Effects of Erythropoietin on Hyperoxic Lung Injury in Neonatal Rats

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ABSTRACT

Pulmonary oxygen toxicity is believed to play a prominent role in the lung injury that leads to the development of bronchopulmonary dysplasia (BPD). To determine whether human recombinant erythropoietin (rhEPO) treatment reduces the risk of developing BPD, we investigated the effect of rhEPO treatment on the histopathologic changes seen in hyperoxia-induced lung injury of BPD. Twenty-five rat pups were divided into four groups: air-exposed control group ($n = 5$), hyperoxia-exposed placebo group ($n = 7$), hyperoxia-exposed rhEPO-treated group ($n = 6$), and air-exposed rhEPO-treated group ($n = 7$). Measurement of alveolar surface area, quantification of secondary crest formation, microvessel count, evaluation of alveolar septal fibrosis, and smooth muscle actin immunostaining were performed to assess hyperoxia-induced changes in lung morphology. Treatment of hyperoxia-exposed animals with rhEPO resulted in a significant increase in the mean alveolar area, number of secondary crests formed, and the microvessel count in comparison with

hyperoxia-exposed placebo-treated animals. There was significantly less fibrosis in rhEPO-treated animals. However, treatment of hyperoxia-exposed animals with rhEPO did not result in a significant change in smooth muscle content compared with hyperoxia-exposed placebo treated animals. Our results suggest treatment with rhEPO during hyperoxia exposure is associated with improved alveolar structure, enhanced vascularity, and decreased fibrosis. Therefore, we conclude that treatment of preterm infants with EPO might reduce the risk of developing BPD. (*Pediatr Res* 58: 38–41, 2005)

Abbreviations

BPD, bronchopulmonary dysplasia
EPO, erythropoietin
rhEPO, human recombinant erythropoietin
SMA, smooth muscle actin

Despite the improvements in preventing acute respiratory disease in preterm infants, the incidence of BPD remains largely unchanged. The pathophysiology of BPD has been extensively studied for several decades, and pulmonary oxygen toxicity is believed to play a prominent role in the lung injury process that leads to the development of BPD (1). Histopathologic characteristics of the lung injury in BPD are lack of increased complexity (a decrease in alveolarization), abnormal capillary morphology, and an interstitium with variable cellularity/fibroproliferation (2).

The treatment goals of BPD focus on minimizing ongoing injury, reducing inflammation, maintaining adequate oxygenation, and facilitating lung growth. A number of groups have used antioxidants to prevent BPD without any significant benefit (3). Corticosteroids facilitate extubation and decrease neonatal respiratory support and oxygen exposure. However, these short-term benefits are achieved at the expense of serious neonatal complications such as poor brain and somatic growth, and substantially

worse neuromotor and developmental outcomes in early childhood (4). Therefore, investigating the effectiveness of new strategies in the treatment of BPD is of great interest.

EPO is a 30.4-kD glycoprotein that regulates the rate of red blood cell production, through binding to its specific cell surface receptors. It has been used for many years to treat anemia of prematurity (5,6). In addition, in various tissues, EPO is not only an angiogenic factor but also an antioxidant due to decreasing the plasma iron concentration and increasing the ability of plasma to inhibit lipid peroxidation (7). Because oxygen toxicity and abnormal vascular growth are thought to be major contributing factors in the pathogenesis in BPD, the benefits of EPO treatment in this disease have been investigated. The aim of the study is, therefore, to evaluate the effect of EPO treatment on the histopathologic characteristics of hyperoxia-induced lung injury in BPD.

METHODS

Animal model and treatment. The present study was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by Animal Care and Use Committee of Dokuz Eylul University, School of Medicine. Wistar rats with dated pregnancies were maintained at the same center and housed in individual cages with free access to water and

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laboratory chow. The rat pups were delivered spontaneously and reared with their dams until the time of the experimentation. The experiment began on postnatal d 3 and continued through postnatal d 13 (day of birth = d 0).

