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Animal models of bronchopulmonary dysplasia. The preterm and term rabbit models

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D’Angio CT, Ryan RM. Animal models of bronchopulmonary dysplasia. The preterm and term rabbit models. Am J Physiol Lung Cell Mol Physiol 307: L959 –L969, 2014. First published October 17, 2014; doi:10.1152/ajplung.00228.2014.—Bronchopulmonary dysplasia (BPD) is an important lung developmental pathophysiology that affects many premature infants each year. Newborn animal models employing both premature and term animals have been used over the years to study various components of BPD. This review describes some of the neonatal rabbit studies that have contributed to the understanding of BPD, including those using term newborn hyperoxia exposure models, premature hyperoxia models, and a term newborn hyperoxia model with recovery in moderate hyperoxia, all designed to emulate aspects of BPD in human infants. Some investigators perturbed these models to include exposure to neonatal infection/inflammation or postnatal malnutrition. The similarities to lung injury in human premature infants include an acute inflammatory response with the production of cytokines, chemokines, and growth factors that have been implicated in human disease, abnormal pulmonary function, disordered lung architecture, and alveolar simplification, development of fibrosis, and abnormal vascular growth factor expression. Neonatal rabbit models have the drawback of limited access to reagents as well as the lack of readily available transgenic models but, unlike smaller rodent models, are able to be manipulated easily and are significantly less expensive than larger animal models.

bronchopulmonary dysplasia; hyperoxia; newborn; premature; rabbit

HYPEROXIA EXPOSURE HAS BEEN used in a number of animal models to recapitulate the inflammation, fibrosis, and developmental arrest seen during the development of human bronchopulmonary dysplasia (BPD) (13, 39, 113). Newborn rabbits have been used in hyperoxia research since the mid-20th century (90, 103). Rabbits have potential specific advantages as a model of newborn lung injury, including developmental similarities to the human newborn, the long-term viability of preterm animals, a size as newborns large enough to permit intensive instrumentation, and relatively low cost. This article will review the use of hyperoxia and other perturbations, such as malnutrition and chorioamnionitis, to reproduce features consistent with human BPD in the term and premature newborn rabbit (Table 1). Studies over the last quarter century have revealed similarities of the responses in these rabbit models to those of human newborns in areas including the acute inflammatory response, cytokine and growth factor production, pulmonary function, lung architecture, and the development of fibrosis (5, 21, 25, 29). The relative advantages and disadvantages of rabbit models, compared with other animals, will also be discussed.

Newborn Rabbit Models of Hyperoxia

Pulmonary oxygen toxicity has been recognized since the time of Lavoisier (68). By the 1960s, investigators had found that neonatal animals of many species, including rabbits, were more resistant to oxygen toxicity than adult animals of the same species (90, 103). Ogawa and Saito (90) reported in 1961 that premature rabbit kits were less resistant to hyperoxia exposure than term rabbit kits. Shanklin (103) noted that the severity of lung injury in a vagotomized newborn rabbit model increased with increasing fractional inspired oxygen concentration (P₂O), with lung injury in P₂O, 0.60 midway between that of P₂O, 0.21 and 1.0. By the 1970s, investigators interested in nutritional research had developed longer-term neonatal rabbit models that could be maintained for up to 10 days by using artificial feeding formulas (6).

The early work in animal hyperoxia exposure suggested that hyperoxia exposure in the newborn rabbit might be a model for recapitulating the role of oxidant stress in the development of...
Table 1. Pulmonary findings in rabbit models of bronchopulmonary dysplasia

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SP, surfactant protein; BAL, bronchoalveolar lavage; SOD, superoxide dismutase; PMN, polymorphonuclear cells; TIMP, tissue inhibitor of metalloproteinases.

BPD in human infants. In a comprehensive study of pulmonary oxygen toxicity reported in 1978, Frank and colleagues (40) compared survival and lung injury between adult and neonatal animals of five species (rat, mouse, rabbit, guinea pig, and hamster). Neonatal guinea pigs and hamsters had no survival advantage over adults. However, nearly all neonatal rats, mice, and rabbits survived 7 days of FIO2 >0.95, whereas nearly all adult animals died before 7 days. Among rabbits, 50% of adults had died by 3.2 days of FIO2 >0.95 and nearly all had died by 5 days, whereas 10/10 newborn rabbits survived a full 7 days. On histological examination, the lungs of the adult rabbits showed alveolar hemorrhage, edema of the interstitial, perivascular, and alveolar spaces and formation of “hyaline-like membranes.” Neonatal lungs, however, showed only occasional alveolar septal widening with mild interstitial edema and/or hypercellularity. The lung architecture among newborns was otherwise reportedly normal. Superoxide dismutase (SOD), catalase, and glutathione peroxidase activities were upregulated in the hyperoxia-exposed newborn rabbits, but not their adult counterparts (40). Similar upregulation of antioxidant enzymes was also seen in the newborn rat and mouse (but not in the guinea pig or hamster), a phenomenon subsequently confirmed by others (15). Later investigations have shown that the relative hyperoxia resistance displayed by newborn rabbits is diminished even if exposure begins 7 days of age and is associated with a decreased ability to upregulate antioxidant enzymes and decreased pulmonary eicosanoids compared with the immediate newborn (47, 48).

