Biochemical Pharmacology, Vol. 46, No. 9, pp. 1565–1572, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

DEXAMETHASONE TREATMENT FAILS TO REDUCE OXYGEN-INDUCED LUNG INJURY IN THE PRETERM GUINEA PIG

EFFECTS ON PULMONARY INFLAMMATION AND ANTIOXIDANT STATUS

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(*Received* 4 *May* 1993; *accepted* 19 *July* 1993)

Abstract—Dexamethasone (10 mg/kg/day) or vehicle was administered in a randomized, controlled fashion to 3-day preterm guinea pigs exposed to either 21% oxygen or 95% oxygen for 72 hr and maintained in room air for a further 96 hr. Treatment with dexamethasone had no effect on survival of preterm pups maintained in either 21% or 95% O_2. Dexamethasone treatment reduced the growth rate of pups, the effect occurring earlier (0–3 days) in 21% O_2-treated pups than in 95% O_2-treated pups (5–7 days). Exposure to 95% O_2 reduced the survival rate of preterm animals (73% vs 100%, $P < 0.05$). Surviving pups developed acute lung injury, characterized by the accumulation of a protein-rich exudate in the alveoli and an infiltration of inflammatory cells, particularly neutrophils into the lung. Dexamethasone treatment attenuated the pulmonary inflammatory cell infiltration, in particular neutrophils, both during oxygen exposure (16.4×10^4 vs 9.4×10^4/mL; $P < 0.05$) and following return to ambient conditions (28.0×10^4 vs 5.1×10^4/mL; $P < 0.05$). Elastase activity in bronchoalveolar lavage fluid, which was primarily of neutrophil origin, was unchanged by dexamethasone treatment. Dexamethasone-treated pups had increased pulmonary antioxidant enzyme activities (Cu/Zn-superoxide dismutase; Mn-superoxide dismutase, catalase and glutathione peroxidase) during recovery from oxidative injury. Although there was both a marked reduction in numbers of neutrophils in the lung and elevated pulmonary antioxidant enzyme activities in dexamethasone-treated pups, the degree of microvascular permeability, as determined by both the lung wet weight/dry weight ratio and the presence of plasma proteins in the lavage fluid, was unchanged. Combined, these results imply that dexamethasone, although capable of blunting the influx of neutrophils to the hyperoxia-exposed lung and inducing antioxidant defences in the immature lung, cannot modify the progression of acute oxygen-induced injury of the immature lung.

The pathogenesis of chronic lung disease (CLD‡) in the preterm infant is complex and almost certainly multifactorial. Contributing factors are thought to include barotrauma associated with positive pressure ventilation [1, 2] and free radical-mediated tissue injury as a consequence of hyperoxic exposure of lungs with deficient antioxidant defences [3–6]. Additional factors probably include pulmonary inflammation [7], inadequate synthesis and release of surfactant [8, 9], and relatively poor nutrition [10, 11].

Lung function of infants with developing or established CLD has been found to improve following the administration of the potent synthetic glucocorticoid dexamethasone [12–14]. The mechanism for such a benefit has yet to be established, although possible effects include an anti-inflammatory action [7], an effect on fluid balance [15, 16], or an enhanced maturation of pulmonary surfactant and antioxidant systems [17, 18]. Progress towards a better understanding of the mechanism of action of dexamethasone in CLD and hence agreement on the most appropriate dosage regimen to be used has been slow, due mainly to the practical and ethical difficulties of working with these very sick infants. Studies are clearly needed both to evaluate the mechanism of corticosteroid action and to determine the optimal strategies for CLD prevention.

We recently reported the development and validation of a small animal model of prematurity using the preterm guinea pig [19]. This model demonstrates a number of parallels with the human preterm infant including susceptibility to respiratory distress following premature delivery, immature antioxidant [20] and surfactant systems [21], and the development of pulmonary inflammation following exposure to high oxygen tensions [19], all of which have been proposed as contributing factors in the development of CLD. In the present study we have

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‡ Abbreviations: BAL, bronchoalveolar lavage; CLD, chronic lung disease; EIC, elastase-inhibitory capacity; GSH-Px, glutathione peroxidase; MEOSAAPVNA, *N*-methoxysuccinyl-ala-ala-pro-val-*p*-nitroanilide; NE, neutrophil elastase activity; PPE, porcine pancreatic lipase; SOD, superoxide dismutase.

used this model to examine the effects of dexamethasone on two of these parameters, pulmonary inflammation and antioxidant expression in the immature lung.

