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MAGNITUDE OF HYPEROXIC STRESS AND DEGREE OF LUNG MATURITY DETERMINE THE NATURE OF PULMONARY ANTIOXIDANT RESPONSE IN THE GUINEA PIG

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The ability of the immature lung to induce antioxidant defences in response to hyperoxic stress was examined. Preterm guinea pigs (65 days gestation, term = 68 d) were exposed to either 21% O_2, 85% O_2 or 95% O_2 for 72 hours. Exposure to 85% O_2 increased lung catalase, glutathione peroxidase and manganese superoxide dismutase activities in comparison to air controls. Exposure to 95% O_2 resulted only in an increase in glutathione peroxidase activity. Bronchoalveolar lavage fluid GSH concentration was increased by a similar amount by both exposure regimes, while lung copper/zinc superoxide dismutase activity was unchanged by either treatment. Comparison of the antioxidant response of term and preterm animals exposed to 85% O_2 for 72 hours indicated a greater response in the lung of the preterm animals. Manganese superoxide dismutase activity was elevated in both term and preterm animals, while catalase and glutathione peroxidase activities were elevated only in preterm animals. The extent of microvascular permeability as indicated by bronchoalveolar lavage fluid protein concentration, was lower in preterm animals than in term animals. We conclude that the immature lung can respond to hyperoxic stress by antioxidant induction and that the nature of the response is dependent, in part, both on the severity of the stress and on the maturity of the lung.

KEY WORDS: Antioxidant enzymes, guinea pigs, prematurity, lung, oxidative stress.

ABBREVIATIONS

ANOVA	analysis of variance
BAL	bronchoalveolar lavage
CAT	catalase
DTNB	5,5′-dithiobis (2-nitrobenzoic acid)
DTPA	diethylenetriamine-pentacetic acid
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
GSSG	oxidised glutathione
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
O_2	oxygen
SOD	superoxide dismutase

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INTRODUCTION

Immediately following birth the lung experiences a number of major changes. Amniotic fluid is rapidly cleared from the airways through expulsion and reabsorption and is replaced by air. As a result, oxygen tension in the lungs increases approximately 5-fold, which in turn probably leads to a rapid increase in oxygen free radical production.[1,2] In full term infants, increased radical production does not appear to produce any serious pulmonary problems as they are adequately protected from oxidative stress by the pre-birth surge in lung antioxidant enzyme defences.[3,4] Following premature delivery however, many infants experience episodes of extensive lung injury, especially those dependent on respiratory support requiring supplemental oxygen.[5] As these infants experience increased oxidative stress it is widely believed that their lung injury results from an imbalance in the production of damaging oxygen free radicals and the availability of protective antioxidant defences.[3,5]

Neonatal animals from several species such as rat, rabbit and mouse, are known to be relatively tolerant to hyperoxic stress. The basis of this tolerance is believed to be their ability to respond to stress by increasing their antioxidant defences.[6–10] Those species which do not respond in this manner, guinea pig and hamster, die after only a few days of hyperoxia.[9,11] Likewise, most adult animals, irrespective of species, tend to die within a short period (3–5 days) following exposure to 95% oxygen with unchanged pulmonary antioxidant activities at the time of death.[9,12] Adult animals can acquire tolerance to 95% oxygen if they are first pre-exposed to a period of 85% oxygen, during which time antioxidant defences are induced.[13–15]

It is currently assumed that the ability to respond to hyperoxic stress through pulmonary antioxidant induction is the basis for decreased oxidative lung injury and subsequent survival during further hyperoxic exposure. The logical extension of this hypothesis is that the ability to withstand oxidative stress is more dependent on the response of antioxidant induction rather than initial antioxidant defence levels.[8,9]

This concept has in turn led investigators to re-examine lung injury in the preterm infant which has low initial antioxidant protection and often experiences oxidative-related lung injury. It is not however presently clear if the immature lung of the preterm infant has the capacity to respond to oxidative stress through antioxidant induction. As it is not possible to address this problem directly it is appropriate to question whether any animal study can provide the answer. Many different species have been used to examine respiratory problems of the newborn. To address this specific question it will be important to use a species whose pattern of lung development closely resembles that of the preterm infant. Recently, we have developed and validated a model of oxidative-induced lung injury in the preterm guinea pig.[16] Of all the small animal species used to study oxidative-induced lung injury the guinea pig is probably the most suitable. Guinea pigs have advanced pre-natal morphological development yet biochemically the surfactant and antioxidant systems remain relatively immature until just prior to birth.[17–21] These features have led several investigators to suggest that the pattern of lung development in the guinea pig more closely resembles human lung development than many other frequently used animal models.[18,19] In the present study we have used the guinea pig model of prematurity to determine if the immature lung is capable of responding to oxidative stress by antioxidant induction.