Other experiments found that physiological changes occur early during neonatal hyperoxia exposure and described more significant histological changes than those reported by Frank and colleagues (40). Wender and colleagues (122) delivered rabbit kits by Caesarean section at 1 day before term (31 days in the rabbit) and exposed the animals to air or FIO2 1.0 for up to 72 h. Kits were fed an artificial formula once daily by gavage. The lungs of hyperoxia-exposed animals displayed focal areas of atelectasis flanked by areas of hyperexpansion. The investigators calculated that only 43% of the lung appeared normal (i.e., without atelectasis or hyperexpansion), as opposed to 82% of the lung in air-exposed animals. The lungs of hyperoxia-exposed animals showed evidence of lipid peroxidation and upregulation of SOD, glutathione peroxidase, and glutathione reductase. The investigators reported that intramuscular injection of vitamin E reversed many of the findings seen in hyperoxia.

Ward and Roberts (119), in a series of experiments, exposed newborn rabbits to FIO2 >0.95 for 48–96 h. Kits were delivered by Caesarean section at term and were gavage fed once daily with a commercial rabbit formula. The investigators found modest but statistically significant disturbances of pressure-volume relationships and maximum lung distensibility in the lungs of newborn rabbits exposed to FIO2 >0.95 for 48 h, when these were compared with normoxia-exposed controls. Phospholipid content in lung lavage was also decreased following 48 or 96 h of hyperoxia, and phosphatidylethanolamine release and turnover were decreased in lung slices from animals exposed to 48 h of hyperoxia (118, 119). Morphometric analysis showed that the number of type I alveolar epithelial cells was decreased and alveolar hemorrhage was increased in the hyperoxia-exposed newborns (120). The administration of vitamin E subcutaneously during exposure prevented the changes in pressure-volume dynamics, phospholipid content, and morphology (120). Similar experiments by the same investigative group, using rabbits exposed to FIO2 >0.90 for 72 h and maintained on subcutaneously administered fluids, showed no effect of hyperoxia on lipid peroxidation but did show an
increased mortality (40%) compared with air-exposed controls (0%) (123).

Experiments performed by Horowitz and colleagues (50, 112), using a similar 96-h exposure to FiO$_2$ >0.95, found that mRNA expression for surfactant protein A (SP-A) and the tissue inhibitor of metalloproteinases (TIMP) was elevated in hyperoxia-exposed animals as opposed to air-exposed controls beginning at 24 h, and metallothionein mRNA expression was elevated at 96 h. Polak and colleagues (93) evaluated surfactant metabolism in lungs from newborn rabbits exposed to FiO$_2$ >0.95 for 72 h and found decreased disaturated phosphatidylcholine synthesis.

Sherman and colleagues (105) also used a 96-h hyperoxia-exposure model with term newborn rabbits but exposed animals to FiO$_2$ 0.21, 0.40, 0.80, or >0.95. Animals treated with FiO$_2$ 0.21 and 0.40, but not those treated with 0.80 and >0.95, showed a steady increase in macrophage yield from bronchoalveolar lavage (BAL) over the course of exposure. The decreased macrophage numbers in hyperoxic animals were shown to be the result of 12-fold lower rates of macrophage replication.

The 96-h hyperoxia exposure model has also been used to evaluate other potential therapeutic approaches to prevention of hyperoxic injury. Kertesz and colleagues (60) exposed 7-day-old rabbits to FiO$_2$ >0.95 to test the effectiveness of an infused leukotriene inhibitor but showed no difference between drug- and vehicle-exposed animals in measures of lung inflammation or edema.

Overall, the data suggest that newborn rabbits display a resistance to hyperoxic injury compared with adults, which wanes quickly following birth. Among newborn rabbits exposed to 48–96 h of hyperoxia, multiple physiological processes, including the surfactant system and control of inflammation and the extracellular matrix, are affected at a time before significant histological changes are always apparent (Fig. 1, Table 1). The models also confirm the feasibility of collecting BAL, performing morphometric analyses, collecting pulmonary function data, and testing interventions including infused agents in newborn rabbits.

**Term Newborn Hyperoxia**

- FiO$_2$ 0.90 – 1.0
- Birth 2 4 6 8 10 Days

**Premature Hyperoxia**

- FiO$_2$ 0.80 – 1.0
- GD 27-29 (Term = 31 days) Days

**Term Newborn Hyperoxia with Moderate Hyperoxia Recovery**

- FiO$_2$ >0.95 to 0.60
- Birth 2 4 6 8 10 22 36 Days

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**Term Newborn Rabbit Models with Extended Hyperoxia Exposure**

In an injury model that went beyond 96 h of hyperoxia, Sherman and Condiotti (104) exposed litters of newborn rabbits to either air or FiO$_2$ >0.95 for up to 7 days (Fig. 1). Mothers were placed into the exposure chamber for feeding for one 6-h period daily. Mortality among newborns was 3% at 96 h and 16% at 7 days of hyperoxia, compared with 1% mortality over 7 days of air exposure. The investigators found that, by 96 h, one-third of the hyperoxia-exposed animals were not growing and that the mean weights of hyperoxia-exposed animals were lower than those of air-exposed controls, consistent with other neonatal hyperoxia models. Histological evaluation showed that hyperoxia-exposed animals had increased numbers of polymorphonuclear cells (PMN) in their airways at 96 h and 7 days. Alveolar macrophages gathered by BAL from the animals had an increased ability to produce superoxide at 48 h of hyperoxia, but this capacity fell by 96 h. Animals exposed to hyperoxia for 96 h or more also showed impaired killing of inhaled staphylococci. These data suggest a disordered inflammatory cell response early in hyperoxia exposure.

