Prenatal Dexamethasone Treatment Improves Survival of Newborn Rats during Prolonged High O₂ Exposure

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ABSTRACT. Prenatal dexamethasone (DEX) treatment is known to accelerate the maturation of both the surfactant system and the fetal lung antioxidant enzyme (AOE) system (Frank L, Lewis P, Sosenko IRS: *Pediatrics* 75:569–574, 1985). Because of this stimulatory effect of prenatal DEX on the normal late gestational development of the AOE system, we questioned whether this treatment might have a salutary effect on the ability of the newborn rat to tolerate early and prolonged exposure to hyperoxia, inasmuch as the AOE are the primary lung defensive system against high O₂ challenge. In nine experiments with term newborn rats in >95% O₂, the composite percentage of survival was significantly greater in the prenatal DEX pups at all time periods in hyperoxia from 7 d [control pups, 67 of 94 (71%); prenatal DEX, 96 of 99 (97%)] to 14 d [controls, 10 of 32 (31%); prenatal DEX, 18 of 33 (55%)] ($p < 0.01$). In addition to survival *per se*, the prenatal DEX pups showed significantly decreased lung wet weight/dry weight ratios, pathologic evidence of pulmonary edema, and lung conjugated dienes *versus* the O₂ control newborn group. Of the many comparative parameters examined, the major difference found between the two groups was in the pulmonary AOE responses to hyperoxia. By 2 d in hyperoxia, the prenatal DEX rat pups showed significantly elevated superoxide dismutase, catalase, and glutathione peroxidase activities compared to air control pups, and at 4 and 7 d in O₂ the AOE levels were consistently greater in the DEX group than the AOE responses in the control O₂ pups. The more rapid and more pronounced AOE response to hyperoxic challenge may help to explain the substantial protective effect of prenatal DEX *versus* newborn O₂ toxicity. (*Pediatr Res* 32: 215–221, 1992)

Abbreviations

DEX, dexamethasone
AOE, antioxidant enzyme
SOD, superoxide dismutase
CAT, catalase
GP, glutathione peroxidase
DSPC, disaturated phosphatidylcholine
BPD, bronchopulmonary dysplasia

Synthetic glucocorticoids have been used extensively in situations of premature labor with threatened delivery of a very

immature infant. Administration of a long-acting glucocorticoid for 48 h before premature delivery may successfully stimulate surfactant production/secretion in the immature fetal lung and lessen the chance of serious respiratory distress syndrome in the prematurely delivered newborn (1).

Previous work from our laboratories has demonstrated that the prenatal administration of DEX to pregnant rats will not only accelerate the late gestational development of the surfactant system in the rat fetuses, but will also accelerate the normal late gestational development of the fetal lung AOE system (2). The late gestational rise in fetal lung SOD, CAT, and GP activities, now demonstrated to be a natural maturational occurrence in five animal species (rat, mouse, rabbit, hamster, and sheep) (3–8), is proposed to be the biochemical means by which the lung is prepared for its safe transition from the quite hypoxic *in utero* environment to the relatively O₂-rich *ex utero* environment it will encounter immediately at birth (3–5, 9).

Because of its marked stimulatory effect on fetal lung AOE system development, we wondered if prenatal DEX treatment might also have a salutary effect on the ability of the newborn animal to tolerate early and prolonged exposure to hyperoxia. We describe herein the findings from a series of hyperoxic exposure experiments that consistently demonstrated an improved resistance to pulmonary O₂ toxicity in the newborn offspring from prenatally DEX-treated dams compared to control newborn rat pups.

MATERIALS AND METHODS

Animals/treatments. We used adult female Sprague-Dawley albino rats (300 g) (originally obtained from Charles River Laboratories, Wilmington, MA), which were in our rat breeding program colonies in the University of Miami Animal Care Facility. Breeding was accomplished by allowing female and male rats to cohabit overnight. Probable pregnancy was determined by a positive sperm smear the next morning, and the onset of gestation was considered to be the midpoint of the 12-h cohabitation period. The length of gestation averages 21.5 d in these animals, with only slight variations ($SD = \pm 0.23$ d) noted in several hundred monitored pregnancies.