MATERIALS AND METHODS

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

Experimental protocol. The guinea pig model of prematurity has been described in detail previously [19]. Briefly, guinea pig pups were delivered by Caesarian section after 65 days gestation (normal term is at 68 days gestation) and were immediately handled and dried in a stream of warm air. Up to six preterm pups and a lactating surrogate female were housed in 25 L sealed perspex cages supplied with dry gas mixtures. Animals were randomly divided into two principal experimental groups, one to receive 95% oxygen for 72 hr and then 21% oxygen for a further 4 days, and the other to receive 21% oxygen for the whole 7 day period.

As the guinea pig is relatively insensitive to the pharmacological effects of corticosteroids [22, 23], we sought to establish a treatment regimen which would provide equivalent physiological and biochemical responses to that seen in human infants. Pilot studies determined that a dose of 10 mg/kg/day resulted in a peripheral blood leukocytosis, neutrophilia and eosinopenia, all well-recognised effects of glucocorticoid administration [24]. Although the dose chosen was 5–10 times that normally administered to the preterm human infant, it had no adverse effect on lung growth (illustrating the insensitivity of the guinea pig to glucocorticoids); however, as expected there was a marked reduction in spleen weight.

One randomly selected subgroup from each arm of the study was treated with a single daily subcutaneous dose of 10 mg/kg dexamethasone phosphate (Organon Laboratories Ltd, Cambridge, U.K.), while the remaining animals received an equivalent volume of saline. Subgroups of animals were removed at random for bronchoalveolar lavage (BAL) (N = 6–9) or determination of lung wet weight/dry weight ratio (N = 3) at 3, 5 and 7 days after delivery.

Wet weight/dry weight ratio. Following anaesthesia the thorax was opened and the lungs dissected free. After washing in saline and blotting dry, the tissue wet weight was determined. The lungs were then placed in tared plastic tubes and freeze-dried, to obtain lung dry weights. The ratio of lung wet weight to dry weight was calculated for each treatment group.

BAL. Guinea pig pups were anaesthetized by intraperitoneal injection of pentobarbital (50 mg/kg). After the 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 the abdominal aortic section and the pulmonary circulation perfused with 5 mL of saline at 37°.

The lungs were lavaged *in situ* with five 2 mL aliquots of sterile saline (37°). The lavage cells were then pelleted by centrifugation at 200 *g* for 10 min at 4°. The supernatant was divided into 0.5 mL aliquots and frozen at −70° for subsequent analysis of total protein, neutrophil elastase activity (NE) and elastase-inhibitory capacity (EIC). The lungs were then dissected from the thoracic cavity, washed in saline and blotted dry, weighed and frozen in liquid nitrogen for later antioxidant enzyme analysis.

Total and differential leukocyte counts. A total nucleated cell count in BAL was performed using a Neubauer haemocytometer. Cytospin preparations were stained with May-Grunwald-Giemsa and differential cells counts performed on 300 cells. Results are expressed as the total number of cells per millilitre of lavage fluid recovered from each animal following BAL. No correction was made for the recovery of lavage as this was uniformly 85–90%. Similarly, total and differential leukocyte counts were performed on blood taken by cardiac puncture prior to exsanguination.

Assessment of microvascular permeability. Changes in vascular permeability were quantified by determining the protein content of lavage fluid. We have previously shown that approximately 50% of total protein recovered in BAL from oxygen-exposed guinea pigs is plasma albumin [19]. Total protein was measured in the unconcentrated lavage fluid using the bicinchoninic acid assay [25]. Absorbance was measured at 562 nm with a Dynatech MR 580 reader using bovine serum albumin standard. Results are expressed as milligrams of protein per millilitre of lavage fluid recovered.

NE. The NE of BAL was assessed using the synthetic substrate *N*-methoxysuccinyl-ala-ala-pro-val-*p*-nitroanilide (MEOSAAPVNA). NE was determined in triplicate against a range of active site-titrated standards of porcine pancreatic elastase (PPE) (0.72–2.16 ng/mL) as described previously [26]. BAL fluid or standard (50 μL) and 100 μL of buffer (0.2 M Tris–HCl, pH 8.0) were preincubated in individual wells of a 96-well microtitre plate at 37° for 15 min. Prewarmed MEOSAAPVNA (50 μL; 2 mM in buffer) was then added to each well and the plate covered with aluminium foil and incubated at 37°. The absorbance at 410 nm was determined at 0, 24, 48 and 72 hr with a Dynatech MR 580 plate reader. Linear regression analysis was performed on the standards, which were linear over the 24 hr period, and a plot of change in absorbance per hour against PPE concentration was obtained. Similar rates were determined for BAL samples and the NE calculated from the standard plot. The data are expressed as picomoles of PPE per millilitre of BAL.

