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ORIGINAL RESEARCH COMMUNICATION

Antenatal Steroids and Antioxidant Enzyme Activity in Preterm Infants: Influence of Gender and Timing

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Abstract

Antenatal steroids have improved the survival of preterm infants; however, the mechanism of action is not fully understood. We aimed to establish an association between antenatal steroids and antioxidant activity and postnatal oxidative stress. In a prospective cohort study, extremely preterm neonates receiving antenatal steroids (CORT) or not (NOCORT) were enrolled. An association between antenatal steroids and activities of antioxidant enzymes and glutathione cycle enzymes in cord blood was found. In addition, reduced oxidative stress (GSH/GSSG ratio, CORT vs. NOCORT, 35.68 ± 12.20 *vs*. 28.38 ± 9.92; $p < 0.01$) and, decreased oxidation of proteins (*ortho*-tyrosine/phenylalanine ratio, CORT *vs*. NOCORT, 8.66 ± 2.45 *vs*. 12.55 ± 4.41; $p < 0.01$) and DNA (8oxodG/2dG ratio, CORT *vs*. NOCORT, 6.73 ± 2.18 *vs*. 9.53 ± 3.83; $p < 0.01$) also was found. Antenatal steroids were associated with reduced oxygen supplementation, mechanical ventilation, and conditions such as bronchopulmonary dysplasia, intra-periventricular hemorrhage, or retinopathy of prematurity. The maximal effectiveness was when steroids were administered 2–4 days before delivery. Female preterm infants had less oxidative stress and increased antioxidant activity and better clinical outcomes than did male infants, independent of receiving or not antenatal steroids. Antenatal steroids are accompanied by a reduction in postnatal oxidative-stress–derived conditions and increased antioxidant enzyme activity. Both these effects seem to be influenced by specific timing and female gender. *Antioxid. Redox Signal.* 11, 2945–2955.

Introduction

Molecular oxygen is essential for the development and growth of multicellular organisms. Mammals have evolved a sophisticated physiological network to maintain oxygen homeostasis at tissue level; this involves capture, binding, transport, and delivery of molecular oxygen (20). Under physiologic conditions, the fetus is persistently hypoxic compared with the adult, and whereas human maternal arterial and venous po_2 is ~12 kPa (90 mm Hg) and 9.3 kPa (70 mm Hg), respectively, the highest arterial or venous po_2 in the late-gestation fetus rarely exceeds 4 kPa (30 mm Hg). However, this situation is partially compensated for by the presence of fetal hemoglobin, which has a greater affinity for oxygen, thus facilitating placental oxygen uptake by fetal blood and increased oxygen saturation for a give partial pressure, as compared with that in the adult (33). Interestingly, oxygen gradients in developing tissues act as a morphogen to determine the differentiation pattern of cells forming human tissues because the balance between the formation and removal of metabolically generated reactive oxygen species can elicit developmental events (2, 24). Of note is that the level and activity of the most-relevant antioxidant enzymes such, as superoxide dismutases (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione peroxidase (GPX; EC 1.11.1.9) change dynamically during development and mature in the last weeks of gestation, preparing the fetus for lung respiration (17, 18, 46). In the fetal-to-neonatal transition, both blood oxygen content and oxygen availability abruptly increase in the first few minutes after birth to adult values, eliciting the generation of a burst of oxygen free radicals (10, 25, 48). Free radicals in the fetal-to-neonatal transition may act as signaling molecules modulating maturation of specific metabolic pathways (16, 35). However, in extremely premature infants, the combination of an immature antioxidant system (8, 51) plus a surfactant-deficient lung (23) and the need for therapy with oxygen supplementation and mechanical ventilation together predispose to oxidative stress, lung-stretch damage, inflammation, and impairment of alveolar development, predisposing to chronic lung disease (29, 49).