Eighteen rat pups were divided into three groups. Group 1 ($n = 5$) served as controls and were kept in air and not subjected to hyperoxia. Groups 2 ($n = 6$) and 3 ($n = 7$) were exposed to oxygen. The pups were maintained in Plexiglas chambers in which the oxygen concentration ($\geq 90\%$) was monitored twice daily. Humidity was maintained at $>80\%$ and CO_2 was removed by soda lime absorption. Pups in group 2 were injected intraperitoneally with rhEPO (400 U/kg) (Eprex, Cilag, Zug, Switzerland) on postnatal d 3 and 5 (rhEPO-treated group). Pups in groups 1 and 3 received 0.9% saline administered in the same way (room air controls and oxygen-exposed placebo group). An additional group, group 4 ($n = 7$) was kept in room air and received rhEPO (400 U/kg, intraperitoneally) on postnatal d 3 and 5.

Nursing mothers were rotated between litters ($n = 4$) in air and hyperoxia every 24 h. All animals were raised in the same room and all other conditions were the same. On postnatal d 14, all animals were killed by intraperitoneal injection of pentobarbital sodium (200 mg/kg).

Tissue preparation. The lungs were resected by thoracotomy and fixed *in situ* at a constant inflation pressure of 20 cm H_2O and then fixed overnight in 10% buffered formalin. Each lobe of the fixed lung tissues was separated, placed in cassettes, and embedded in paraffin after tissue processing. Sections at 4 μm thickness were cut from the paraffin blocks and evaluated by a single observer (E.O.) in a blinded fashion.

Histologic assessment of fibrosis. For fibrosis assessment, lung sections from the right middle and left lobes were stained with hematoxylin and eosin. At $\times 10$ magnification, three nonoverlapping lung sections per animal and a minimum of 30 lung fields were examined. Fields in which large vessels and airways were present were not included. Each lung was evaluated histologically for the absence (score 0) or presence and amount of alveolar septal fibrosis as mild alveolar septal fibrosis limited to a few scattered alveoli (score 1); score 2 for moderates; and score 3 for marked, if fibrosis was present in most alveoli throughout the lung section (8).

Morphometric measurement of alveolar surface area. To measure the internal space of alveoli, sections from the right middle lobe were stained with hematoxylin and eosin. The area was measured using a computer-assisted image analyzer system consisting of a microscope (Labophot-2, Nikon, Tokyo, Japan) equipped with a high-resolution video camera (VKC220E, Hitachi, Tokyo, Japan). The images were processed using an IBM-compatible personal computer, high-resolution video monitor, and image analysis software (BS 200Docu Version 2.0, BAB Imaging Systems, Ankara, Turkey). Briefly, the images were grabbed with the video camera at $\times 10$ magnification and contiguous airspaces within 300 μm of the pleura in each of six nonoverlapping fields were measured as described previously (9).

Quantification of secondary crest formation. For quantification of secondary crest formation, sections from the approximate center of the right caudal lobe and left lobes were stained with Verhoeff's stain for elastic fibers. Five nonoverlapping microscopic fields per lung lobe (10 fields per animal) were chosen on each section. Secondary crests were identified by their characteristic structure as small ridges that extend out from both sides of the primary alveolar septa and counted. When choosing fields for analysis care was taken to avoid underinflated areas and fields containing large vessels and airways (10).

Immunohistochemical assessment of SMA expression. SMA expression was visualized using the avidin-biotin-peroxidase method. Embedded lung tissues from the right caudal and left lobes were sectioned on poly-L-lysine-coated slides. The sections were deparaffinized in xylene, rehydrated, and immersed in distilled water. Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxidase in PBS. The primary antibody against α -SMA (prediluted, DAKO, Glostrup, Denmark) was applied for 30 min at room temperature and washed in PBS. Linking antibody and streptavidin-peroxidase complex (NeoMarkers, Fremont, CA) were added consecutively for 10 min at room temperature and washed in PBS. The peroxidase activity was visualized with 0.03% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) applied for 5 min. After rinsing in deionized water and counterstaining with hematoxylin, the slides were dehydrated and mounted. Appropriate tissue sections as positive and negative controls for the primary antibody were also labeled.