EIC. The capacity of BAL to inhibit the degradation of the synthetic substrate succinyl-l-trialanine *p*-nitroanilide (SLAPN) by exogenously administered PPE was determined as described previously [19].

Lung antioxidant enzyme analysis. Lung samples were prepared as described previously [20]. Following preparation, 80–90% of enzyme activities are typically recovered. Glutathione peroxidase (GSH-Px) activity was determined by the method of Beutler [27]. Total superoxide dismutase (SOD) (Cu/Zn-SOD and Mn-SOD) and Mn-SOD activities were determined by the pyrogallol autoxidation method of Marklund [28]. Cu/Zn-SOD was calculated as the

Table 1. Growth rates of preterm guinea pigs following hyperoxic exposure and dexamethasone treatment

	21% Oxygen		95% Oxygen	
Days	Saline	Dexa	Saline	Dexa
	(%/day)			
0–3	−0.9 ± 0.5	−3.3 ± 0.4[a]	−3.8 ± 0.5[a]	−2.7 ± 0.5[a]
3–5	4.6 ± 0.5[a]	4.7 ± 0.4[b]	6.4 ± 0.6[c]	5.8 ± 0.6[d]
5–7	8.4 ± 0.7[a,e]	9.3 ± 0.8[b,f]	8.0 ± 0.7[c]	4.0 ± 0.5[d,g,h]

Values are means ± SD for 6–17 animals per group.

Superscripts indicate statistical differences ($P < 0.05$ or better) as follows: [a]different from 0–3 days, 21% O_2, saline; [b]different from 0–3 days, 21% O_2, dexa; [c]different from 0–3 days, 95% O_2, saline; [d]different from 0–3 days, 95% O_2, dexa; [e]different from 3–5 days, 21% O_2, saline; [f]different from 3–5 days, 21% O_2, dexa; [g]different from 5–7 days, 21% O_2, saline or dexa; [h]different from 5–7 days, 95% O_2, saline.

difference between total and Mn-SOD activities. Catalase activity was measured using the method of Aebi [29] in which the initial rate of hydrogen peroxide decomposition is determined. Antioxidant enzyme activities are expressed per unit DNA. DNA was analysed using the fluorochrome Hoechst 33258 [30]. Coefficients of variation for antioxidant enzymes were: catalase, 9.2%; total SOD, 11.6%; Mn-SOD, 13% and GSH-Px, 2.7%.

Microbiology. Fresh BAL from a random selection of animals was submitted for microbiological assessment for the presence of bacterial or Mycoplasma infection.

Statistical method. The data was analysed using a three-way analysis of variance (ANOVA) for an unbalanced design. Log transformation was used to stabilize the variance and make the data more normally distributed. Where significant interactions were indicated statistical probability was determined using Student's unpaired *t*-test. Survival curves were compared using the Mantel–Haenszel test. A probability value of less than 0.05 was taken as statistically significant.

RESULTS

Survival

As reported previously [19], exposure to hyperoxia had an adverse effect on survival: 95% O_2, saline, cumulative survival at 72 hr, 73%, N = 27; 21% O_2, saline, 100%, N = 13; ($P < 0.05$). Dexamethasone treatment had no apparent effect on survival with 96% of the pups surviving in 21% O_2, N = 17 and 67% of the pups surviving in 95% O_2, N = 23 ($P < 0.05$). No further animals died in any group following return to ambient conditions.

Growth

As reported previously, preterm guinea pigs initially lose weight but this tends to have been reversed by 96 hr [19]. In the present study, those pups treated with dexamethasone in the presence or absence of hyperoxia lost more weight than those maintained in 21% oxygen and treated with saline ($P < 0.05$). Combined dexamethasone and hyperoxia treatment did not result in greater body weight loss than either treatment alone (Table 1). In the early recovery period, i.e. days 3–5, pups in all groups gained approximately 5–6% body weight/day. During days 5–7, growth increased to 8–9%/day in all animals except those that had been previously exposed to hyperoxia and treated with dexamethasone where the growth rate was only 4%/day ($P < 0.05$).