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First experimentally and thereafter in a randomized trial in humans, Liggins and co-workers (30, 31) demonstrated the effectiveness of antenatal corticosteroids (ACs) to prevent respiratory distress syndrome (RDS) due to surfactant deficiency. Meta-analysis of the clinical trials performed has confirmed the effectiveness of AC when administered to the mother within 1 week before delivery to reduce neonatal mortality, incidence or severity of bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), respiratory distress syndrome (RDS), intracranial hemorrhage (ICH), retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC) (11, 28). Of note is that all these neonatal conditions are associated with free radical production (33).

We hypothesized that AC effectiveness could be related to enhancement of the expression of antioxidant enzymes and subsequent attenuation of oxidative stress. To evaluate our hypothesis, we launched a prospective clinical study enrolling extremely low-gestational-age neonates (ELGANs) whose mothers had or not received a full course of antenatal corticosteroids and compared oxidant status (reduced-to-oxidized glutathione ratio); antioxidant enzyme activity of SOD, CAT, and GPX; glutathione redox cycle enzymes; and biomarkers of oxidative damage to protein (*ortho*-tyrosine) and DNA (8 oxo-2′-hydroxy-deoxyguanosine). Oxidation of phenylalanine (phenyl) by hydroxyl radicals produces *ortho*-tyrosine (o-tyr), a metabolite that is not produced by physiologic metabolic pathways and is eliminated in the urine (44). Moreover, oxidation of nucleoside/bases of DNA produces a significant number of metabolites, which are eliminated in the urine. One of the most reliable markers is 8-hydroxy-2′-deoxyguanosine (8oxodG), and the ratio with 2′-deoxyguanosine (2dG) has been widely used as a reference for oxidative aggression to DNA (44). In addition, clinical (RDS, BPD, ROP, IPVH) and therapeutic outcomes (days on oxygen and days on mechanical ventilation) also were recorded.

Materials and Methods

Study design

This is a prospective observational-cohort clinical study performed in a tertiary referral center (University Hospital La Fe, Valencia, Spain) during a 24-month period (January 2003 through January 2005), enrolling extremely low-gestational-age neonates (ELGANs), defined as having 28 weeks or less gestational age. The trial was registered in the Clinical Government Trial Registry with the number NCT00791687. Biologic samples were blinded for the Research Laboratory (Department of Physiology, Faculty of Pharmacy, University of Valencia, Spain). Ethical and Scientific Committee approval was obtained, and parents of all recruited infants signed the informed consent.

Population

The enrolling criteria were (a) gestational age, 28 weeks or less gestation, as determined by obstetric ultrasound or last menstrual bleeding or both; (b) reliable information regarding the administration of antenatal betamethasone, as defined by the National Institute of Health Consensus Statement 2000 (4); thus, babies who had received incomplete treatment or treatment >7 days before delivery were not enrolled; (c) born in the obstetric ward of the site hospital; and (d) fulfillment of the study protocol for analytic determinations. Exclusion criteria were (a) uncertainty about gestational age; (b) use of pro- or antioxidant drugs by the mother during gestation (protracted used of nonsteroidal antiinflammatory drugs; *N*-acetyl-cysteine, vitamin C, vitamin A, or other); (c) overt clinical or analytic signs (white cell count and differential, C-reactive protein, interleukin 6), or prenatal inflammation or infection (*e.g.*, chorioamnionitis); and (d) major congenital malformations or chromosomopathies. Power calculation, based on the assumption that antenatal steroids would cause an increment of 20% of the GSH/GSSG ratio, indicated that 20 or more babies would have to be recruited per group.

From a total of 93 eligible ELGANs, 36 did not complete the study, as shown in the flow diagram depicted in Fig. 1. Thus, 57 babies were finally studied: the CORT group ($n = 37$), which included those babies whose mothers had received a full course of antenatal betamethasone within the week before delivery, and the NOCORT group ($n = 20$), which included those babies whose mothers had not received antenatal corticosteroids.