At 48 and 24 h before the expected time of delivery, pregnant animals (two or four per experiment) were given intraperitoneal injections of DEX, 0.4 mg/kg/d (dexamethasone sodium phosphate; Henry Schein, Inc., Woodbury, NY) diluted in physiologic saline, or were handled in the exact manner and given intraperitoneal injections of physiologic saline (two or four per experiment). Shortly after delivery, all the newborn pups were removed from the mothers, marked by toe-clipping (DEX or saline pre-treated), and pooled together before being randomly redistributed to the newly delivered mother rats, one half of which were then placed in the O₂ exposure chambers and one half of which remained in room air. Litter sizes were 10 to 12 pups per dam.

Exposures to hyperoxia were conducted in clear plastic 3.5-ft³

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exposure chambers with continuous monitoring of O₂ concentration (96 to 98% O₂; oxygen analyzer, model OM-11; Beckman Instruments, Inc., Fullerton, CA), CO₂ concentration (<0.5%, Beckman medical gas analyzer, model LB-2), temperature (23–25°C, in-chamber thermometers), and humidity (50 to 70%, in-chamber hygrometers). Exposures to >95% O₂ were continuous for 7 to 14 d except for a daily 15- to 20-min period when the chambers were opened for animal maintenance purposes and for switching the dams from O₂ litters to air litters to prevent the development of O₂ toxicity in the nurturing dams. Daily litter weights were also obtained to check for possible poor nursing by specific dams, but no replacement dams were ever required. The experimental animal protocol for these studies was preapproved by the University's Animal Research Use Committee.

Biochemical analyses. For lung AOE analysis, some of the experimental pups were killed by an intraperitoneal overdose of pentobarbital after variable periods in hyperoxia or room air. After the great vessels in the abdomen were severed to exsanguinate the animals, their lungs were rapidly perfused *in situ* with ice-cold isotonic buffer (0.1 M potassium phosphate, 0.15 M potassium chloride, pH 7.4) via the pulmonary artery after removing the left atrial appendage for ease of drainage. The dissected lungs were then homogenized in ice-cold 0.005 M potassium phosphate buffer, pH 7.8 (1:20 wt/vol) with a Brinkmann Polytron homogenizer (Brinkmann Instruments, Co., Westbury, NY). SOD activity was measured on fresh homogenates using the spectrophotometric ferricytochrome reduction method (10). The crude lung homogenate was centrifuged at 15 000 × *g* for 10 min at 5°C and the supernatant fluid was removed and frozen overnight. CAT activity (11) and GP activity (12) in the supernatant fluid were measured spectrophotometrically the next day. The CAT assay monitors the rate of loss of H₂O₂ substrate at 240 nm; the GP assay follows the rate of NADPH oxidation in the presence of cumene hydroperoxide substrate at 340 nm. Purified enzyme standards were obtained from Sigma Chemical Co., St. Louis, MO (SOD and CAT) and Boehringer Mannheim Co., Indianapolis, IN (GP). Lung protein was determined with purified bovine albumin (Sigma) as standard (13), and lung DNA was determined with calf thymus DNA (Sigma) as standard (14). Lung enzyme activity was calculated on the basis of activity per g lung, per mg protein, and per mg DNA, with the latter values used for statistical comparisons because this expression of sp act best reflects the enzyme activity per lung cell, which is what is most meaningful in lung cell protective capacity *versus* oxidant stress.