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MATERIALS AND METHODS

Reagents

All reagents were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.) unless otherwise stated.

Animals

Virgin female Hartley strain guinea pigs (500 g) obtained from our own colony were caged in pairs in a room controlled for temperature (22°C) and light (12 h), with free access to food and water. Timed pregnancies (± 1 day) were achieved by taking daily vaginal smears from each female to establish her oestrus cycle and by introduction of a male prior to the next ovulation. The date of ovulation in the successfully fertilized cycle was taken as day zero. The normal gestational period ends on day 68.

Guinea pig pups were delivered by Caesarian section at day 65 of gestation (normal gestation is 68 days) under halothane anaesthesia (2–4%) maintained with nitrous oxide (0.4 l/min) and oxygen (1.5 l/min). Pups were delivered by hysterotomy, the oropharynx cleared and the umbilical cord double clamped and cut. The dam was then sacrificed by exsanguination. Following delivery the pups were dried in a stream of warm air, weighed and placed with a lactating surrogate dam into 25 l perspex chambers containing ample food, water and hay. Cages were changed daily, the pups spending no more than 2 minutes out of the chamber. Where appropriate, a number of pregnancies were allowed to proceed to day 68 and the pups (term animals) delivered by Caesarian section were used for comparison to preterm (day 65) pups.

Experimental protocols

Experiment 1. Preterm pups were randomly allocated to hyperoxic (85% O_2 or 95% O_2) or normoxic (21% O_2) exposure for 72 h. Oxygen exposures were achieved by supplying the cage with humidified oxygen (BOC; medical grade). Oxygen concentration was monitored continuously (IL 407 apparatus; Instruments Laboratory, Lexington, MA, USA) and set by blending in appropriate flows of nitrogen to achieve a final flow rate 3 litres/min. The CO_2 concentration was determined 1 h after commencement of the experiment and just before the termination of each exposure; it never exceeded 0.5% (PA 404; Servomax, Crowborough, Sussex, UK). Relative humidity ranged from 50–70% and temperature between 23 and 25°C. Control animals were kept in identical cages supplied with room air at 3 litres/min. As adult animals are very susceptible to 95% O_2, dams in these experiments were changed daily to avoid fatal oxygen toxicity.

Experiment 2. Term and preterm pups were exposed to either 85% O_2 or 21% O_2 for 72 h, for determination of their relative ability to mount an induction of antioxidant enzymes, and to assess their relative susceptibility to hyperoxic lung damage.

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Bronchoalveolar lavage (BAL)

At the end of the 72 h period, guinea pig pups were anaesthetized by intraperitoneal injection of pentobarbital (50 mg/kg). Following onset of adequate anaesthesia, the trachea was exposed, a tracheotomy performed and a 14 gauge cannula inserted and secured. The animals were then exsanguinated by abdominal aortic section and the pulmonary circulation perfused with 5 ml of 0.9% NaCl (saline) at 37°C. The lungs were then lavaged *in situ* with five, 2 ml aliquots of sterile saline at 37°C, of which 80–90% was recovered. Lavage cells were pelleted by centrifugation at 200 g for 10 min at 4°C. The resultant supernatant was divided into 0.5 ml aliquots, two of which were used immediately for the determination of total GSH and GSSG concentrations while the remainder was frozen at −70°C for subsequent analysis of total protein content.[22] The lungs were then dissected from the thoracic cavity, washed in saline, blotted dry, weighed and frozen at −70°C for subsequent analysis of DNA concentration and antioxidant enzyme activities.

In those instances where fetal tissue was required for antioxidant reference points (65 and 68 day) pups were anaesthetized 30 minutes following delivery by Caesarian section. These pups were then lavaged as described above and the tissue frozen at −70°C for subsequent antioxidant enzyme analysis.