Ten nonoverlapping microscopic fields were selected at $\times 10$ magnification in a random manner for immunohistochemical scoring. The degree of positive staining was evaluated by semiquantitative scoring on a scale of 1 to 4 for intensity (I) and for distribution (D). Tissues with $I \times D \leq 4$ were considered weakly positive, and those with $I \times D > 4$ were designated strongly positive (11). In addition, the scoring was also further simplified in positive (one or more stained cells) or negative (no stained cells).

Quantification of microvessel count. The avidin-biotin-peroxidase method was performed using the primary antibody against CD31 (1:50 dilution, NeoMarkers) for labeling vascular structures. The paraffin-embedded lung tissues from the right caudal and left lobes were sectioned on poly-L-lysine-coated slides. Briefly, the sections were deparaffinized and endogenous per-

oxidase activity was blocked using a 0.3% solution of hydrogen peroxidase in PBS at room temperature for 10 min. After microwave treatment, primary antibody was applied for 30 min at room temperature and washed in PBS. Linking antibody and streptavidin-peroxidase complex (NeoMarkers) were added consecutively for 10 min at room temperature and washed in PBS. The peroxidase activity was visualized with 0.03% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) applied for 5 min. Appropriate positive and negative controls were also labeled with the primary antibodies.

Three nonoverlapping "vascular hot spots" were identified by scanning the whole section under low magnification and microvessels were counted at $\times 10$ magnification in a random manner (12).

Statistics. The data were statistically analyzed using computer software (SPSS 10.0, Chicago IL). A level of $p \leq 0.05$ was chosen to represent statistical significance. Experimental group means and standard deviations were determined using descriptive statistics. Immunohistochemical scores were assessed by the χ^2 test and Fisher's exact test. Treatment groups were compared using a one-way ANOVA and significance was determined using Bonferroni's correction for multiple comparisons.

To assess interobserver variability for each of the methods used, assessments were performed by another observer (S.O.), blinded to the results of the first observer. There was no significant difference in the results of the two observers (paired samples t test, $p > 0.05$). To test the significance of the areas selected for assessments, the results of three randomly selected animals were statistically compared with the average of the results obtained from ten randomly selected areas. There was no significant difference by paired t test analysis.

RESULTS

Animal weight gain. Although there was no difference in average birth weight between the four groups, animals nursed in hyperoxia showed a significant decrease in body weight ($p < 0.05$, 21.1 ± 1.7 g). Body weights of the rhEPO-treated, hyperoxia-exposed group were not significantly different from those of the control group ($p < 0.05$, 25.1 ± 1.9 g and 27.2 ± 1.8 g, respectively).

Alveolar surface area measurements and quantification of secondary crest formation. As shown in Figure 1, relative to animals in the hyperoxia-exposed placebo group, treatment of hyperoxia-exposed animals with rhEPO resulted in a marked difference in lung morphology. The alveolar surface areas and number of crests were significantly different between the groups (Table 1). Exposure to hyperoxia resulted in a significant decrease in mean alveolar surface area and number of

Figure 1. Lung morphology. Representative light micrographs showing morphology of rat lung. (A) Control group; (B) hyperoxia-exposed, placebo-treated group; (C) hyperoxia-exposed, rhEPO-treated group; and (D) air-exposed, rhEPO-treated group. Note that rhEPO treatment results in increased alveolar surface area and decreased fibrosis (hematoxylin-eosin staining).

Table 1. Alveolar surface area (μm^2), number of crests (per one high-power field), and microvessel count (at $\times 10$ magnification)

	Air	Hyperoxia + EPO	Hyperoxia + placebo	Air + EPO
Surface area	8345 $\pm$ 218.5	8031.6 $\pm$ 291.9	5300 $\pm$ 1622.4	8437.8 $\pm$ 154.7
Crests	71.4 $\pm$ 3.3	65.1 $\pm$ 7.9	47.2 $\pm$ 6.8	72.4 $\pm$ 3.7
Microvessels	4.4 $\pm$ 0.2	6.7 $\pm$ 2.0	2.1 $\pm$ 0.3	6.1 $\pm$ 0.3

Data given as mean $\pm$ SD.

crests formed compared with air-exposed animals ($p = 0.001$ and $p = 0.002$, respectively). Treatment of hyperoxia-exposed animals with rhEPO resulted in a significant increase in mean alveolar surface area and increased number of secondary crest in comparison with hyperoxia-exposed, placebo-treated animals ($p = 0.001$ and $p = 0.01$, respectively).