For DSPC determinations, aliquots of the homogenates were subjected to lipid extraction using the method of Bligh and Dyer (15). The extract was dried under nitrogen, reconstituted with chloroform:methanol (2:1), and assayed for total lipid phosphorus following the method of Morrison (16). A portion of the dried lipid extract was used to estimate DSPC using the osmium tetroxide method described by Mason *et al.* (17) and then assayed for inorganic phosphorus as described above (16). A known quantity of [¹⁴C]-dipalmitoylphosphatidylcholine (New England Nuclear, North Billerica, MA) was added before lipid extraction to estimate and correct for sequential losses. DSPC was expressed as mg/g of wet lung weight.

Lung lipid peroxidation was assayed by conjugated diene measurements in air- and O₂-exposed rat lungs (18). This assay involves homogenizing the perfused lungs in isotonic phosphate buffer containing 0.001 M EDTA. After centrifugation, the homogenate is extracted with 2 volumes of 45°C methanol:chloroform (1:2) two times, then once with H₂O:methanol:chloroform (1:1:2). After centrifugation, the methanol-H₂O upper phase is aspirated off and a 2-mL aliquot of the chloroform extract is dried in a water bath at 40–50°C under N₂. The lipid residual is redissolved in 3 mL of heptane and the absorption spectrum at 223 nm is recorded against a heptane blank. Results are expressed as absorbance units per g wet lung and per mg DNA.

Lung elastin was assayed by the spectrophotometric method of Naum and Morgan (19). After homogenizing the lungs in 0.15 M NaCl and centrifugation, 1.0 mL of 5 M guanidine HCl, pH 7.0, is added to the pellet. Guanidine extraction continues for 24 h at room temperature in a shaking water bath. After centrifugation and a second 24-h extraction with guanidine, the residue is suspended in 1.0 mL H₂O and then autoclaved at 15 lb pressure for 45 min. After centrifugation, the precipitate is mixed with 1.0 mL H₂O and 1.0 mL elastase solution (0.1 mg purified elastase in 0.1 M veronal acetate buffer, pH 8.8) and incubated for 30 min at 37°C in a shaking water bath. Aliquots are then assayed for protein (elastin) content (13). Purified standards of elastin (Sigma) are treated as above for generation of a standard assay curve.

Lung morphometry. For microscopic studies, animals were killed as above and then had their lungs fixed *in situ* via a tracheal cannula using a constant inflation pressure of 20 cm H₂O (fixative = 10% buffered formalin, pH 7.4). After fixation in the inflated state for 24–48 h, the tissue is processed for hematoxylin and eosin staining. Similarly oriented sections of left lung and right middle and lower lobe are used.

Light level morphometric assessment was done on coded slides for comparison of lung structural development (alveolarization) in air- and O₂-exposed neonatal animals of both experimental groups. We determined mean linear intercept, % air/% tissue space, internal surface area (ISA), and specific internal surface area (ISA/100 g body wt) as we have reported previously (20), using ×450 magnification and an eyepiece with a simple square grid pattern (square grid with seven horizontal lines and 42 intercept bars, model CPLW 10X/18 eyepiece; Zeiss Optical, Hanover, MD). A minimum of 30 lung fields per animal were examined. To calculate the mean linear intercept (L_M) (representing the average distance between air space walls, or average airspace diameter), we used the formula $L_M = n \cdot L / \epsilon_i$, where *n* is the number of lines counted, *L* is the length of the line, and ϵ_i is the sum of septal (walls of air spaces) intercepts. To calculate the ISA, we use the formula $ISA = 4 \cdot VL / L_M$, where VL is the postfixation lung volume by water displacement (21, 22).

Pathologic assessment was done on additional coded lung sections, looking for evidence of interstitial, periarterial/peribronchiolar edema and intraalveolar edema in the O₂-exposed rat lungs. Pulmonary edema was also assessed by lung wet weight/dry weight ratios [80°C oven; lungs dried until constant weight found (48 h)].