Blood leukocyte counts

Hyperoxia treatment caused a marked increase ($P < 0.05$) in total blood leukocytes in all pups whether or not they were receiving dexamethasone treatment (21% O_2, saline, 13.7 ± 2.1; 95% O_2, saline, 19.8 ± 1.5; 21% O_2, dexamethasone, 14.4 ± 2.9; 95% O_2, dexamethasone, 25.2 ± 3.9 × 10^5/mL). Blood leukocyte numbers remained significantly elevated 2 days following cessation of oxygen exposure but returned to normal levels following 4 days recovery in 21% O_2. Treatment with either hyperoxia or dexamethasone resulted in blood neutrophilia which was still evident four days following return to ambient conditions (21% O_2, saline, 3.8 ± 0.6; 21% O_2, dexamethasone, 9.8 ± 0.8; 95% O_2, saline, 3.8 ± 0.6; 95% O_2, dexamethasone, 8.3 ± 0.9 × 10^5/mL).

Leukocytes in BAL fluid

The administration of dexamethasone had no effect on any of the cellular components in BAL from 21% oxygen-treated animals at any time point apart from the BAL eosinophil count, which was lower in the saline-treated group at 5 and 7 days. For clarity, only the 21% O_2 saline results are presented for comparison with the 95% O_2 saline and dexamethasone groups (Fig. 1). It should be noted that considerable variation in BAL leukocyte numbers were seen from animal to animal and this is reflected in the relatively large standard deviations of these data.

Exposure of preterm animals to 95% O_2 resulted in elevated BAL total leukocyte numbers both during (day 3) and following (day 5) oxygen exposure (Fig. 1). At both time points this was due primarily to an increase in the number of neutrophils, although at day 5, the number of macrophages was also significantly increased. Inflammatory cell numbers

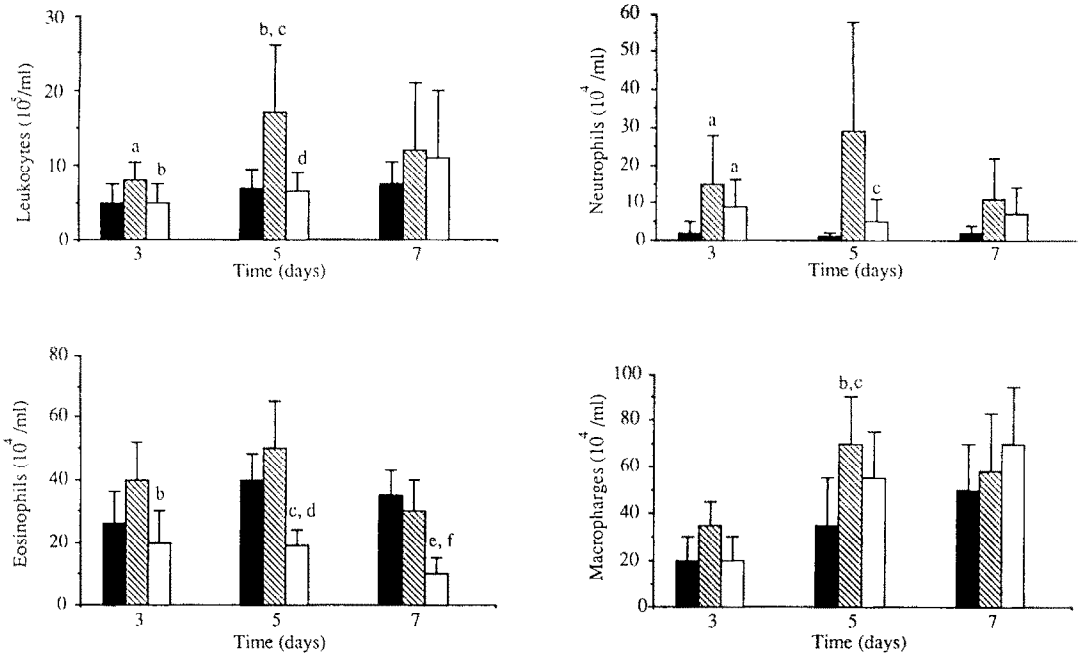

Fig. 1. Histograms showing total leukocyte, neutrophil, eosinophil and macrophage numbers in BAL for 21% O_2, saline (■); 95% O_2, saline (▨) and 95% O_2, dexamethasone (□). Results are expressed as mean values (error bars represent 1 SD), N = 6–9 per group. Superscripts indicate significant differences ($P < 0.05$ or better) as follows: [a]different from day 3, 21% O_2, saline or dexa; [b]different from day 3, 95% O_2, saline; [c]different from day 5, 21% O_2, saline or dexa; [d]different from day 5, 95% O_2, saline; [e]different from day 7, 21% O_2, saline or dexa; [f]different from day 7, 95% O_2, saline.

were elevated maximally at 5 days and had returned to control levels by day 7.