Glutathione analysis

Fresh BAL fluid supernatant was assayed undiluted for total GSH (reduced and oxidised) and GSSG concentrations by the method of Griffith.[23] Briefly, 0.2 ml of sample or freshly made standard (0.1–5.0 nmol/ml) in duplicate were equilibrated with 0.1 ml 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.7 ml NADPH at 30°C for 5 min. Glutathione reductase (0.9 Unit) was added to each sample, vortexed for 5 sec and the formation of TNB monitored as an increase in absorbance at 412 nm over 1 min at 30°C, pH 7.5 in a Ultraspec II spectrophotometer (LKB, S. Croydon, Surrey). For the measurement of GSSG alone, samples were incubated with 0.01 ml of 2-vinyl pyridine at room temperature for one hour and then assayed as described for total GSH. The reduced glutathione content of the samples was calculated by subtracting the GSSG content from the total glutathione content.

Total and differential leukocyte counts

Total nucleated cell count in BAL was performed using a Neubauer haemocytometer. Cytospin preparations were stained with May-Grunwald-Giemsa and differential cell counts performed on 300 cells. Results are expressed as the total number of cells per ml of lavage recovered from each animal.

Tissue preparation

Frozen tissue samples (200 mg) in 10 vols of 0.01 M potassium phosphate, 0.03 potassium chloride buffer were homogenized on ice by two 15 sec bursts of an Ultraturrax (Janke & Kunkel, Staufen, Sweden). The resultant homogenate was then

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sonicated (MSE Soniprep) at 14 microns with six 10 sec bursts and an aliquot removed for DNA analysis.[24] The remaining homogenate was then incubated for 30 min with 1% (v/v) absolute alcohol and mixed with 1% (v/v) Triton x-100. Following centrifugation (10,000 g, 5 min), supernatants were removed and frozen overnight at −70°C before analysis of antioxidant enzymes.

Antioxidant enzyme analysis

Total SOD activity, that is both Cu/Zn-SOD and Mn-SOD, were determined by the pyrogallol autoxidation method.[25] The final reaction mixture contained 0.1 M Tris-HCl buffer (pH 8.2), 1 mM diethylenetriaminepentacetic acid (DTPA), 0.1 μM CAT and 0.88 μM pyrogallol in 0.01 mM HCl. The autoxidation of pyrogallol was followed continuously in a reaction rate spectrophotometer (LKB Mk II Kinetic analyzer, Croydon, Surrey) at 25°C for 2 min. Activities were determined by calculating the percent inhibition of pyrogallol autoxidation. Percent inhibition values for the tissues were converted to activities using a purified Cu/Zn-SOD standard of known activity. One unit of SOD was defined as the amount of enzyme required to halve the rate of substrate oxidation. Mn-SOD activities were determined by the addition of cyanide, at a final concentration of 2 mM, to the assay buffer, this inhibited Cu/Zn-SOD by greater than 90%. The percent inhibition values were determined and converted to activities using a Mn-SOD standard (E. Coli Mn-SOD).

GSH-Px activity was determined using a coupled assay in which enzyme activity was proportional to the rate of NADPH oxidation.[26] The final reaction mixture contained 0.01 M Tris-HCl (pH 8.0), 5 mM EDTA, 0.23 mM NADPH, 2 mM GSH, 1 unit/ml glutathione reductase and 0.33 mM t-butylhydroperoxide. The oxidation of NADPH was followed at 360 nm with a reaction rate analyzer (LKB Mk II Kinetic Analyser, S. Croydon, Surrey) at 37°C for 2 minutes. The activity of GSH-Px was calculated using the molar extinction coefficient for NADPH ($\varepsilon = 6.22 \times 10^3$ $M^{-1}\,cm^{-1}$. One unit of enzyme activity was defined as one μmol of NADPH oxidised/min at 37°C.

CAT activity of tissue supernatants was determined by following the catalytic reduction of hydrogen peroxide at 240 nm.[27] The decomposition of hydrogen peroxide was monitored continuously using a spectrophotometer (LKB Ultraspec II, Croydon, Surrey, UK). The final reaction mixture contained 0.01 M sodium phosphate buffer (pH 7.4) and 10 mM H_2O_2. Units of CAT were calculated from the extinction coefficient of H_2O_2 at 240 nm ($\varepsilon = 43.6\,M^{-1}\,cm^{-1}$). CAT units were defined as μmols of H_2O_2 decomposed/min at 20°C.

All enzyme activities were determined within 3 months of tissue collection and storage at −70°C during which time activity losses were less than 10%. The following coefficients of variation were determined for each enzyme assay: CAT, 9.2%; total SOD, 11.6%; Mn-SOD, 13%; GSH-Px, 2.7%.

Statistical methods

When two groups were compared an unpaired t-test was used. For comparison of more than two groups, analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used. Survival curves were compared using a Mantel-Haenszel test. A p value of less than 0.05 was considered statistically significant.