In a preliminary study, Dobkin and colleagues (37) exposed newborn rabbits to FiO$_2$ 0.55–0.65 for up to 10 days. Compared with air-exposed animals, hyperoxia-exposed animals experienced an earlier (days 1–4) and higher peak of transforming growth factor (TGF)-β protein content in whole lung homogenate, which may be associated with stimulating the development of fibrosis.

Ahmed and colleagues (1) reported administering a human extracellular SOD cDNA by nebulization to newborn rabbits exposed to air or FiO$_2$ 0.95 (with 5% CO$_2$) for 3 or 7 days. Lungs from hyperoxia-exposed rabbits had increased immunostaining for nuclear factor-κB (NF-κB) at 7 days, compared with air-exposed control lungs. Transient transfection with SOD maintained the availability of nitric oxide and decreased NF-κB activity in the hyperoxia lungs, indicating a possible protective effect against oxidative stress (1).
Overall, the results of longer hyperoxia exposures in newborn indicate that such exposures are feasible with acceptable mortality. The inflammatory response seen early in hyperoxia exposure continues to progress, and the effect of hyperoxia on weight gain becomes apparent (Table 1). Abnormalities in bacterial killing and TGF-β, NF-κB, and nitric oxide production are also detected during these exposures. Exposure to moderate hyperoxia (FiO₂ 0.55–0.65) as well as to higher FiO₂ also produces effects, at least on potential profibrotic pathways. The experiments also demonstrate the feasibility of administering nebulized therapy in the neonatal rabbit model.

**Term Newborn Rabbit Model Involving Hyperoxia and Recovery**

In a series of experiments, D’Angio, Ryan, and colleagues (20, 26, 27, 72, 110, 121) developed a model of acute severe hyperoxia, followed by recovery in moderate hyperoxia, to mimic the acute injury followed by recovery in the setting of continued oxidative stress that is experienced by some human infants developing BPD (Fig. 1). Pregnant rabbits delivered vaginally at term and the litter was placed in a separate exposure chamber. Litters were exposed as a whole to either humidified oxygen or humidified room air. Among hyperoxia-exposed litters, the FiO₂ was maintained at >0.95 for 8–9 days and then reduced to 0.60 for a total exposure of up to 36 days. Mothers were placed in the exposure chamber once daily for 30 min to feed the litters, with the chamber returned to the exposure condition (air or hyperoxia) during feeding. Animals exposed beyond 22 days were weaned at 3–4 wk of age.

**Physiological and histological findings.** In initial experiments, litters were exposed to FiO₂ >0.95 until 50% of animals had died or had been killed electively to avoid further suffering (LD₅₀). The time to LD₅₀ ranged from 7–11 days, with a median of 9 days, which was used to establish the timing of the switch to FiO₂ 0.60 (26). Rabbit kits exposed to hyperoxia gained weight poorly, with lower weights than controls by day 7 that persisted over 36 days of exposure. BAL protein was elevated in hyperoxia-exposed animals at the LD₅₀ time point (7–11 days) and at 22 days (26). Hyperoxia-exposed animals developed mild alveolar edema by 4 days of exposure and significant alveolitis, with PMN and mononuclear cell infiltrates, septal edema and thickening, hyaline membranes, and type II alveolar epithelial cell hyperplasia by 10 days (26, 27). Type II cell proliferation was increased at 8–12 days in hyperoxia animals (20). The alveolar architecture appeared simplified at 4 and 10 days of hyperoxia exposure (26, 27). By 14–22 days of total exposure (that is, 5–14 days of recovery in FiO₂ 0.60), hyperoxia-exposed animals showed resolving septal edema and persistent mild alveolitis, with a predominance of intra-alveolar macrophages. Alveolar septal thickness and septal collagen content were elevated at 22 days (26). By 36 days of hyperoxia, patchy mild septal thickening persisted, but cellular infiltrates had largely resolved and overall septal thickness no longer differed from air-exposed animals (26).

**Chemokines and cytokines.** Proinflammatory cytokine mRNA expression for monocyte chemoattractant protein (MCP)-1 (CCL2) (localized to type II cells), interleukin (IL)-1β (localized to mononuclear cells), IL-8 (CXCL8) (localized to PMN), and growth-regulated protein (Gro) B (CXCL2) mRNA expression was elevated in hyperoxia-exposed animals, peaking between 6 and 10 days of exposure (27, 110). IL-8 protein in lung lavage fluid was elevated in a similar pattern. Tumor necrosis factor (TNF)-α mRNA was not detected in lung tissue and TNF bioactivity could not be detected in lung lavage fluid (110). Cytokine expression decreased rapidly once FiO₂ was decreased to 0.60.