Methods

The nurse in charge of the delivery room was informed of every pregnancy admitted to the Obstetrics ward with suspected or proven gestational length of 28 weeks or less. When they met the entry criteria, parents signed the informed consent, and the patient was admitted into the study. The patient was assigned to the CORT group if the mother had received a full course of prenatal corticosteroids (betamethasone) within 7 days before delivery, and to the NOCORT group, if not. Immediately after birth, a first sample of 2 ml of blood was drawn from the umbilical cord. A second 2-ml sample was collected at 24 h after birth from a peripheral vein, coinciding with routine analysis performed in the NICU. From the total volume of blood drawn, 1 ml of blood was processed for reduced (GSH) and oxidized glutathione (GSSG), as previously described (25), and 1 ml was immediately centrifuged for 10 min at 500 *g* and 4°C, and the supernatant was deep frozen (−80°C). Urine was collected at day 1 by using a Hollister U-bag collector under sterile conditions and, after aliquoting to 2-ml Eppendorf tubes, was frozen at −80°C until processed.

Analytic assays

GSH and GSSG analyses were performed with high-performance liquid chromatography (HPLC), as previously described by Asensi *et al.* (6, 7). Glutathione peroxidase, glutathione reductase, glutathione *S*-transferase, and superoxide dismutases activities were determined, as described by Flohé and Güzzler (15), Akerboom and Sies (1), Habig *et al.* (22), and Flohé and Otting (14), respectively. Blood in heparinized tubes was immediately centrifuged for 10 min at 500 *g* and 4°C. Plasma was removed, and erythrocytes were washed twice with 0.9% sodium chloride. The supernatant was aspirated, and the cell pellet was hemolyzed with distilled water. Enzyme activities were assayed in the hemolysate and were expressed per gram of hemoglobin content. The O-tyr/phenyl ratio, indicative of the rate of hydroxyl radical production and amino acid oxidation, was determined with mass

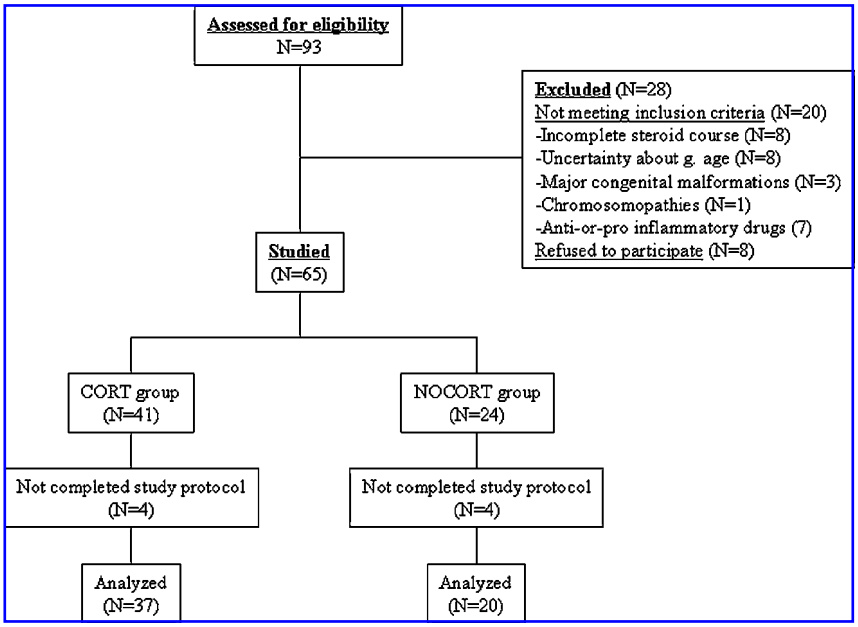

FIG. 1. Flow diagram describing assessment for eligibility, exclusion criteria, and number of patients finally included in the study. Several mothers had more than one exclusion criterion.

spectrometry, as previously described (44). The 8oxodG/2dG ratio was determined in urine with mass spectrometry, as previously described (44).