Statistical analyses. For quantitative comparisons of the DEX *versus* control groups, *t* testing was done. For four group comparisons (control air + O₂, DEX air + O₂), one-way analysis of variance testing was done, followed by Duncan's multiple-range testing, using a *p* < 0.05 value for statistical significance. Survival data were compared for significance using χ^2 testing (23).

RESULTS

Prenatal treatment effects on newborn offspring. Tables 1 and 2 provide comparative gravimetric, biochemical, and lung morphometric data on the newborn offspring of the DEX- and saline-treated dams. Prenatal DEX treatment resulted in significantly reduced body weight and lung weight in the newborn pups as noted before by us and many other investigators (2, 24–26). However, the lung weight/body weight ratio was no different than for the control (saline) newborns. Prenatal DEX treatment also resulted in significant reductions in lung protein and DNA content compared to the control group of newborns, but when calculated as mg/g lung weight, the group values were not significantly different.

In this study, as was found in our earlier study (2), newborn pups of both treatment groups had essentially similar lung surfactant (DSPC) contents as well as AOE activities (SOD, CAT, and GP) at birth (Fig. 1). Although prenatal DEX treatment elevates the levels of surfactant and AOE in the premature animal

Table 1. Comparative parameters in newborn rat pups after prenatal DEX or saline treatment*

Treatment group	Body wt (g)	Left lung wt (g)	Lung wt/body wt	Protein (mg/lung)	DNA (mg/lung)	Protein/DNA
Saline (n = 10)	5.96 ± 0.41	0.054 ± 0.006	0.906 ± 0.072	2.39 ± 0.12	0.83 ± 0.09	2.89 ± 0.12
DEX (n = 12)	4.93 ± 0.41 (↓17%)	0.040 ± 0.003† (↓26%)	0.828 ± 0.112 (↓9%)	1.87 ± 0.31† (↓22%)	0.67 ± 0.11† (↓19%)	2.80 ± 0.29 (↓3%)

* Newborn offspring from pregnant rats treated at 48 and 24 h before delivery with DEX 0.4 mg/kg or equivolume saline and killed at the time of birth. Values are mean ± 1 SD for n rat pups/group.

† p < 0.05 for DEX vs saline values.

Table 2. Comparative morphometry of newborn rat lungs after prenatal DEX or saline treatment*

Treatment group	Body wt (g)	LV (mL)	LV/100 g	L _M (μm)	ISA (cm ²)	ISA/100 g
Saline (n = 8)	6.15 ± 0.60	0.30 ± 0.03	4.83 ± 0.25	55.4 ± 2.2	210 ± 30	3489 ± 135
DEX (n = 6)	4.75 ± 0.35† (↓23%)	0.22 ± 0.02† (↓27%)	4.73 ± 0.19 (↓2%)	57.7 ± 2.2 (↑4%)	154 ± 16† (↓27%)	3284 ± 153 (↓5%)

* Newborn offspring from pregnant rats treated at 48 and 24 h before delivery with DEX 0.4 mg/kg or equivolume saline and killed at the time of birth. Values are means ± 1 SD for n lungs per group. LV, lung volume; L_M, mean linear intercept (average airspace diameter); and ISA, internal surface area.

† p < 0.05 for DEX vs saline values.

Fig. 1. Pulmonary AOE and lung surfactant (DSPPC) content in newborn rat pups prenatally treated with DEX (black bars) or saline (hatched bars) and killed at the time of birth. No differences between groups are seen. Values = mean ± 1 SD for 12 pups per group.

lung, greater than normal full-term lung levels are not found at the time of birth.

Lung morphometric analysis of newborn lung structure (Table 2) showed DEX treatment effects on absolute lung volume and lung internal surface area. However, when normalized to body weight, these values were similar for both experimental groups, as were the mean airspace diameters (L_M). Lung elastin contents were similarly unchanged in the DEX (0.177 ± 0.015 mg/lung) versus the control pups (0.190 ± 0.003 mg/lung, n = 5 per group, p > 0.10).