**Growth factors.** Keratinocyte growth factor (KGF) mRNA expression was noted to be elevated at 6 days in hyperoxia-exposed animals (20). TGF-α protein in BAL was increased at 6–8 days of hyperoxia, whereas the appearance of lower-molecular-weight, mature forms of TGF-α was delayed, rising above control levels at 22 and 36 days (114). BAL concentrations of parathyroid hormone-related protein (PTHrP), a putative regulator of type II cells, were increased during acute hyperoxia, fell at the LD₅₀ time point and rebounded during recovery, and was inversely correlated with the number of type II cells expressing proliferating cell nuclear antigen (PCNA) (46). Vascular endothelial growth factor (VEGF)-1 mRNA abundance (expressed primarily by type II cells) decreased during exposure to FiO₂ >0.95, reaching a nadir at 9 days, and returned to normal during recovery in FiO₂ 0.60 (72). VEGF-1 protein immunostaining followed a similar pattern, whereas VEGF-1 protein in lung lavage also decreased during FiO₂ >0.95, but became elevated above room air levels during recovery in FiO₂ 0.60. The relative abundances of the splice variants of VEGF-1 were also altered by exposure to FiO₂ >0.95, with a marked relative decrease of the 189-amino-acid variant, which is primarily bound to the extracellular matrix (121).

**Surfactant and surfactant proteins.** In keeping with the findings of others, surfactant function was diminished at the LD₅₀ (26). Surfactant protein (SP)-A mRNA expression was elevated in LD₅₀ animals compared with controls and appeared to be localized in type II cells (26). SP-B and SP-C expression was unaffected.

Overall, the acute injury/recovery model reproduced the findings of other investigators regarding the inflammation, architectural changes, and surfactant system dysfunction accompanying acute hyperoxia (Table 1). The model revealed that the histological and physiological changes were closely associated with changes of proinflammatory cytokines and growth factors that are potential mediators of the hyperoxia effect. However, the model did not result in apparent long-term changes in alveolar size or structure. The changes seen during acute hyperoxia largely resolved in an atmosphere of moderate hyperoxia and the observed changes in growth factors such as VEGF-1 and PTHrP may facilitate the repair process. In addition to adding extensive characterization of hyperoxia-induced injury in the newborn rabbit, the injury/recovery model may provide a reasonable reproduction of the human experience of injury followed by recovery under continued oxidative stress.

**Premature Newborn Rabbit Models of Bronchopulmonary Dysplasia**

Early work in oxygen exposure showed that premature rabbits have increased sensitivity to hyperoxia, compared with term newborn rabbits (90). Frank and colleagues (42, 43) evaluated the development of the antioxidant systems during fetal life and found that in rabbits, as in several other species,
the antioxidant system develops quickly in concert with the surfactant system during the last 10–15% of pregnancy, with lung antioxidant levels in the fetal rabbit rising 110–200% in the 3–5 days before the end of a typical 31-day gestation. Lung structure matures from the early saccular to early to mid alveolar phase over the same period (39, 43, 67, 77). The same investigators exposed premature (delivered at 29 days of gestation) and term newborn rabbit kits to $F_{O_2} > 0.90$ for 48–72 h (44). Animals were fed formula daily by gavage. Term rabbits responded to hyperoxia with elevations in activities of SOD, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase, whereas premature rabbits did not show similar elevations. The premature rabbits displayed increases in BAL protein content and conjugated diene levels and more severe lung pathology, compared with term rabbits exposed to the same degree of hyperoxia. In subsequent experiments, the investigators found that subcutaneous administration of endotoxin at birth and 24 h to 29-day-gestation rabbits exposed to $F_{O_2}$ for 48 h improved SOD mRNA expression and activity, BAL protein content, and survival (91%), compared with hyperoxia alone (survival 76%) (107). Walther and colleagues (115) confirmed a similar lack of upregulation of catalase and SOD in 28- or 29-day-gestation rabbit kits exposed to 24 h of $F_{O_2}$ 1.0 and showed that administering surfactant liposomes containing CuZn-SOD and catalase increased lung SOD and catalase activity in both preterm and term rabbit kits. They also found that, despite the lack of increase in measured enzyme activity, both CuZn-SOD and catalase mRNA expression in 28-day-gestation rabbits was increased following 24 h of hyperoxia exposure (116).

Using a similar model, Bany-Mohammed and colleagues (9) exposed 29-day-gestation rabbit kits delivered by hysterotomy to either air or $F_{O_2}$ 1.0, providing nutrition by daily gavage feeding of formula. Animals received recombinant human erythropoietin or vehicle subcutaneously on days 0 and 2. Hyperoxia for 72–96 h increased BAL protein, decreased the ability of BAL to prevent lipid peroxidation, and caused alveolar thickening and alveolar proteinaceous exudate, compared with air-exposed controls. Erythropoietin administration decreased plasma iron and decreased iron saturation of transferrin and mitigated the BAL and histological findings. The same investigative group also tested the administration of intravenous transferrin in hyperoxic 29-day-gestation rabbits and showed similar improvements in BAL protein and evidence of lipid peroxidation at 2 to 4 days (102). Inhaled nitric oxide (14 ppm) was also found to mitigate the negative effects of a brief, 20-h period of $F_{O_2}$ 0.98 in 29-day-gestation kits on BAL surfactant composition and activity, surfactant protein B content, lipid peroxidation, and glutathione content (54).