The administration of dexamethasone caused a marked attenuation in the number of BAL fluid inflammatory cells. Differential cell counting revealed significantly lower neutrophil counts both during oxygen exposure, 16.4 vs 9.4 10^4/mL (day 3), and following return to ambient conditions, 28.0 vs 5.1 10^4/mL (day 5). Eosinophil numbers were also significantly reduced, both during and following hyperoxic exposure (Fig. 1).

Lung wet weight/dry weight ratio

Following 72 hr exposure to 95% O_2 the lung wet wt/dry wt ratio increased significantly ($P < 0.05$) both in the presence and absence of dexamethasone. Corticosteroid treatment alone did not influence the lung wet wt/dry wt ratio. Following 48 hr recovery in 21% O_2, the tissue wet wt/dry wt ratio had returned to normal (Table 2).

Table 2. Lung wet weight/dry weight ratio of preterm guinea pigs following hyperoxia exposure and dexamethasone treatment

	21% Oxygen		95% Oxygen	
Day	Saline	Dexa	Saline	Dexa
3	$4.4 \pm 0.2^{a,b}$	$4.6 \pm 0.3^{a,b}$	5.6 ± 0.3	5.7 ± 0.3
5	$4.3 \pm 0.2^{a,b}$	$4.4 \pm 0.3^{a,b}$	$4.5 \pm 0.2^{a,b}$	$4.6 \pm 0.3^{a,b}$
7	$4.5 \pm 0.3^{a,b}$	$4.6 \pm 0.3^{a,b}$	$4.6 \pm 0.3^{a,b}$	$4.6 \pm 0.3^{a,b}$

Ratios are expressed as g/g and represent means ± SD for four or five animals per group. Where indicated, significance values are at least $P < 0.05$.

[a]Different from day 3, 95% O_2, saline; [b]different from day 3, 95% O_2, dexa.

Microvascular permeability

The protein concentration of BAL fluid from saline-treated pups exposed to 95% oxygen for 72 hr was increased 2.5-fold ($P < 0.01$) with respect to 21% O_2 controls (Fig. 2). Dexamethasone administration appeared to increase alveolar capillary permability even further, although considerable variation between animals was noted in this group. At the 5 day time point, even though the animals had been returned to ambient conditions for 48 hr, significantly increased protein concentrations were detected in BAL fluid obtained from pups that had earlier breathed 95% O_2 and received either saline or dexamethasone (Fig. 2). Similar differences were also noted at the 7 day time point.

NE in BAL fluid

Preliminary experiments using [^{3}H]elastin indicated that no free elastase activity was present in BAL fluid from any treatment group at any time point. Elastase activity was evident in the presence

