>
<td>Wistar</td>
<td>62% at 13d</td>
</tr>
<tr>
<td>Condition</td>
<td>Species</td>
<td>Survival at 14d</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>100% O₂, birth to 10d, or 100% O₂, birth to 9d followed by room air to 18d</td>
<td>Wistar</td>
<td>(a) 61% at 10d, (b) 80% at 9d, 80% of survivors at 9d survived to 18d</td>
</tr>
<tr>
<td>95% O₂, birth to 14d</td>
<td>Sprague Dawley</td>
<td>55% at 14d</td>
</tr>
<tr>
<td>95% O₂, birth to 14d followed by room air to 21d</td>
<td>Sprague Dawley</td>
<td>50% at 14d, 100% of survivors at 14d survived to 21d</td>
</tr>
<tr>
<td>100% O₂, birth to 10d, or 100% O₂, birth to 9d followed by room air to 18d</td>
<td>Wistar</td>
<td>(a) 40% at 10d, (b) 60% at 9d, 100% of survivors at 9d survived to 18d</td>
</tr>
<tr>
<td>95% O₂, birth to 14d, or 95% O₂, birth to 7d followed by 60% O₂, 7-14d, or 60% O₂, birth to 14d</td>
<td>Sprague Dawley</td>
<td>(a) 40% at 14d, (b) 56% at 14d, (c) 81% at 14d</td>
</tr>
<tr>
<td>&gt;95% O₂, birth to 10d</td>
<td>Sprague Dawley</td>
<td>33% at 10d</td>
</tr>
<tr>
<td>&gt;95% O₂, preterm birth (at ~E21d) to 14d</td>
<td>Sprague Dawley</td>
<td>33% at 14d</td>
</tr>
<tr>
<td>&gt;95% O₂, birth to 14d</td>
<td>Sprague Dawley</td>
<td>31% at 14d</td>
</tr>
</tbody>
</table>
**Figure 1. Stages and gestational ages of lung development in humans and rats**

Schematic depicting stages of lung development in the human and the rat. Preterm infants at risk of developing BPD are born during the late-canalicular to early-saccular phase of lung development. The neonatal rat pup is born during the saccular phase of lung development. The alveolar phase occurs entirely postnatally in rats. Common hyperoxia-exposure timeframes in the rat model of BPD commence in the saccular phase and continue into the alveolar phase. A, alveolar; C, canalicular.
References


64. **Wagenaar GT, Laghmani el H, de Visser YP, Sengers RM, Steendijk P, Baelde HJ, and Walther FJ.** Ambrisentan reduces pulmonary arterial hypertension but does not stimulate alveolar and vascular development in


70. **Xu D, Perez RE, Rezaiekhaligh MH, Bourdi M, and Truog WE.** Knockdown of ERp57 increases BiP/GRP78 induction and protects against


Human

Birth of extremely preterm infants at risk for BPD

Embryonic

Pseudoglandular

Canalicular

Saccular

Alveolar

Rat

Common timeframes of hyperoxia exposure in the rat BPD model

Embryonic

Pseudoglandular

C

95% O₂

P7

95% O₂

P14

60% O₂

P7

95% O₂

P14/P28

Days of Gestation

Weeks of Gestation

Postnatal Days

term

0 10 20 24 28 30

term (38-40)

0 12 14 16 18 20 24 26

term

12 14 16 18 20

95% O₂

60% O₂

P14/P28