Using a longer hyperoxia exposure of 11 days, Mascreatti and colleagues (77) assessed lung histology and morphology following premature birth in rabbits. Twenty-eight-day-gestation preterm rabbits were delivered by Caesarean section and placed in either air or $F_{O_2} > 95\%$. Animals were fed twice daily by gavage with a rabbit formula and treated with prophylactic vitamin K and antibiotics during exposure. Mortality was higher (89%) in hyperoxia-exposed animals than in air-exposed animals (69%). The lungs of hyperoxia-exposed animals showed a reduction in alveolar number and thickening of the interstitium with inflammatory cell infiltration. Morphometric analysis confirmed an increase in the mean linear intercept and a decrease in alveolar number. The number of collagen fibers was decreased and both collagen and elastic fibers were disorganized. Subsequent work by the same group compared 29-day-gestation rabbits exposed to $F_{O_2}$ 0.80 for 11 days to 28-day gestation rabbits exposed to $F_{O_2} > 0.95$ for the same time (75). Both hyperoxia groups showed impaired alveolar development and septal thickening and, in contrast to earlier work, elevated proportions of collagen and elastic fibers, compared with age-matched, air-exposed controls. Although the early survival was higher in the 29-day $F_{O_2}$ 0.80 group than the 28-day $F_{O_2} > 0.95$ group, mortality by day 11 approached 90% in both groups, compared with 40–70% in the air-exposed groups.

Richter and colleagues (95) have recently reported results of a 7-day hyperoxia exposure among preterm rabbits. Preterm kits were delivered at 28 days gestation by Caesarean section and term controls were allowed to deliver spontaneously at term. Preterm animals were exposed to either air or $F_{O_2} > 0.95$ for 7 days. They were fed twice daily by gavage with a rabbit formula and treated with vitamin K and antibiotics. Term controls remained in air with their mothers for the duration of the exposure. Survival to 7 days was 83% among air-exposed preterm rabbits and 56% among hyperoxia-exposed preterm rabbits (and 84% among term controls). The investigators performed extensive pulmonary function testing using whole body plethysmography and forced oscillation. Hyperoxia-exposed animals had multiple lung function abnormalities, including decreased minute volume, decreased total lung capacity, decreased static compliance and increased tissue elasticity, compared with air-exposed preterm rabbits (who were similar to term controls). In keeping with other models, histological and morphometric comparisons showed increased alveolar size and septal thickening with edema and inflammatory cell infiltration in the hyperoxia-exposed animals. Hyperoxia-exposed animals also had higher numbers of proliferating cells in the lung. Sirius red staining showed increased collagen deposition in hyperoxia-exposed lungs.

Consistent, controlled morphometric measurements such as those described in the aforementioned studies (75, 95) are particularly important to provide reproducible, easily comparable measures, since differing models of differing lung diseases may require the use of differing morphometric techniques (83, 89). For instance, Mühlfeld and Ochs (83), in a recent review of quantitative microscopy of the lung, note that the appropriate stereological techniques to assess changes in blood vessels differ from those appropriate to assessing emphysematous changes; indeed, techniques that measure alveolar size may not be appropriate for assessing alveolar septa. The appropriate tissue preparation and sectioning for each morphometric technique may differ, requiring separate fixation, processing, and sectioning for various measures (89).

In summary, the lungs of premature rabbits have many similarities to those of human premature infants. Premature rabbit models of hyperoxia exposure show that preterm rabbits display diminished antioxidant response, compared with term newborns, and develop lung pathology similar to that described in term rabbits. Although hyperoxia exposures as long as 11 days have been described, mortality appears unacceptably high with exposures beyond 7 days (Fig. 1). Work to date in premature rabbit models of hyperoxia shows the feasibility of moderately prolonged hyperoxia exposure and their suitability.
for extensive physiological and morphometric testing and intratracheal and intravenous drug administration, although less mechanistic work has been done in prematurity than in the term models. The models tend to require supportive measures, such as gavage feeding and the administration of vitamin K and antibiotics. Overall, however, premature rabbit models of hyperoxia are practical and may be more relevant to human BPD than term newborn rabbit models.