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RESULTS

Experiment 1. Comparison of 85% and 95% O_2 exposure regimes in preterm guinea pigs

Survival. All preterm pups delivered at 65 days gestation (3 days premature) developed respiratory distress, characterized by tachypnoea (98 ± 10 breaths/min), which usually resolved within 4–6 h of delivery (71 ± 5 breaths/min). Exposure of preterm pups to 21% or 85% O_2 for 72 h did not result in any further breathing difficulties. Exposure to 95% O_2 for longer than 24 h resulted in renewed respiratory distress characterized by tachypnoea (139 ± 27 breaths/min), cyanosis and rib retraction. No difference in the survival rates of preterm animals exposed to 21% or 85% O_2 was noted, with 10/12 and 8/11 pups surviving 72 h respectively. Mortality of pups exposed to 95% O_2 was higher as 5/12 animals died, but this failed to reach statistical significance.

Lung injury. BAL fluid protein content was used as an index of pulmonary microvascular permeability and hence tissue injury. We have previously shown in this model, that following acute lung injury there is an influx of plasma proteins, primarily albumin, to the airways.[16] In the present study the protein content of BAL fluid from preterm animals exposed to 95% O_2 for 72 h was significantly higher than that of pups exposed to 21% or 85% O_2 for the same period (Table I). Pups exposed to 85% O_2 did not show any increase in BAL fluid protein content. Consistent with this apparent lack of lung injury the lung weights of animals in 85% O_2 were similar to those of 21% O_2 exposed pups (Table I). In 95% O_2, lung weight was significantly increased ($P < 0.002$). The number of neutrophils recovered in lavage fluid was significantly increased ($p < 0.05$) in pups exposed to both 85% and 95% O_2 (Table I).

Antioxidant response. Lung Mn-SOD activity fell 29% *in utero* over the final three days of gestation (Figure 1). If pups were delivered prematurely and maintained in 21% O_2 or 85% O_2 for 72 hours, lung Mn-SOD activity decreased by 64% and

TABLE I
Lung weight and BAL fluid protein concentration and neutrophil number in preterm pups following 72 h exposure to different oxygen concentrations

O_2 exposure	n	Lung wet weight (g)	Protein (μg/ml)	Neutrophils (10^4/ml)
21%	(10)	1.84 ±0.10	240.9 ±48.2	1.11 ±0.81
85%	(8)	1.96 ±0.14	252.7† ±57.1	4.2* ±2.2
95%	(7)	2.30** ±0.12	1096.7** ±588.8	4.4* ±2.1

Values are means ± S.D. for (n) samples per group.
*$P < 0.05$, **$P < 0.002$ for pups in hyperoxic vs air.
†$P < 0.005$ for 85% O_2 vs 95% O_2.
ANOVA: BAL fluid protein; 95% significantly different to 21% and 85% (2,19). $F = 16.18$, $P < 0.001$.
BAL fluid neutrophils; 95% and 85% significantly different to 21% (2,21). $F = 4.23$, $P < 0.05$.
Lung weight; 95% significantly different to 21% and 85% (2,24) $F = 30.25$, $P < 0.001$.

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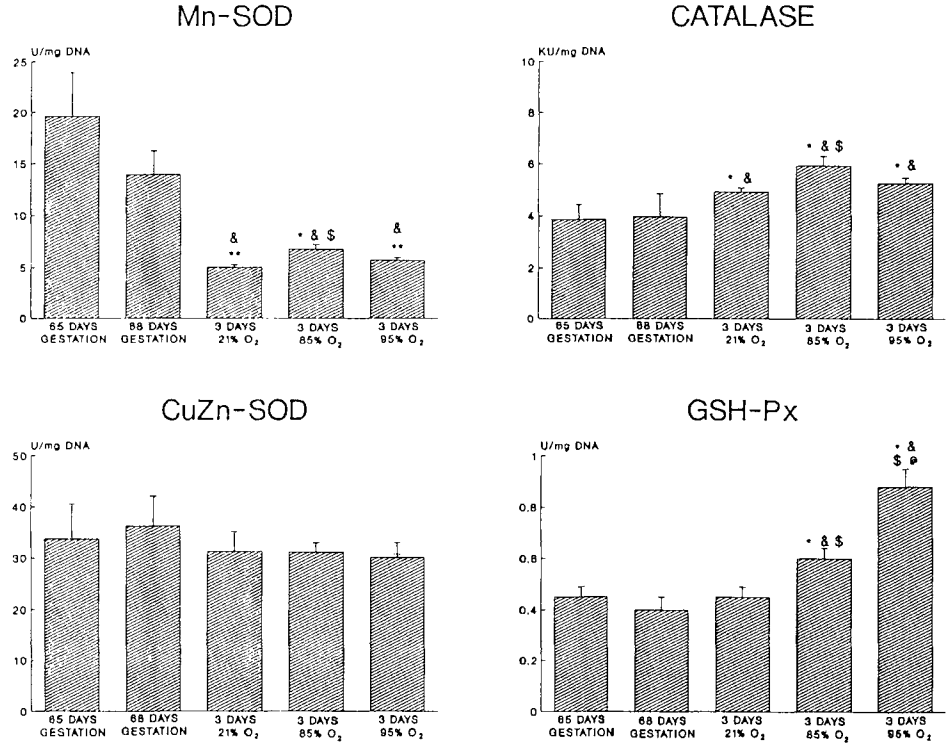