Survival in hyperoxia. In each of nine separate hyperoxia exposure experiments, the prenatal DEX-treated pups demonstrated a greater percentage of survival than the simultaneously exposed control pups in O₂. Figure 2 illustrates the composite survival percentages.

The DEX-treated pups had significantly improved O₂ tolerance between exposure d 7 and 14 inclusive (p < 0.01 or greater by χ² testing).

In addition to survival *per se*, the DEX group had other indications of superior O₂ tolerance by comparative lung wet weight/dry weight ratios (reflection of pulmonary edema), comparative conjugated diene levels (as an index of O₂ radical-induced lipid peroxidation), and comparative light microscopic

Fig. 2. Survival of prenatal DEX-treated and prenatal saline-treated (control) newborn pups in >95% O₂ for 14 d (numbers in parentheses = number alive/number put in O₂). O₂-DEX pup survival rate is significantly improved compared to O₂ controls at all time periods from 7 to 14 d in hyperoxia (*, p < 0.01; **, p < 0.05; χ²).

Fig. 3. Comparative evidence for pulmonary O₂ toxicity in prenatal DEX-treated and saline-treated (control) newborn rats after 7 d in >95% O₂. DEX-treated group values in air were similar for all parameters to the control (saline) air group values ($n = 6$), which are illustrated here. Lung wet weight/dry weight values = mean $\pm$ 1 SD for 10 pups per O₂ group. Lung edema by microscopic pathology (%) = number with intraalveolar edema fluid per total number of lung sections examined (= 73 per group). Conjugated dienes, values = mean $\pm$ 1 SD for six samples per group. *, $p < 0.05$ for O₂-DEX vs O₂-saline group.

pathologic findings (Fig. 3). The lung wet weight/dry weight ratio averaged 35% higher than the air control value in the O₂-saline pups versus 15% higher than the air control values in the O₂-DEX pups ($p < 0.05$). Conjugated dienes in the lung were increased 46% in the O₂-saline group versus 11% in the O₂-DEX pups ($p < 0.05$). On light microscopic examination, all the O₂-exposed pups had perivascular/peribronchiolar edema present after 7 d in O₂. However, as evidence of more advanced O₂ toxicity, intraalveolar edema was present in four of 73 (5.5%) O₂-DEX lung sections compared to 38 of 73 (52%) O₂ control group lung sections ($p < 0.001$) (Fig. 3).

Lung responses to hyperoxia. The responses of the surfactant system to 7 d in O₂ were very similar in the two treatment groups, with the O₂ control group showing a $37 \pm 4\%$ increase in lung tissue DSPC content in response to hyperoxia versus a $39 \pm 8\%$ increase in the DEX-O₂ pup lungs (both values $p < 0.05$ versus respective air controls). Figure 4 illustrates the comparative responses of the pulmonary AOE to hyperoxic exposure in the two groups of offspring at 2, 4, and 7 d of >95% O₂ exposure. After 2 d in hyperoxia, a trend toward a greater AOE response was seen in the DEX group, with SOD, CAT, and GP enzymes already significantly elevated in O₂ versus the air control group values at this early time point. Only CAT activity showed elevation at the 2-d time point in the O₂ control group. Although both newborn groups manifested significant increases in SOD, CAT, and GP activities (compared to air control values) at 4 and 7 d of high O₂ exposure, at both time points the comparative increases in AOE were significantly enhanced in the O₂-DEX group versus the changes in the O₂ control group levels in response to hyperoxia ($p < 0.05$).

The lung morphometric changes induced by prolonged >95% O₂ exposure in both groups of neonates are shown in Table 3. The improved survival of the DEX offspring in hyperoxia was not accompanied by any differences in the degree of O₂-induced inhibition of normal lung structural maturation (alveolarization and increase in respiratory surface area) that occurred during hyperoxia. Compared to the air control values, the lungs of both O₂-exposed groups had significantly enlarged mean airspace diameters and significantly reduced respiratory exchange surface area and surface area/100 g body wt. Figure 5 shows comparative lung micrographs illustrating the marked inhibition of alveolarization in the lungs of both O₂-exposed groups of pups. Addi-

tionally, the typical presence of alveolar edema in the O₂ control group versus the prenatal DEX-O₂ group is demonstrated in these light-level micrographs.