Other Newborn Rabbit Models

Mataloun and colleagues (78) have explored a model that combines the effects of malnutrition and hyperoxia in the preterm rabbit kit. Kits were delivered at 28 days gestation by Caesarean section and fed twice daily for 7 days by gavage with either a routine rabbit formula or a diet with 30% fewer calories than the routine diet. Malnutrition resulted in lower body weights, lower lung weights, lower lung water content, decreased alveolar numbers, decreased numbers of elastic fibers, and decreased lung collagen. Animals in each group were also exposed to either air or FiO₂ >0.95. Hyperoxia among control animals decreased alveolar number, elastic fibers, and lung collagen and produced septal thickening. Combined exposure to malnutrition and hyperoxia further reduced alveolar number, elastic fibers, and lung collagen. These data are in agreement with earlier reports of malnutrition exacerbating hyperoxic lung injury in newborn rats (41). Spontaneous feeding rabbit models of hyperoxia may need to be evaluated for a nutritional deprivation component of their effect, because animals might feed less well as their lung function worsened.

Newborn rabbits have also been used to assess the effect of chorioamnionitis on pulmonary development. Gras-Le Guen and colleagues (45) inoculated the uteruses of pregnant rabbits at gestational day 29 with Escherichia coli, treated the mothers with antibiotics beginning 8 h after inoculation, and allowed kits born spontaneously 60–84 h later to remain with their mothers for up to 15 days. Stillbirth and neonatal mortality were higher in E. coli-exposed pregnancies than in vehicle-treated pregnancies. Compared with kits from vehicle-injected controls, surviving E. coli-exposed kits had lower body weights and absolute lung volumes by days 8–15 after birth. Alveolarization in the E. coli-exposed kits was impaired. Vehicle- and E. coli-exposed kits showed no differences on measures of inflammation or apoptosis. The findings in this model are reminiscent of the alveolar simplification that has been described in the “new” BPD among humans (55, 56).

Both term and preterm rabbits (delivered between 27 and 29 days of gestation) have been used in short-term (~16 h) experiments involving mechanical ventilation. These models have tested the effects of the administration of surfactant (24, 30, 64, 86), of varying conventional mechanical ventilation and oxygen strategies (52), and of conventional vs. high-frequency oscillatory ventilation (88), as well as the effects of conventional ventilation on SP-A and KGF (36). With animal weights ranging from ~25 to 35 g at 27 days gestation, the preterm model allowed ventilation via an 18-gauge catheter inserted into the trachea (30, 88).

The effect of 14 days in a heliox (21% oxygen, 79% helium) environment has been tested in 4-day-old rabbit kits. No differences in weight, growth factors, tissue/organ weights, blood chemistries, or muscle enzyme activity were detected between heliox- and air-exposed animals (106). This finding has relevance for human infants treated with heliox to decrease gas viscosity in obstructive lung conditions.

Fetal rabbits have been used to study various aspects of lung development and conditions such as lung growth following tracheal occlusion (34, 42, 61). Instrumented pregnant rabbits have allowed study of the complex interactions between surfactant present in the amniotic fluid and vernix caseosa in the subsequent stimulation and maturation of intestinal enterocytes (85). Newborn rabbits have also been used to study other conditions and disorders relevant to the human newborn, such as meconium aspiration, methods of resuscitation, lung liquid clearance, and surgically created diaphragmatic hernia. (22, 38, 49, 124, 125), but these models are not directly relevant to BPD.

Overall, newborn rabbit models including malnutrition and infection stress the need to consider these factors in the pathogenesis of experimental BPD and point to potential mechanisms for human disease. Newborn rabbit models have also been shown to be versatile, allowing instrumentation and mechanical ventilation, as well as the study of multiple other perturbations.

Other Relevant Rabbit Models of Lung Disease

It would be difficult in the course of a single review to detail the many uses to which the rabbit has been put to study the pathogenesis of lung disease, but a few examples bear mention. In a study of juvenile, rather than newborn, animals, 8- to 10-wk-old tracheostomized, ventilated rabbits have been used as a model for perflurochemical-enhanced delivery of recombinant human SOD in acute lung injury (14).

Adult rabbits have been used particularly extensively to model human asthma, since they are phylogenetically more closely related to humans than are rodents, have lungs that are more anatomically similar to humans, share qualities such as IgE mediation of anaphylaxis and relative capsaicin unresponsiveness with humans, and can model both early- and late-phase airway responses (58, 59). Recent work in a rabbit asthma model has elucidated the role of the proinflammatory cytokine IL-13 in producing impaired endogenous glucocorticoid activity via the upregulation of 11β-hydroxysteroid dehydrogenase, showing the value of well-developed rabbit models for dissecting the pathogenesis of human asthma (57).

Adult rabbits also have been used for the study of innate immunity in the lungs in response to mechanical ventilation and/or sepsis (76), an example of the many adult rabbit models of acute lung injury, including some of the earliest groundbreaking work on transfusion-associated lung injury (71). Rabbit models have contributed to air pollution research. In a recent article, Miyata and colleagues (82) described the ability of lovastatin to counter the systemic and bone marrow inflammatory responses to ambient particulates, showing a delay in the release of PMN from bone marrow pools, a reduction in retention of PMN in the lung, and a reduction in plasma IL-6. In areas beyond the lung proper, rabbits have been used to model the pleural response to injury (66). In a recent study, Komissarov and colleagues (65) described the role of α-macroglobulin as a “molecular cage” for urokinase used in fibrinolytic therapy following experimental pleural injury with tetracycline in rabbits.
Table 2. Onset of alveolar stage of lung development in animals used in bronchopulmonary dysplasia models