Microvessel count. The microvessel count for each of the groups is shown in Table 1. As shown in Figure 2, the microvessel count was found to be significantly increased in the rhEPO-treated group compared with the animals in the hyperoxia-exposed placebo group and the controls ($p < 0.001$ and $p = 0.02$, respectively). There was a significantly increased microvessel count in the air-exposed, rhEPO-treated group compared with controls ($p = 0.004$), however all other comparisons were insignificant (Table 1).

Histologic assessment of fibrosis and SMA immunostaining. There was a significant difference in the degree of fibrosis between the two groups. Fibrosis was absent in all air-exposed animals. In contrast, exposure to hyperoxia resulted in fibrosis in all of the animals in the placebo group, whereas only one animal in the rhEPO-treated, hyperoxia-exposed group showed weak fibrosis ($p = 0.01$).

Immunostaining for SMA demonstrated a marked increase in smooth muscle content in hyperoxia-exposed animals (Fig. 3). However, treatment of the hyperoxia-exposed animals with rhEPO did not result in a significant change in smooth muscle content compared with hyperoxia-exposed placebo animals.

DISCUSSION

During normal intrauterine development, lung volume and surface area dramatically increase in the late canalicular and early saccular stages. Secondary septation forms new alveoli in the late fetus and newborn, and continues at a slower rate during the first 2–3 y of life. Parallel increases in vascular growth are closely synchronized with alveolarization during the same time periods, but molecular signals that link distal airspace growth with angiogenesis are uncertain (13). Preterm infants likely to develop BPD are born during the canalicular

Figure 2. Marked angiogenic activity and increased microvessel count (arrows) in the rhEPO-treated group. (A) Hyperoxia-exposed placebo group; (B) hyperoxia-exposed, rhEPO-treated group (immunoperoxidase staining).

Figure 3. Increased SMA immunostaining in the hyperoxia-exposed rats. (A) Control group; (B) hyperoxia-exposed, placebo-treated group (immunoperoxidase staining).

phase of lung development at 24–26 wk, a time when alveolar and distal vascular development commences.

The histopathologic changes of severe airway injury and alternating sites of overinflation and fibrosis which used to be seen in older forms of BPD have been replaced by a milder form characterized by alveolar and capillary hypoplasia and variable interstitial cellularity and/or fibroproliferation. The alveolar and capillary hypoplasia of this newer form of BPD will require the development of specific therapies (2,14). It has been shown that oxidative stress plays a major role in the development of BPD, therefore, antioxidant therapies are likely to improve lung morphology (3). However, there has not been any improvement in antioxidant therapy in newborn infants.

Iron is an important catalyst for free oxygen radicals and lipid peroxidation reactions. During the early neonatal period, extracellular iron is available in excessive amounts. Free iron could be scavenged, EPO being one compound that may mobilize iron from the plasma and hence inhibit its toxic effects. In premature rabbits exposed to hyperoxia, rhEPO decreased the plasma iron concentration and increased the ability of plasma to inhibit lipid peroxidation (7). In addition, EPO may increase the activity of protective enzymes of oxidative injury and modulate the production of nitric oxide, a major mediator of tissue injury (15,16).

There is evidence from animal models that exposure of the developing lung to high concentrations of oxygen inhibits alveolus formation and decreases lung surface area. The mechanisms by which hyperoxia affects alveolar size in developing lungs are unclear. Prematurely born infants with structurally immature lungs are often exposed to hyperoxia (10). Because of the potential for hyperoxia to alter normal lung development, the identification of agents such as EPO that may prevent hyperoxia-induced effects would be important in preserving normal alveolus formation.