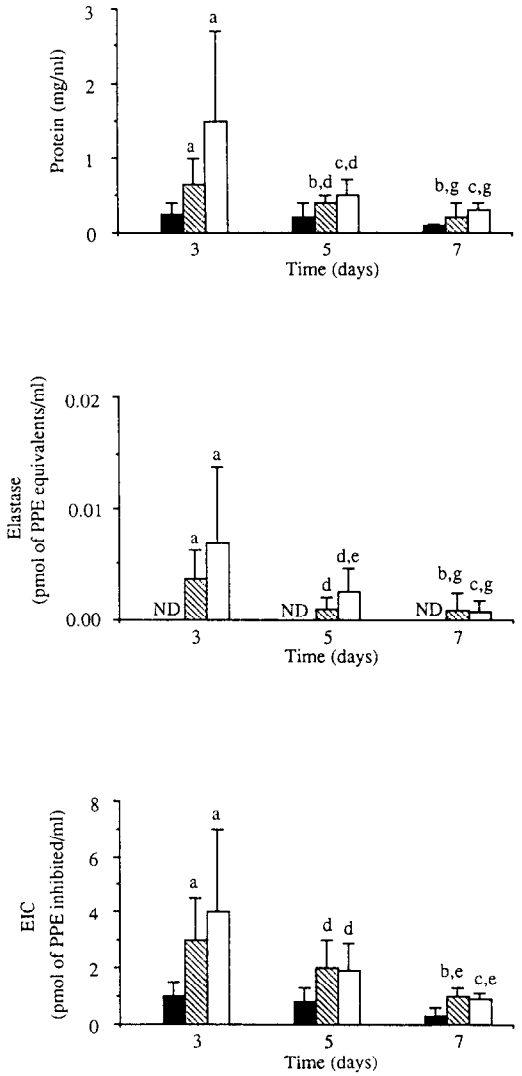

Fig. 2. BAL protein (a), NE (b) and EIC (c) from 21% O_2 saline (■), 95% O_2 saline (▨) and 95% O_2 dexamethasone (□)-treated animals. Results are expressed as mean ± SD, N = 6–9 per group. Superscripts indicate significant differences ($P < 0.05$ or better) as follows: [a]different from day 3, 21% O_2, saline or dexa; [b]different from day 3, 95% O_2, saline; [c]different from day 3, 95% O_2, dexa; [d]different from day 5, 21% O_2, saline or dexa; [e]different from day 5, 95% O_2, saline; [f]different from day 5, 95% O_2, dexa; [g]different from day 7, 21% O_2, saline or dexa.

of small synthetic substrates such as MEOSAAPVNA or SLAPN. Detection of elastase activity in this manner most likely reflects elastase which is complexed with α-2 macroglobulin and therefore not currently active. It is unknown if this elastase has previously been active in the lung.

Using the substrate MEOSAAPVNA which is specific for NE no activity was detected in 21% O_2-exposed pups (Fig. 2). Following exposure to 95% O_2 NE was detected in BAL fluid at 72 hr and continued to be present for at least a further 96 hr after the animals had been returned to ambient conditions (Fig. 2). Pups treated with dexamethasone had significantly higher NE at the 5 day time point.

EIC in BAL fluid

As found previously [19], small amounts of EIC are present in BAL fluid obtained from control (21% O_2) animals. Exposure to 95% O_2 significantly elevated EIC and it remained elevated throughout the course of the study (Fig. 2). Dexamethasone treatment did not influence EIC at any time point considered.

Pulmonary antioxidants

Lung antioxidant enzyme expression was found to be influenced by all three parameters considered, i.e. time, dexamethasone and elevated oxygen (Fig. 3). With the exception of GSH-Px, little change in antioxidant enzyme activity was observed at day 3. Notable changes were seen at days 5 and 7, that is, 2 and 4 days into recovery. The pattern of response was not, however, uniform between the four antioxidants considered. At day 5, Cu/Zn-SOD activity was elevated in those animals treated with dexamethasone and exposed to hyperoxia, in contrast to those treated with dexamethasone and maintained in 21% oxygen. At day 7 Cu/Zn-SOD activity was significantly elevated in only those animals that had been exposed to hyperoxia alone. Mn-SOD activity was found to be elevated at days 5 and 7 in those animals exposed to hyperoxia and treated with dexamethasone, whereas at day 7 Mn-SOD activity was also significantly elevated in those pups exposed to hyperoxia alone (Fig. 3). In general, GSH-Px appeared to be more sensitive to hyperoxic exposure rather than dexamethasone treatment. On the other hand, catalase was found to be sensitive to both hyperoxic exposure and dexamethasone treatment as significantly elevated pulmonary catalase activities were observed at both days 5 and 7.

Microbiology

A total of 35 specimens (i.e. 35%) were submitted for microbiological culture, randomly selected from each of the four experimental groups. The majority of specimens submitted grew a small number of *Pseudomonas* colonies, at least three separate species based on colony morphology and antibiograms. These organisms were not formally identified other than to establish that they were environmental organisms and were not thought to be pathogenic. No other bacterial contaminants were present. Immunofluorescence staining for Mycoplasma was negative in all samples.

DISCUSSION

Recent studies [31] performed in human neonates with ventilator-dependent CLD have provided evidence of a link between the anti-inflammatory action of dexamethasone and improved pulmonary function. Yoder *et al.* [31] demonstrated that dexamethasone reduced indices of pulmonary inflammation in tracheo-bronchial lavage fluid. These data are similar to those of Gerdes *et al.* [7] and provide some insight into the mechanisms of