FIGURE 1 Comparative antioxidant enzyme responses of the immature lung of 3 day premature guinea pigs exposed to either 21%, 85% or 95% O_2 for 72 h. The 65 day and 68 day *in utero* activities are provided for comparison. Results are expressed as means ± S.D., n = 7–9 per group. *$p < 0.05$, **$p < 0.01$ compared with 65 day *in utero*; [&] $p < 0.05$ compared with 68 day *in utero*; [$] $p < 0.05$ compared to 21% O_2; [@] $p < 0.05$ compared with 85% O_2. ANOVA: Mn-SOD, significant effect of treatment (3,32) $F = 47.12$, $P < 0.001$. CAT, significant effect of treatment (3,38) $F = 9.30$, $P < 0.001$. GSH-Px, significant effect of treatment (3,28) $F = 3.25$, $P < 0.05$.

51% with respect to the 68 d *in utero* value ($p < 0.05$). The decrease in lung Mn-SOD activity in 85% O_2 was significantly less than in air ($p < 0.05$). Exposure of preterm animals to 95% O_2 for 72 h resulted in a 59% decrease in Mn-SOD activity (Figure 1). No change in lung Cu/Zn-SOD activity was observed either *in utero* between 65 and 68 days gestation, or following premature delivery at 65 days gestation and maintenance in 21%, 85% or 95% O_2 (Figure 1).

Pulmonary catalase activity did not change over the final three days of gestation *in utero* (Figure 1). Following premature delivery and maintenance in either 21% O_2 or 85% O_2 for 72 hours, lung catalase activity increased 24% and 49% respectively, the increase being significant in both cases ($p < 0.05$). Maintenance of preterm pups in 95% O_2 also significantly increased pulmonary catalase activity with respect to the activity at birth ($p < 0.05$).

Lung GSH-Px was not significantly altered from the day 65 activity following either 3 further days *in utero*, or 3 days in 21% O_2 (Figure 1). Exposure to 85% O_2 or 95% O_2 for 3 days increased lung GSH-Px activity 50% and 120% respectively

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with respect to the 68 d *in utero* value (Figure 1; $p < 0.05$). Pups placed in 85% O_2 or 95% O_2 at birth showed a rapid rise in BAL fluid glutathione content (Figure 2). This increase was due primarily to an increase in GSH concentration ($p < 0.05$). GSSG accounted for approximately 5% of the total glutathione concentration and the ratio of GSH/GSSG remained relatively constant in all groups (BAL GSSG: 21% O_2, 0.010 ± 0.001 μM; 85% O_2, 0.018 ± 0.002 μM; 95% O_2, 0.027 ± 0.003 μM).

Experiment 2. Comparison of the antioxidant response in preterm and term pups exposed to 85% O_2

Survival. No significant difference in survival rates of preterm and term animals exposed to 85% O_2 was noted with 6/8 and 10/12 pups surviving the 72 h exposure period. In 21% O_2 one preterm died, while all term pups survived.

Lung injury. Following exposure to 85% O_2, BAL fluid protein content and lung weight were unaltered in preterm pups. As found previously, there was an influx of neutrophils to the lung (Table II). In term animals, the same hyperoxic stress resulted in elevated BAL fluid protein content ($P < 0.05$), increased lung weight ($P < 0.01$) and an influx of neutrophils to the lung ($P < 0.01$).