In the present study, we found that treatment of newborn rats exposed to hyperoxia with EPO was associated with increased alveolar surface area and number of secondary crests formed compared with the hyperoxia-placebo group. Secondary crests are characteristic ridges growing out from the primary alveolar wall that will subsequently become secondary septa dividing the lung saccule into alveoli, a process that occurs primarily in

active alveolar formation (10). In the present study, EPO treatment reversed the negative effect of hyperoxia that was observed in the hyperoxia-placebo group resulting in increased alveolar surface area and secondary crests formed.

The most prominent effect of EPO observed in this study was the decreased fibrosis in the alveolar walls of hyperoxia-exposed, EPO-treated rats. The mechanisms by which EPO treatment alters fibrosis remain unknown. Recent pathologic reports have subdivided human BPD into a new milder form with minimal small airway abnormality, but a predominance of alveolar wall thickening caused by fibrosis and associated with variable interstitial fibroproliferation and smooth muscle hyperplasia (2).

Pulmonary inflammation is a key feature of fibrosis in the pathogenesis of fibrosis in BPD. An imbalance between pro-inflammatory and anti-inflammatory factors might result in lung injury that affects normal alveolarization and pulmonary vascular development in preterm infants with BPD. Besides pro-inflammatory mediators having a direct effect on cell integrity and inducing apoptosis, toxic oxygen radicals might also be responsible for lung injury (3,17). Therefore, although it is speculative, our finding that EPO treatment was associated with decreased fibrosis in the alveolar walls of hyperoxia-exposed rats might be explained by antioxidant and anti-apoptotic effects. In particular, the latter might result from a signaling pathway triggering anti-apoptosis mechanisms (18,19). However, further studies aiming to investigate apoptosis by either TUNEL staining or analysis of apoptotic proteins and the antioxidant mechanism by assessment of hematologic variables such as free iron will help to clarify this further. In addition, in the present study, although we did not investigate the effect of EPO on fibroblast proliferation, SMA immunostaining revealed that EPO treatment does not appear to have an effect on smooth muscle hyperplasia.

Jakkula *et al.* (20) suggested that angiogenesis is necessary for alveolarization during normal lung development and that injury to the developing pulmonary circulation during a critical period of growth can also contribute to lung hypoplasia. They also demonstrated that treatment of newborn rats with anti-angiogenic drugs inhibits vascular growth and decreases alveolarization. New strategies that stimulate vascular growth may provide an alternative approach to the treatment of abnormal lung growth in clinical disorders such as BPD.

Although classically described as a hormone that stimulates erythroid precursors, EPO is now known to have effects on many cells and tissues. Functional EPO receptors are present on endothelial cells. Stimulation of endothelial cells by EPO increases cell migration and proliferation, endothelin-1 production and release, enhanced vascular sensitivity to norepinephrine, and induction of angiogenesis (5). Stimulation of cultured endothelial cells with EPO can induce this pro-angiogenic phenotype. EPO alone can stimulate neovascularization *in vivo* and *in vitro* (5). EPO also interacts synergistically with vascular endothelial growth factor, a potent angiogenic factor, to potentiate its vascular activity (21). Based on this evidence, we hypothesized that EPO treatment might prevent lung injury seen with hyperoxia by stimulating angiogenesis. In accordance with our hypothesis, EPO treatment significantly increased vascularization in EPO-treated hyperoxia-exposed rats.

In conclusion, due to its angiogenic effect, treatment of preterm infants with EPO may reduce the risk of the development of BPD. However, recent evidence suggests that great

caution should be exercised in the use of hematopoietic growth factors, including EPO, because of their potential side effects (22). Further studies investigating the anti-apoptotic and antioxidant mechanisms of EPO and clinical trials in patients with BPD will provide additional data about the potential use of rhEPO in the prevention of this disease. Although our results suggest treatment with rhEPO is associated with improved alveolar structure, enhanced vascularity, and decreased fibrosis, they should be interpreted cautiously due to the small number of animals included. Addition of a new group might have also led to flaws because environmental conditions of this group might be different from those of the remaining groups. However, to our knowledge, this is the first study investigating histologic changes associated with the antioxidant and angiogenic effects of EPO treatment in hyperoxic lung injury.