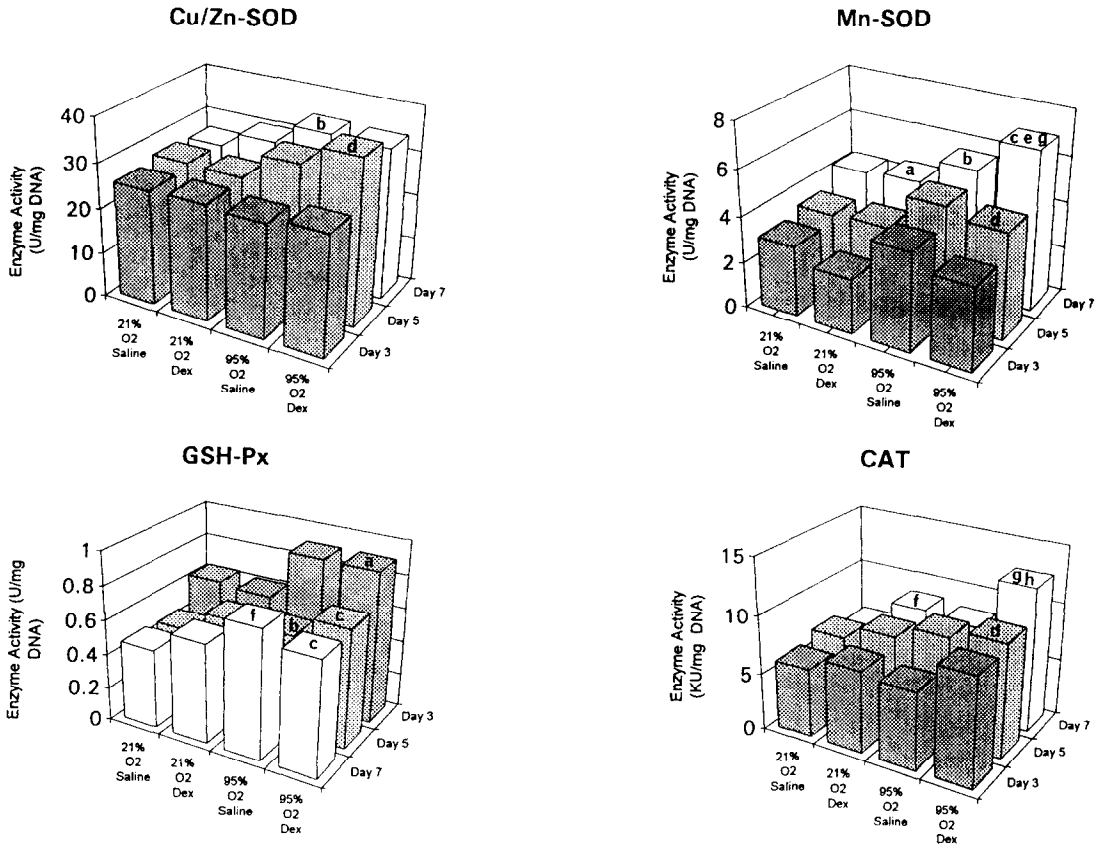

Fig. 3. Pulmonary antioxidant enzyme activities in preterm guinea pigs exposed to hyperoxia and dexamethasone. Results are expressed as average values, N = 6–9 animals per group. For clarity, the results for GSH-Px have been drawn in reverse order, i.e. day 7–3. Superscripts indicate significant differences ($P < 0.05$ or better) as follows: [a]different from day 3, 21% O_2, dexa; [b]different from day 3, 95% O_2, saline; [c]different from day 3, 95% O_2, dexa; [d]different from day 5, 21% O_2, dexa; [e]different from day 5, 95% O_2, dexa; [f]different from day 7, 21% O_2, saline; [g]different from day 7, 21% O_2, dexa; [h]different from day 7, 95% O_2, saline. CAT, catalase.

action of corticosteroids in human infants with CLD. In the present study these findings were investigated further in an animal model of prematurity.

Three day, preterm guinea pigs exposed to 95% O_2 for 72 hr developed acute lung injury, characterized by a protein-rich exudate and the accumulation of inflammatory cells, primarily neutrophils in the lung. Dexamethasone attenuated the oxygen-induced rise in leukocytes in BAL fluid, in particular, preventing the increase in BAL neutrophils. This occurred despite the presence of peripheral blood leukocytosis and neutrophilia. The reduction in BAL fluid neutrophils implies that dexamethasone blocked the recruitment of these inflammatory cells from the pulmonary circulation into the oxygen-damaged lungs of the preterm guinea pig. This may have occurred as a result of reduced amounts of chemoattractants, or a depressed responsiveness of neutrophils to these chemoattractants, or both. These proposed mechanisms are consistent with known effects of dexamethasone [32–34]. Histological examination of the lung was not performed in the present study, as BAL fluid contents have been shown to reflect tissue interstitial events in this model [19]. Although neutrophil recruitment to the lung was depressed by dexamethasone treatment, elastase activity, which represented primarily neutrophil-derived serine elastase, was increased in BAL fluid from these pups. It should be noted that the synthetic substrate used in these studies (MEOSAAPVNA) can be utilized by both free (uncomplexed) and α-2 macroglobulin-bound neutrophil elastase and as no free elastase activity could be detected using [^{3}H]-elastin, it must be assumed that the NE activity detected was bound to α-2 macroglobulin. Indeed, using gel fractionation techniques we have previously shown that the majority of elastase-like activity in BAL fluid from oxygen-exposed pups is present in a high molecular weight complex likely to be α-2 macroglobulin [19]. These data suggest that dexamethasone did not prevent the activation of those neutrophils attracted to, or already present in the lung. Paradoxically, dexamethasone also increased pulmonary microvascular permeability in hyperoxia-treated pups.