Statistical analysis

Descriptive statistics were determined by using SPSS 13 (SPSS Inc., Chicago, IL). Statistical analysis was performed in two steps. Initially, we performed a one-factor analysis of variance (ANOVA), and when the overall comparison of groups was significant, differences between individual groups were investigated with the Tukey method. Differences were considered significant at $p \leq 0.05$.

Nonparametric statistics were used to compare nonnormally distributed variables. Therefore, the Mann–Whitney *U* test was used for comparison of nonpaired samples, and the Kruskall–Wallis test was used for paired comparisons. Data obtained across time (day of antenatal corticosteroid administration) were compared by using the log-rank test, which allowed us to obtain a *p* value at each time point and to assess differences between groups. Qualitative nonnormally distributed variables were compared by using χ^2 analysis. In addition, we performed logistic regression analysis to correlate antenatal steroid (positive or negative) administration with the GSH/GSSG ratio and antioxidant enzyme activities (41).

Table 1. Maternal and Obstetric Factors Associated with Acceleration of Delivery Progression in Extremely Low-Gestational-Age Neonates Receiving (CORT) or Not (NOCORT) Antenatal Corticosteroids

	NONCORT group (n = *20*)	*CORT group* (n = *37*)	*Significance*
Maternal age (yr)[a]	24 (17–32)	27 (19–35)	NS
Level of education[b]	2.3 (1, 3)	2.7 (1, 3)	NS
Maternal overweight[c]	4 (20.0%)	6 (16.2%)	NS
Inadequate prenatal care[d]	8 (40.0%)	4 (10.8%)	$p < 0.01$
Pregnancy-induced hypertension (%)	5 (25.0%)	3 (8.1%)	$p < 0.05$
Time of rupture of membranes[e] (h)	6 (1, 18)	12 (1, 28)	$p < 0.05$
Abruptio placentae (%)	4 (20.0%)	6 (16.2%)	NS
Postnatal infection[f] (%)	5 (25.0%)	7 (18.9%)	NS
Smoking[g]	3 (15.0%)	5 (13.5%)	NS
Alcohol ingestion[h]	8 (40.0%)	12 (32.4%)	NS

[a]Expressed in median plus 25–75% centiles in parentheses.
[b]Scoring was calculated according to achieved level of education: primary school, 1 point; secondary school, 2 points; college, 3 points; PhD/MD or similar, 4 points. Expressed in median plus 25–75% centiles in parentheses.
[c]Defined as body mass index [weight (kg)/height (m^2)] >25 according to standard Spanish reference tables [SEEDO. Med Clin (Barc) 107: 782–787, 1996].
[d]Defined as fewer than two visits to the obstetrician/midwife during pregnancy.
[e]Defined as time elapsed between rupture of membranes and delivery, expressed in median plus 25–75% centiles in parentheses.
[f]Defined as infections with clinical symptoms and positive cultures.
[g]Defined as smoking more than one cigarette/day during gestation.
[h]Defined as drinking alcohol-containing beverages 1 day per week during gestation.

Table 2. General Characteristics of Extremely Low-Gestational-Age Neonates (≤28 Weeks' Gestation) Receiving (CORT) or Not (NOCORT) a Full Course of Antenatal Corticosteroids (Betamethasone) According to the Recommendations of the United States National Institutes of Health Consensus Statement (24)