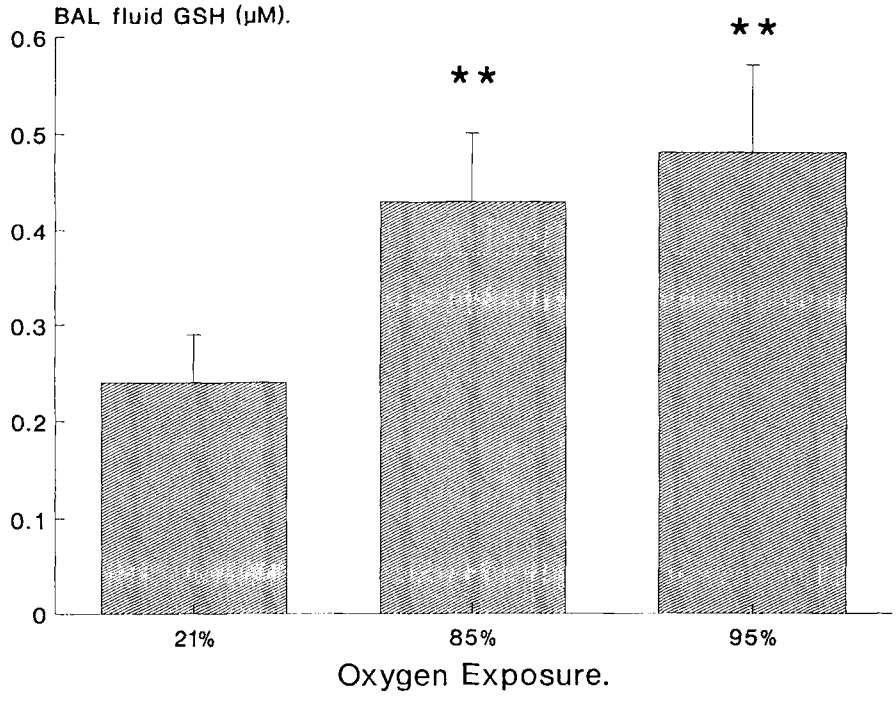

FIGURE 2 Comparison of the BAL fluid glutathione content in the immature lung of 3 day premature guinea pig pups exposed to 21%, 85% or 95% O_2. Results are expressed as means ± S.D., n = 7–10 per group. **$p < 0.002$ for pups in hyperoxia vs air. ANOVA: 85% and 95% significantly different to 21% (2,19) $F = 16.18$, $P < 0.001$.

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TABLE II
Lung weights and BAL fluid protein concentration and neutrophil number in preterm and term pups following 72 h exposure to either 21% of 85% O_2

	O_2 exposure	n	Lung wt. (g)	Protein (mg/ml)	Neutrophils (10^4/ml)
Preterm	21%	(7)	1.77 ±0.08	187.8 ±53.6	0.78 ±0.41
	85%	(6)	1.78 ±0.11	237.4 ±41.6	3.89 ±1.76
Term	21%	(5)	2.04† ±0.13	111.4 ±22.8	0.53 ±0.26
	85%	(10)	2.41** ±0.10	514.5* ±238.8	2.84* ±2.49

Values are means ± S.D. for (n) samples per group.
*$P < 0.05$, **$P < 0.01$ for pups in hyperoxia vs air.
†$P < 0.05$ for preterm in air vs term in air.
ANOVA: BAL fluid protein; Significant effect of O_2 (1,27) $F = 15.00$, $P < 0.001$. Interaction between O_2 exposure and gestation, (1,27) $F = 9.17$, $P < 0.01$.
BAL, neutrophils; Significant effect of O_2 (1,27) $F = 5.79$, $P < 0.05$.
Lung weight; Significant effect of O_2 (1,26) $F = 5.77$, $P < 0.05$. Significant effect of gestation (1,26) $F = 28.97$, $P < 0.001$.

Antioxidant response. Exposure of term guinea pigs to 85% O_2 for 72 h produced a significant increase in pulmonary Mn-SOD activity ($p < 0.05$). Lung Cu/Zn-SOD, CAT and GSH-Px were unchanged (Figure 3). In comparison, 72 h exposure to 85% O_2 in preterm pups lead to significant increases in GSH-Px and CAT activities ($p < 0.05$). Pulmonary Mn-SOD activity was 35% greater in pups exposed to 85% O_2 versus 21% O_2 ($p < 0.001$). However, as shown in Experiment 1 this did not represent an induction but rather a smaller decrease in Mn-SOD activity with respect to the *in utero* value.