These observations agree well with the majority of other studies, conducted in a variety of more mature animals, which have failed to document any benefit of corticosteroid therapy in oxidative-induced lung injury [35–37]. They differ however from those of Koizumi *et al.* [38] who found improved survival and a small but significant reduction in protein exudation in rats exposed to hyperoxia and treated with dexamethasone. This difference may be accounted for by the different species used, age of animals or protocol followed. Koizumi *et al.* [38] administered dexamethasone following 48 hr hyperoxic exposure, a time when the inflammatory response was gaining momentum. In a similar study in sheep which were exposed to 96 hr hyperoxia, methylprednisolone had no effect on pulmonary lymph protein content whether given for the initial or latter 48 hr period [35].

In view of the critical role that antioxidant defences appear to play in preventing oxygen-induced lung injury, the influence of dexamethasone on their expression may be an important determinant of the eventual outcome. At present the literature contains reports of both positive and negative effects of glucocorticoids on antioxidant enzyme expression. Yam and Roberts [39], reported that dexamethasone-treated adults rats had significantly decreased pulmonary enzyme activities and that they sustained greater oxygen-induced lung damage. Conversely, Frank *et al.* [40] reported that dexamethasone treatment had no effect on antioxidant enzyme activities in oxygen-exposed new-born rats.

In the immature lung of the preterm guinea pig we found that dexamethasone treatment influenced the activities of several antioxidant enzymes. With the exception of GSH-Px, elevated antioxidant activities were not however noted until several days into the recovery phase from oxidative injury. This may explain why changes in pulmonary antioxidant enzymes activities were not observed in dexamethasone-treated newborn rats which were monitored only during the hyperoxic exposure [40]. Alternatively, this may be an age-specific response as dexamethasone is known to promote cellular differentiation at the expense of proliferation [18]. Hence, these effects may represent accelerated normal developmental events, rather than a change in the nature of fetal lung maturation. As premature infants are deficient in pulmonary catalase [41], it is possible that treatment with dexamethasone may accelerate catalase expression. However, the results of the present study suggest that this response alone will not improve oxidative-induced lung injury in the short term.

No overt evidence of any adverse effects of dexamethasone was observed in this study. The principal concern with regard to the use of dexamethasone in human inflammatory disorders, including neonatal lung disease, has been the potential for the immunosuppressive effect of this agent to contribute to an increased risk of pulmonary infection. Despite this, the evidence from controlled studies in humans is that dexamethasone therapy does not appear to be associated with an increased incidence of infection [13, 14]. In the present study we screened for the presence of pulmonary infection but apart from a sparse growth of environmental *Pseudomonas* species in most animals tested, no other respiratory pathogens were detected. Another major concern with the use of dexamethasone is the negative effects it has on nitrogen balance and growth. In the present study, combined dexamethasone and hyperoxic treatment was observed to reduce growth when compared with either treatment alone.

In conclusion, dexamethasone treatment reduced the numbers of neutrophils recruited to the hyperoxia-injured immature lung. However, neutrophils recruited to the lung were activated and released significant amounts of elastase which probably contributed to the increased microvascular injury. Likewise, even though dexamethasone treatment resulted in increased antioxidant enzyme activity, the response took several days to occur and is therefore unlikely to explain the rapid benefits observed in human infants treated with dexamethasone. In summary, it is not presently clear how the results obtained in animal studies, including this one, relate to the clinical setting where dexamethasone therapy is perceived to have a beneficial effect. In babies, dexamethasone is administered against a background of established lung injury which includes fibrotic changes. To date, animal studies have not reproduced these conditions exactly and as such may not represent the true clinical setting. Such an approach warrants testing to elucidate hopefully the mechanism of action of dexamethasone under these conditions.

Acknowledgements—We wish to thank Mr C. Bunce and the University animal facility staff. This work was supported by the Wellcome Trust, the Medical Research Council and the Chest, Heart and Stroke Association.

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