Parameter	*CORT* n = *37*	*NOCORT* n = *20*	*Comparison CORT vs. NOCORT*
Gestational age (postconception wk)[a]	27 (24, 28)	27 (24, 28)	NS
Birth weight (g)[b]	786.5 ± 122.8	755.4 ± 109.3	NS
Gender (m/f)	20/17	12/8	NS
Cord acid/base status[b]	7.12 ± 0.16	7.09 ± 0.12	NS
Apgar 1 min[a]	5 (2, 7)	5 (3, 7)	NS
Apgar 5 min[a]	7 (5, 9)	7 (5, 9)	NS
Type of delivery (vg/cs)	12/25	7/13	NS
Oxygen in the delivery room	78.5%	80.8%	NS
Intubation in the delivery room	55%	58%	NS
Days on oxygen	8 (2, 55)	12 (5, 68)[b]	$p < 0.01$
Surfactant-replacement therapy	21 (56.7%)	18 (90.0%)	$p < 0.01$
Mechanical ventilation including CPAP (days)	11 (4, 45)	15 (7, 62)	$p < 0.01$
Bronchopulmonary dysplasia (BPD)[c]	9 (24.3%)	11 (55%)	$p < 0.01$
Patent ductus arteriosus (PAD)	22 (59.5%)	15 (5.0%)	$p < 0.01$
Intra-periventricular hemorrhage grades III/IV (IPVH)[c]	8 (21.6%)	11 (55.0%)	$p < 0.01$
Retinopathy of prematurity (ROP)[c]	4 (10.8%)	7 (35.0%)	$p < 0.05$

[a]Expressed as median CI, 95%.
[b]Expressed as mean ± standard deviation.
[c]BPD definition: (33); IPVH grading: (34); ROP grading: (35).

Results

Characteristics of mothers and newborn infants

Table 1 reflects obstetric and maternal conditions capable of inducing premature labor or accelerating delivery or both, hindering administration of corticosteroids. The mothers of the NONCORT group had a higher incidence of deficient prenatal care ($p < 0.01$), pregnancy-induced hypertension ($p < 0.05$), abruptio placentae ($p < 0.05$), and premature rupture of membranes ($p < 0.05$). However, no differences in overweight, educational level, smoking, alcohol ingestion, or the incidence of overt postnatal infections were detected. Table 2 includes the description of the general characteristics of the population, as well as type of delivery, interventions in the delivery room (DR) and in the NICU, and long-term outcomes such as BPD (27), PDA, IPVH (39), and ROP (26). No differences regarding gestational age, birth weight, gender, cord pH, or Apgar scores were detected between both groups. However, patients in the NONCORT group spent more days on oxygen and mechanical ventilation ($p < 0.01$) and had a higher incidence of BPD, PDA, IPVH, and ROP ($p < 0.01$).

Antioxidant enzymes and GSH-cycle enzymes

Figure 2 shows the activity expressed in International Units per gram of hemoglobin of superoxide dismutases (SOD) and catalase (CAT). Both SOD and CAT activity in the CORT group were significantly higher in cord blood ($p < 0.05$) and at 24 h than were those in the NOCORT group ($p < 0.01$). Figure 3 depicts the activity of glutathione redox-cycle enzymes, glutathione peroxidase (GPX), glutathione *S*-transferase (GST), and glutathione reductase (GSR). No differences regarding GPX activity were found between CORT and NOCORT groups at the different time points; however, at 24 h,