DISCUSSION

The effects of prenatal glucocorticoid treatment on the maturation of the fetal lung continues to be extensively explored both *in vivo* and *in vitro*. Most experimental work continues to focus on the important effect of prenatal glucocorticoids on accelerating the maturation of the surfactant system (and its associated biosynthetic enzymes). Newer work has centered on the effects of glucocorticoids on development changes in the surfactant-related proteins, SP-A, SP-B, and SP-C (26). This very extensive research effort complements the widespread use of prenatal glucocorticoid treatment in threatened human premature labor to try to stimulate early surfactant production and secretion by the premature fetus and prevent serious respiratory distress syndrome if premature delivery eventuates.

Newborn infants who are born too soon, especially those < 1000 g (very low birth weight), are excessively prone to developing early and severe lung damage secondary to barotrauma and required high O₂ therapy, which all too often may progress to chronic lung disease or BPD. The more premature the infant, the more distressingly high becomes the clinical incidence of BPD (27). Protection of the lung from the injurious effects of high O₂ exposure depends on a variety of known and still unknown factors. The pulmonary AOE have been consistently shown to be the primary defensive system against the increased O₂ free radicals that are produced in cells under hyperoxic conditions (4, 28, 29).

In the last couple of years, some interesting information has been garnered that indicates that in parallel to the developmental profile of the fetal lung surfactant system in late gestation the AOE system of the fetal lung also demonstrates marked increases in activity during the final *in utero* period before birth. This late gestational increase in fetal lung AOE activities has been found to occur in all species examined, including the rat, hamster, guinea pig, rabbit, and sheep (3-8). A single report from a small sampling of human infants suggests that late gestational AOE changes in the human fetal lung may also be a normal phenomenon (30). The similar chronology of development of the fetal

Fig. 4. Comparative AOE responses to hyperoxia in prenatal DEX-treated (white bars) and prenatal saline-treated (hatched bars) newborn rats at 2, 4, and 7 d of >95% O₂ exposure. Lung AOE values in the air-exposed prenatal DEX-treated group were equivalent to the air-exposed control (saline) group values, which are illustrated here (black bars). AOE values = mean $\pm$ 1 SD for four air control and six to eight O₂ lung samples per group at each time period. Note that ordinate values do not start at zero activity. *, $p < 0.05$ for O₂ group vs air group. **, $p < 0.05$ for O₂-DEX vs O₂-saline and air group.

Table 3. Comparative morphometry of newborn rat pups after 10 d in >95% O₂ or room air*

Treatment group	Body wt (g)	LV (mL)	LV/100 g	L _M (μ m)	ISA (cm ²)	ISA/100 g
Air control (n = 6)	19.8 $\pm$ 1.0	0.99 $\pm$ 0.06	5.01 $\pm$ 0.21	46.6 $\pm$ 1.4	850 $\pm$ 56	4303 $\pm$ 238
O ₂ -saline (n = 5)	17.2 $\pm$ 1.8†	0.76 $\pm$ 0.07†	4.50 $\pm$ 0.19	68.4 $\pm$ 5.1†	458 $\pm$ 18†	2726 $\pm$ 248†
O ₂ -DEX (n = 9)	17.3 $\pm$ 1.9†	0.79 $\pm$ 0.08†	4.61 $\pm$ 0.57	66.5 $\pm$ 3.8†	476 $\pm$ 48†	2769 $\pm$ 416†

* Ten-d-old rat pups from pregnant rats prenatally treated at 48 and 24 h before delivery with DEX or equivolume saline. Values are mean $\pm$ 1 SD for n rat lungs per group. Air DEX group values were statistically similar to the air control (saline) values shown. LV, lung volume; L_M, mean linear intercept (average airspace diameter); and ISA, lung internal surface area.