DISCUSSION

The primary aim of this study was to determine if the lung of the preterm guinea pig was capable of responding to hyperoxic stress by antioxidant enzyme induction. Our findings indicate that the immature lung can mount a protective response to oxidative stress and that the nature of the response is dependent, in part, on the magnitude of the stress and on the degree of lung maturity. Excluding the alveolar glutathione pool, the antioxidant changes observed in the preterm animal following exposure to 85% O_2 exceeded those following a similar period of 95% O_2. The magnitude of the stress is therefore particularly important in dictating the acute pulmonary response. Although survival as an end point was not considered in the present study, evidence from previous studies would indicate that those animals which responded to the oxidative stress by antioxidant induction would be in a better position to withstand further oxidative stress.[8,9,11] In the present study, lung injury, as determined by increased microvascular permeability and lung weight was noted in those pups exposed to 95% O_2, but not those exposed to 85% O_2 in which, with the exception of the glutathione pool, the greater antioxidant response was observed.

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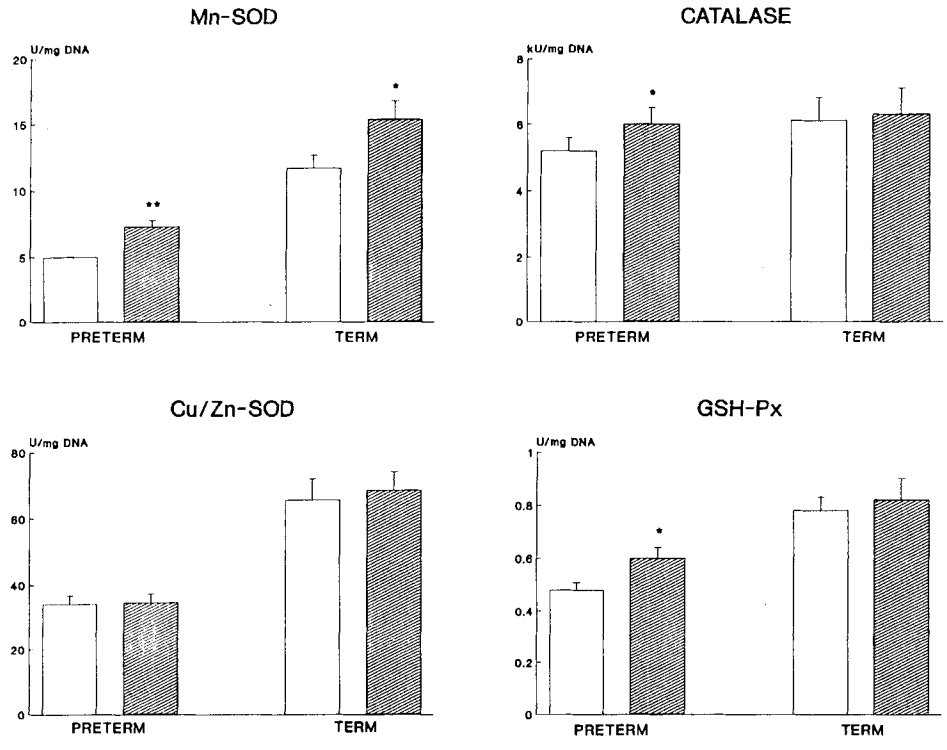

FIGURE 3 Comparative pulmonary antioxidant enzyme responses of preterm and term guinea pigs to either 21% O_2 (open blocks) or 85% O_2 (closed block). Results are expressed as means ± S.D., n = 6–8 group. *$p < 0.05$, **$p < 0.001$ 21% O_2 vs 85% O_2.

The observed increases in the extracellular concentration of lung glutathione in the present study agree well with previous work in the developing rat lung.[28]

Both exposure regimes (85% and 95% O_2) led to a marked influx of neutrophils to the lung, the magnitude of the response being similar in each case. Although it has been widely suggested that polymorphonuclear leukocytes contribute to oxidative induced lung injury, through both the generation of reactive oxygen species and the release of proteolytic enzymes[29,30] there is equally compelling evidence to suggest that they are not involved in acute lung injury.[31,32] In view of the observation that increased neutrophil numbers occur in the absence of lung injury the results of the present study do not support a role for the neutrophil in oxidative-induced acute pulmonary injury of the immature lung.