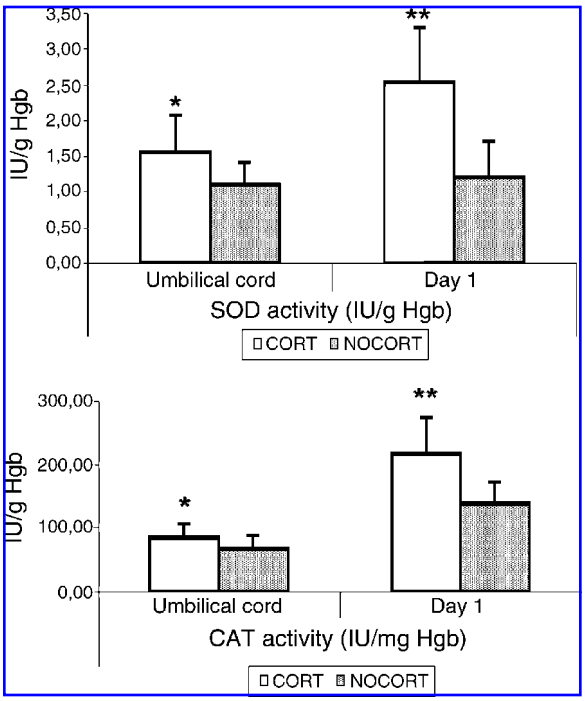

FIG. 2. Superoxide dismutase (SOD) and catalase (CAT) activities in umbilical cord and day 1 in extremely low gestational-age neonates receiving (CORT group) or not receiving (NOCORT group) a full course of antenatal steroids. Activities are expressed in International Units per gram hemoglobin.