† $p < 0.05$ for O₂-exposed group vs air-controls.

lung surfactant and AOE systems suggested that perhaps both systems share common regulatory mechanisms. And, when pregnant rats were administered DEX at 48 and 24 h before delivery, it was found that rat fetuses delivered prematurely at 20 and 21 d gestation (term = 22 d) demonstrated significantly elevated lung levels of DSPC (expected), as well as significantly elevated SOD, CAT, and GP activities (hypothesized) compared to saline-treated control fetuses (2).

Because of this stimulatory effect of prenatal DEX treatment on the protective AOE system of the immature lung, we questioned whether prenatal DEX treatment might result in some more prolonged beneficial effects on the ability of the newborn animal to tolerate prolonged hyperoxia. And, as we report herein, the hyperoxic tolerance effect of prenatal DEX treatment resulted in superior survival of the prenatal DEX-exposed pups in >95% O₂ in nine of nine experiments compared to control newborn rats and significantly increased the composite percentage of survival from d 7 through 14 of O₂ exposure (Fig. 2). Similarly, signs of classic O₂ toxicity including lung edema and associated lipid peroxidation were significantly reduced in the prenatal DEX O₂ group compared to the control newborns in hyperoxia (Fig. 3).

To try to explain the basis for the improved O₂ tolerance of the prenatally treated DEX pups, we examined a variety of comparative parameters both at the time of birth and during hyperoxic exposure. The data in Tables 1 and 2 and Figure 1 fail to provide any explanation for the improved O₂ tolerance of the prenatal DEX newborns. There was no evidence at the time of

beginning high O₂ exposure for any group differences in lung structural maturation (alveolarization), lung tissue DSPC content, or pulmonary AOE activities.

During exposure to >95% O₂, we found similar increases in lung DSPC content in both groups of neonates. Similarly, the quantitative morphometric data, after 10 d in O₂, showed very similar O₂-induced lung alterations in the two experimental groups. The inhibition of normal lung development (alveolarization) in hyperoxia is considered a growth-specific effect of high O₂ rather than a classic pathologic finding. It's believed to be related to the inhibitory effect of O₂ on DNA, protein, and elastin synthesis and may occur in early postnatal lungs that are relatively free of severe O₂ toxicity pathology (31).

The key difference that was found between the prenatal DEX and the saline pups in O₂ was in the pulmonary AOE responses induced by hyperoxic exposure (Fig. 4). Although both experimental groups of newborns were able to mount an AOE response to O₂ challenge, the prenatal DEX pups demonstrated an earlier, more consistently enhanced AOE response to hyperoxia than the O₂ saline pups at each time interval tested. Thus, based on the various parameters that we examined, a possible explanation for the significantly improved O₂ tolerance of the prenatally DEX-treated newborns is the accelerated and increased AOE responsiveness of their lungs to >95% O₂ exposure. Hyperoxia disrupts the normal oxidant-antioxidant balance in (lung) cells because of markedly increased rates of O₂ free radical production (32, 33). The more rapidly and more completely this oxidant-antioxidant imbalance can be restored to normal by increasing the

Fig. 5. Light micrographs of lungs of air controls (A), O₂-prenatal DEX-treated rats (B), and O₂ control rats (C). Both groups of O₂-exposed rat pups demonstrate O₂-induced inhibition of normal lung structural development (alveolarization) compared to air controls. The lungs of the pups maintained in air (A) have many small alveolar airspaces apparent compared to the larger, more saccular airspaces of the O₂-exposed rat pups (>95% O₂; 10 d). In addition, lung of the O₂ control group (C) shows evidence of intraalveolar edema, which is absent from lung of O₂-prenatal DEX group lungs (B). Original magnification $\times 450$; hematoxylin and eosin stain.