<table>
<thead>
<tr>
<th>Species</th>
<th>Days of gestation</th>
<th>Percent of completed gestation</th>
</tr>
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<tbody>
<tr>
<td>Mouse (92, 109, 126)</td>
<td>PD 1–5</td>
<td>N/A</td>
</tr>
<tr>
<td>Rat (17, 92, 109, 126)</td>
<td>PD 1–7</td>
<td>N/A</td>
</tr>
<tr>
<td>Rabbit (62, 67, 92, 126)</td>
<td>28–30 days</td>
<td>90–99%</td>
</tr>
<tr>
<td>Sheep (4, 92, 126)</td>
<td>120 days</td>
<td>80%</td>
</tr>
<tr>
<td>Primate (baboon) (23, 92)</td>
<td>155–165 days</td>
<td>84–90%</td>
</tr>
<tr>
<td>Human (92, 126)</td>
<td>252 days</td>
<td>90%</td>
</tr>
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PD, postnatal day; N/A, not applicable.

Overall, the extensive use of the adult rabbit as a model for human disease and the increasingly mechanistic studies possible in the rabbit bode well for the extension of similar techniques into newborn rabbit models.

Rabbit Models in Context

Rabbits are born with lungs in the early to middle alveolar stage, similar to the lungs of newborn human infants, have mature surfactant and antioxidant systems, and have postnatal lung development similar to humans (39, 67) (Table 2). This is in contrast to mice and rats, which are born at term with lungs in the saccular stage, similar to premature human newborns, but which have mature surfactant and antioxidant systems and air exchange (39). Other animal models, such as the sheep and primates, are also born in the alveolar (rather than saccular) stage of development, but lack the small size and lower cost of rabbits and other small animal models (4, 23, 92, 126) (Table 2). Preterm rabbits at 28 or 29 days of gestation are in the early saccular phase of lung development and have immature surfactant and antioxidant systems, making them potentially valuable models of the premature human newborn, in that they possess both structural and functional similarities to the human (43, 77). Overall, further development of such a premature model may offer significant advantages over both term rabbit models and term rodent (rat, mouse) models of BPD.

Rabbits, rats, mice, and several other species, including sheep, pigs, and baboons, all develop similar inflammation and alveolar simplification following hyperoxia exposure (13, 32, 35, 94, 113) (Table 1). Some potential mechanisms for these effects, such as the elaboration of proinflammatory cytokines and chemokines, appear to be relatively similar across species ranging from mouse to rabbit to baboon to human, lending support to the hypothesis that these are common, central mediators of the response to hyperoxia (23, 27, 98, 110). Similarly, elevations in TGF-α and/or TGF-β have been reported in multiple animal models of newborn lung injury, including rabbits, rats, and lambs (12, 18, 53). Protective effects of the exogenous administration or overexpression of various forms of SOD similar to those seen in the rabbit have been observed in a number of newborn animal models of lung injury, including mice, sheep, and piglets (1, 2, 63, 81, 84, 91, 97, 115). Each these animal models may shed light on the protective effect reported in some studies of exogenous administration of SOD to human premature newborns (31, 33, 99, 100, 108). Other phenomena, however, such as VEGF expression and production, vary in direction and magnitude in response to hyperoxia across species from rodents to rabbits to humans (7, 8, 10, 11, 16, 28, 51, 69, 72–74, 80, 121). This led Buczyński and colleagues (16) in a recent review to propose that interspecies differences in lung development, as well as timing and methods of VEGF measurement, might be at the root of such disparities.

Recent reviews of animal models in various species, including humans and some of the rabbit models here described, have extensively explored recent progress in areas such as the mechanisms of acute lung injury in preterm fetuses and newborns (53), the mechanisms of derangement of late lung development and its relationship to BPD (70, 79), the use of large animal (sheep and baboon) models (3), the role of strain in the alveolus (96), the mechanism of hyperoxic injury in both animal models (16) and humans (101), and the role of stem cells in the pathogenesis of BPD (87). It is only through close evaluation of the similarities and differences among the various models and between the models and human disease that the most appropriate model for each aspect of the pathogenesis of BPD will become apparent.

Advantages, Disadvantages, and Conclusions

Neonatal rabbit models of hyperoxia exposure for varying periods in both term and preterm rabbits appear to recapitulate many of the patterns seen in human neonatal lung disease and bronchopulmonary dysplasia (Table 1, Fig. 1). These similarities include an acute inflammatory response with the production of cytokines, chemokines, and growth factors that have been implicated in human disease, abnormal pulmonary function, disordered lung architecture and alveolar simplification, development of fibrosis, and abnormal vascular growth factor expression (5, 21, 25, 29). In a model involving recovery after acute hyperoxia, many of these abnormalities improve during recovery from the acute injury, despite the continuing moderate hyperoxia, the sort of conditions an infant developing the “new” BPD might encounter (26, 55, 56). Models involving malnutrition and infection, conditions also likely to be experienced by human premature infants, also show abnormalities (45, 78). Malnutrition alone causes alveolar simplification and collagen and elastin abnormalities, reminiscent of the new BPD; hyperoxia exacerbates this process (78). Prenatal infection alone also produces alveolar simplification (45).