Of the four antioxidant enzymes studied, Cu/Zn-SOD was found to be the least responsive to oxidative stress. No change in Cu/Zn-SOD activity was noted following exposure to either 85% or 95% O_2. In contrast, Mn-SOD activity was increased following both 85% and 95% O_2 compared to 21% O_2. Comparison of Mn-SOD activity with the *in utero* values at 65 days or 68 days gestation revealed that in fact all *ex utero* treatments resulted in a marked decrease in Mn-SOD activity. Exposure to 85% O_2 simply reduced the loss of Mn-SOD activity as opposed to increasing

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the activity of this antioxidant. We have previously observed similar complicated changes in pulmonary Mn-SOD activity just prior to, and following birth in the guinea pig.[21]

A critical balance point, therefore, appears to exist around 90% O_2. Below this oxygen concentration, the stress appears sufficient to trigger an improvement in antioxidant defences which in turn help to reduce lung injury. Above this oxygen concentration, the antioxidant response is less and lung injury develops more rapidly. It is however worth remembering that the lung is an extremely complex tissue containing up to 40 different cell types, any number of which may be contributing to these observed changes in antioxidant enzyme status. In the present study no attempt was made to identify which cell type(s) were responsible for the changes in antioxidant activity.

Previously, Sosenko and Frank[20] investigating the relative intolerance of new born guinea pigs to hyperoxia (>95% O_2), reported that term guinea pigs did not respond to hyperoxic stress by antioxidant induction but that preterm guinea pigs did. In keeping with the antioxidant induction theory, preterm animals had superior tolerance to hyperoxia than term animals. The difference in response to hyperoxic stress was attributed by these investigators to the difference in lung maturity between the two ages of animal examined. Although only a matter of days older, lung development in the term guinea pigs is considerably advanced over the preterm animals as rapid maturation of both the surfactant and antioxidant systems occur over the final few days of gestation.[20,21,23] As the extent of the hyperoxic stress is apparently important in determining the nature of the pulmonary antioxidant response in preterm pups we investigated whether a reduced (i.e. 85% O_2) hyperoxic stress would lead to antioxidant induction in term animals with more mature lungs.

Hyperoxic stress provoked an antioxidant response in the preterm animal as discussed above, with induction of CAT and GSH-Px, and a reduced loss of Mn-SOD activity relative to day 68 (seen as an apparent increase compared to 21% O_2 exposed animals). In term pups the response was less marked, with only an induction of Mn-SOD being noted. Lung injury as determined by the protein content of BAL fluid, was evident in term animals but not in preterm animals following 72 hours 85% O_2. However, both age groups had a marked influx of neutrophils to the lung. It would therefore appear that in the lungs of preterm pups an induction of antioxidant enzymes occurs during 85% O_2 exposure, which is of sufficient magnitude to protect the lungs from hyperoxic insult. In the term animal the absence of an adequate antioxidant response leads to an increased lung injury.

Frank and Sosenko[34] recently examined the ability of preterm and term rabbits to respond to oxidative stress (<90% O_2). They found that while term rabbits exhibited antioxidant enzyme induction, preterm rabbits did not and subsequently exhibited signs of increased lung injury. Frank and Sosenko used these findings to suggest that antioxidant induction does not occur in the lung of the preterm infant and that this may be why these infants are excessively prone to oxygen-induced lung injury and subsequently, develop chronic lung disease. This hypothesis depends heavily on the suitability of the preterm rabbit as a model of oxidative injury in the human infant. Previous studies have shown that the lung of the rabbit is less mature than that of the human at birth.[35] With the observations made in the current study and those made previously by Sosenko and Frank[34] and many others[6–10] it would appear that the stage of lung development is very important in determining the nature of pulmonary antioxidant response to hyperoxic stress.

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A second study, recently published by Jenkinson and colleagues[36] examined the ability of preterm baboons ventilated with 100% O_2 to induce pulmonary antioxidant enzymes. The findings of this study, in what is probably the most appropriate model of oxidative lung injury in the preterm infant, provide further evidence that the immature lung is unresponsive, in respect of antioxidant induction, to very high oxidative loads (i.e. 100% O_2). Interestingly, they did find that antioxidant induction did occur in those premature baboons treated with allopurinol, a xanthine oxidase inhibitor.

In summary, our present findings indicate that the immature lung of the guinea pig can mount a protective antioxidant response. The nature of this response is dependent in part on the magnitude of the stress and the state of lung maturity. If these findings can be applied to the preterm infant they support the common sense approach already in operation in special care units, that the minimum amount of oxygen should be used to sustain these infants. This approach will not only maintain viability but will provide the infant with the greatest chance of increasing its endogenous pulmonary antioxidant defences to protect itself against the detrimental side effects of prolonged oxygen therapy.

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