endogenous antioxidant defenses, the more effectively O₂-induced lung injury can be ameliorated. However, even animals with elevated pulmonary AOE may die of progressive lung damage after more prolonged periods in hyperoxia. This is true of newborn rats (including the prenatal DEX treatment group)

as well as adult rats treated with exogenous AOE (liposomal SOD, CAT) to extend their survival time in >95% O₂. Presumably, the elevated AOE may still be insufficient to detoxify the increased quantity of O₂ radicals generated in >95% O₂, and/or specific vital intracellular sites of O₂ radical generation or injury (e.g. nuclear DNA) may be inaccessible to the protection offered by increased cytosolic or mitochondrial AOE activity (31, 34, 35).

Because of the protean nature of glucocorticoid actions, there may be other explanations for why prenatal DEX treatment can protect against the pathologic lung changes associated with hyperoxia (Fig. 3) and improve the survival of newborn rats in hyperoxia. Also, although prenatal DEX treatment results in a persistently more pronounced newborn animal AOE response to hyperoxic challenge, how this response in the postnatal period is mediated by exposure to DEX in the prenatal period is an unanswered question. An interesting study by Schellenberg *et al.* (36), who investigated the ontogeny of elastin and collagen development in the late fetal to 30-d-old rat lung, essentially raised this same question. When these investigators tested the effect of prenatal DEX treatment on the normal course of postnatal mesenchymal tissue development, they found that their prenatally DEX-exposed pups showed normal lung elastin contents at birth and at age 5 d, but significant decreases in lung elastin content spanning the period of postnatal d 10 to 20. Because, as in our newborns in O₂, this could not possibly be related to exogenous DEX still in the circulation ($t_{1/2}$ = 5 h in the rat), these authors referred in their discussion to the earlier ideas of Davies and Reid (37) that prenatal hormonal treatment can interfere at later times with the normal "developmental program" of the lung. Similarly, delayed onset CNS maturation effects associated with prenatal DEX treatment have recently been discussed in this same vein (38). Not only DEX, but any exogenous agent that either inhibits or stimulates immature (lung) development may serve to alter some facet of later (lung) development or (lung) responses to stressors (37).

Finally, one can speculate on the possible meaning of these experimental newborn animal studies of pulmonary O₂ toxicity in relation to the clinical situation. Prenatal glucocorticoid treatment for imminent premature birth may actually have a dual beneficial effect in accelerating fetal lung maturation of both the surfactant and the AOE system. The DEX-treated prematurely born infant would thus have an important antioxidant advantage over those equally premature infants born before DEX treatment could be used.

In this light, two recent clinical reports are of interest. These studies specifically looked at whether prenatal glucocorticoid treatment had any effect on the comparative incidence of BPD in treated *versus* untreated premature newborns (39, 40). Both reports clearly demonstrated a reduced incidence of BPD in relationship to prenatal glucocorticoid therapy, especially in infants of 1000–1500 g birth weight. Both authors speculate whether surfactant stimulation alone could really account for the reduced incidence of chronic lung disease, suggesting, again, that glucocorticoids may have additional effects on the premature lung including structural changes and diverse biochemical changes, many of which still remain unknown.

Addendum. In view of the large number of reports appearing in the recent pediatric literature about the clinical use of DEX postnatally to try to reduce the incidence and severity of BPD (41–44), it is very important to point out here that DEX treatment appears to act very differently on the course of experimental pulmonary O₂ toxicity when it is administered prenatally (present studies) than when it is administered postnatally during the period of high O₂ exposure. When used as a treatment agent during hyperoxia, there is very convincing evidence from animal studies in a variety of species that DEX treatment, rather than having a protective effect, in fact tends to markedly accelerate the development of O₂-induced lung damage and lethality (31, 45–47).

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