Specific advantages of rabbit models include their relatively brief course, compared with larger animals (Table 3). Rabbits

Table 3. Potential advantages and disadvantages of rabbit models of BPD

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Mimic many findings in human BPD</td>
<td>Term models do not reflect preterm human anatomical or functional immaturity</td>
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<tr>
<td>Term models mimic lung maturity of term human newborns</td>
<td>Lack of standardized model</td>
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<tr>
<td>Preterm models mimic lung maturity of premature human newborns</td>
<td>Fewer reagents and transgenic models</td>
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<td>Relatively brief time from newborn to adulthood</td>
<td>BPD, bronchopulmonary dysplasia,</td>
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<td>Once daily feeding behavior (term newborns)</td>
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reach adolescence by the close of exposure at 5–6 wk (although, unlike the human, their alveoli are still developing rapidly at that stage), allowing early evaluation of near-adult animals following a neonatal perturbation (67). Rabbit models are also cost effective compared with larger animal models, but large enough to permit perturbations, including nebulized treatments, mechanical ventilation, and pulmonary function testing, and easy isolation of individual cells, even among newborn or premature animals (1, 30, 36, 52, 64, 86, 88, 95). Neonatal rabbits weigh ~50 g, slightly above the size of an adult mouse, and even premature (27–29 days) rabbits have weights in the range of a mouse’s 30 g. The once-daily feeding behavior of term rabbits allows mothers to be placed in the exposure chamber for brief periods once daily, avoiding the need for gavage feeding or cross-fostering and rotating mothers to prevent oxygen toxicity to the mothers. Preterm rabbits appear to grow well on twice-daily gavage feedings, also minimizing the need for interrupting exposure and handling the animals. Rabbits are relatively easy to breed in the local vivarium, and the cost of maintaining a colony is not prohibitive.

The developmental stage of rabbits at birth is both an asset and a liability to the use of rabbits to model BPD. As noted previously, compared with species such as the mouse or rat, rabbits are more similar to humans at term, since both their alveolarization and surfactant and antioxidant systems have similar levels of maturity to the human. Although this is an advantage for modeling the term human newborn, BPD overwhelmingly occurs among premature infants, restricting the value of the term rabbit as a model. It is not clear, however, that term small rodent models, with discordant anatomic and functional maturation compared with the human, serve the purpose better. Preterm rabbits in late gestation (28–29 wk) are similar to premature human newborns in terms of maturity and appear capable both of surviving and of being instrumented, which make them perhaps better models for the human premature infant than either term rodents or term rabbits. The addition to the premature model of recovery in moderate hyperoxia may further improve the fidelity to the human experience. Since rabbits are used less commonly than rats or mice for modeling BPD, no truly standard rabbit model has emerged. Described models vary by the type of exposure, length of exposure, gestational age of the animals, mode of delivery, type of feeding, and ancillary components such as humidity and the use of vitamins and antibiotics. Hyperoxia is the most common reported exposure, but investigators have used FiO2 ranging from 0.55 to 1.0 for the acute injury portion of their models (37, 75, 122). Acute hyperoxia exposures have varied in length from short-term models under 24 h to up to 11 days of exposure, with exposures from 7 to 11 days producing similar results (26, 52, 54, 77, 104, 112). Bacterial exposure and malnutrition as models have been explored infrequently, although the findings from those experiments suggest that investigators should be sensitive to the effects of nutrition and infection on their models and that these factors may be important in the human newborn (45, 78).

Rabbit models, but survival has been low in some investigators’ hands. A term newborn model of acute hyperoxia exposure and recovery has been described, but, as noted, it may not faithfully reflect the developmental state of the human premature newborn (26). Furthermore, the term newborn recovery model has not been critically evaluated for morphometric similarities to or differences from the human infant recovering from BPD. Premature rabbit kits have been maintained briefly on mechanical ventilation, showing evidence that it is possible to manipulate the model in this manner (30, 36, 52, 64, 86, 88). However, there are no published longer-term exposures to both hyperoxia and mechanical ventilation that would mimic more fully the exposures faced by the human premature infant.

A notable disadvantage of newborn rabbit models of lung injury is the relative paucity of reagents and transgenic models specific to the rabbit, particularly as the tools available to study the mouse have proliferated during the early 21st century (58). siRNA technology has been used in rabbits (117), rabbit-specific qPCR primers are now commercially available and there are now companies devoted to producing antibodies specific to rabbit proteins, but these resources remain rarer than those for humans or mice. Transgenic rabbit models also remain rare (58).

An appropriate place for rabbit models of BPD among other models of neonatal lung injury might be for the relatively rapid and inexpensive initial testing of hypotheses that require instrumentation or other active intervention in preterm or term animals. Promising hypotheses could then undergo further mechanistic testing in mouse models and/or be moved into larger animal models as a step toward human interventions.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

C.T.D. prepared figures; C.T.D. drafted manuscript; C.T.D. and R.M.R. edited and revised manuscript; C.T.D. and R.M.R. approved final version of manuscript.

REFERENCES