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REFERENCES

1. Welty SE 2001 Is there a role for antioxidant therapy in bronchopulmonary dysplasia? *J Nutr* 131:947S-950S
2. Coalson JJ 2003 Pathology of new bronchopulmonary dysplasia. *Semin Neonatol* 8:73-81
3. Saugstad OD 2003 Bronchopulmonary dysplasia-oxidative stress and antioxidants. *Semin Neonatol* 8:39-49
4. Vaucher YE 2002 Bronchopulmonary dysplasia: an enduring challenge. *Pediatr Res* 23:349-358
5. Juul SE 2000 Nonerythropoietic roles of erythropoietin in the fetus and neonate. *Clin Perinatol* 27:527-541
6. Ohls RK 2000 The use of erythropoietin in neonates. *Clin Perinatol* 27:681-696
7. Bany-Mohammad FM, Slivka S, Hallman M 1996 Recombinant human erythropoietin: possible role as an antioxidant in premature rabbits. *Pediatr Res* 40:381-387
8. Stocker JT 1986 Pathologic features of long-standing "healed" bronchopulmonary dysplasia: a study of 28 3- to 40-month-old infants. *Hum Pathol* 17:943-961
9. Veness-Meehan KA, Moats-Staats BM, Price WA, Stiles AD 1997 Re-emergence of a fetal pattern of insulin-like growth factor expression during hyperoxic rat lung injury. *Am J Respir Cell Mol Biol* 16:538-548
10. Veness-Meehan KA, Bottone FG Jr., Stiles AD 2000 Effects of retinoic acid on airspace development and lung collagen in hyperoxia-exposed newborn rats. *Pediatr Res* 48:434-444
11. Ozer E, Sis B, Ozen E, Sakizli M, Canda T, Sarioglu S 2000 BRCA1, c-erbB-2 and H-ras gene expressions in young woman with breast cancer. An immunohistochemical study. *Appl Immunohistochem Mol Morphol* 8:12-18
12. Costello P, McCann A, Carney DN, Dervan PA 1995 Prognostic significance of microvessel density in lymph node negative breast carcinoma. *Hum Pathol* 26:1181-1184
13. Abman SH 2001 Bronchopulmonary dysplasia: "A vascular hypothesis." *Am J Respir Crit Care Med* 164:1755-1756
14. Bancalari E, Claure N, Sosenko IR 2003 Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. *Semin Neonatol* 8:63-71
15. Sasaki R, Masuda S, Nagao M 2000 Erythropoietin: multiple physiological functions and regulation of biosynthesis. *Biosci Biotechnol Biochem* 64:1775-1793
16. Kumral A, Baskin H, Duman N, Yilmaz O, Tatli M, Ozer E, Gokmen N, Genc S, Ozkan H 2003 Erythropoietin protects against necrotizing enterocolitis of newborn rats by the inhibiting nitric oxide formation. *Biol Neonate* 84:325-329
17. Speer CP 2003 Inflammation and bronchopulmonary dysplasia. *Semin Neonatol* 8:29-38
18. Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Caobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, Ghezzi P 2001 Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci U S A* 98:4044-4049
19. Wen TC, Sadamoto Y, Tanaka J, Zhu PX, Nakata K, Ma YJ, Hata R, Sakanaka M 2002 Erythropoietin protects neurons against chemical hypoxia and cerebral ischemic injury by up-regulating Bcl-xL expression. *J Neurosci Res* 67:795-803
20. Jakkula M, Le Cras TD, Gebb S, Hirth KP, Tudor RM, Voelkel NF, Abman SH 2000 Inhibition of angiogenesis decreases alveolarization in the developing rat lung. *Am J Physiol Lung Cell Mol Physiol* 279:L600-L607
21. Marti HH 2004 Erythropoietin and the hypoxic brain. *J Exp Biol* 207:3233-3242
22. Zipursky A 2002 The risk of hematopoietic growth factor therapy in newborn infants. *Pediatr Res* 51:549