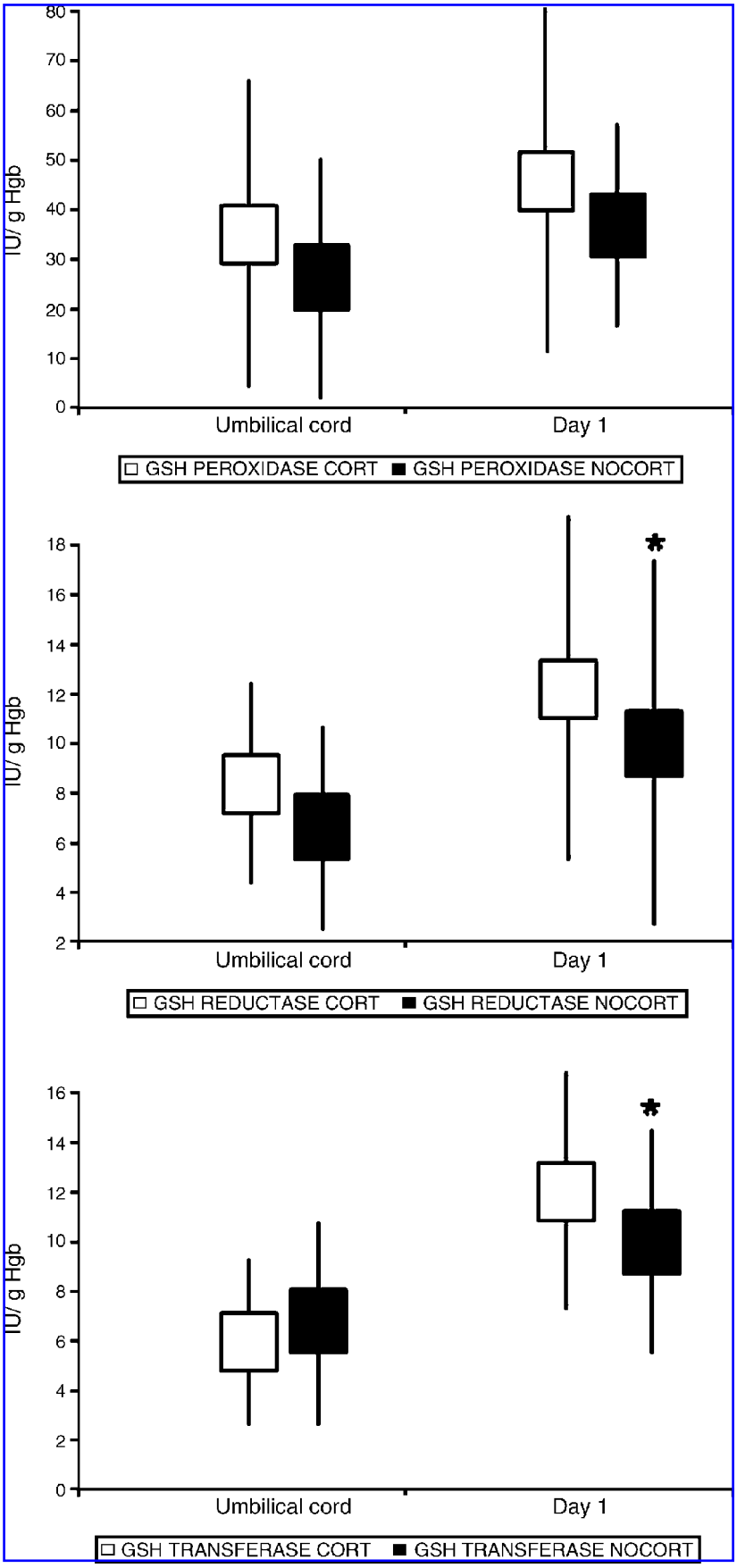

FIG. 3. Activities of glutathione redox cycle enzymes (glutathione peroxidase, glutathione reductase, glutathione *S*-transferase) in umbilical cord and at day 1 in extremely low-gestational-age neonates receiving (CORT group) or not receiving (NOCORT group) a full course of antenatal steroids. Activities are expressed in International Units per gram hemoglobin.

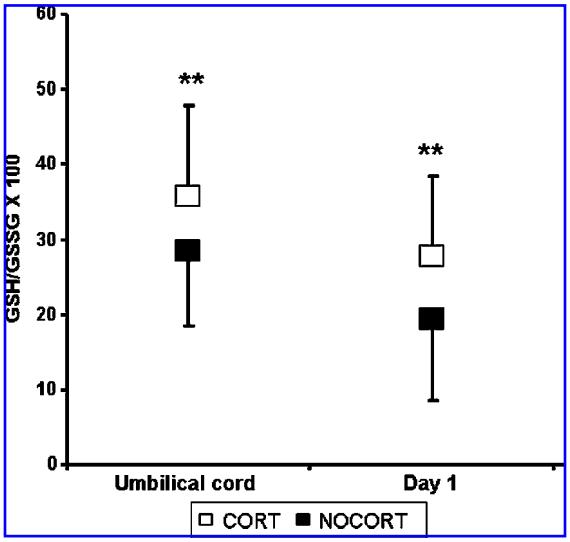

FIG. 4. Reduced (GSH)-to-oxidized (GSSG) glutathione × 100 ratio in umbilical cord and at day 1 in extremely low-gestational-age neonates receiving (CORT group) or not receiving (NOCORT group) a full course of antenatal steroids.

the activities of GSR and GST were significantly higher in the CORT than in the NOCORT group ($p < 0.05$).

Biomarkers of oxidative stress

Figure 4 depicts the reduced-to-oxidized glutathione (GSH/GSSG) ratio in whole blood at birth and on day 1. The GSH/GSSG ratio was significantly higher in neonates receiving prenatal corticosteroids both at birth ($p < 0.01$) and 24 h thereafter ($p < 0.01$). In addition, Fig. 5 depicts *o*-tyr/phenyl elimination in urine during the first 24 h of postnatal life, which was significantly higher in the NOCORT than in the CORT group ($p < 0.01$). Moreover, Fig. 5 also shows that elimination *ortho*-tyrosine was significantly greater ($p < 0.05$) in boys of the CORT and NOCORT groups as compared with girls. Figure 6 depicts 8 oxodG/2dG elimination of urine during the first 24 h of postnatal life, which was significantly higher in the NOCORT than in the CORT group ($p < 0.01$). Boys had a significantly higher elimination of this biomarker in both groups ($p < 0.05$). Interestingly, elimination of urinary 8 oxodG in girls of the NOCORT group did not differ from that of boys in the CORT group.

Correlation between gender, timing of corticoid administration before delivery, and antioxidant enzyme activities

Figure 7A and B represents SOD and CAT activities according to the gender and timing of last dose of corticoid administration expressed as days before delivery. SOD and CAT activities were determined in cord blood. A polynomial adjustment regression curve for boys and girls has been calculated and plotted. Thus, for SOD activity in boys (y, $-0.0531x^2 + 0.3517x + 0.7765$; R^2, 0.8258) and in girls (y, $-0.00772x^2 + 0.5527x + 0.7198$; R^2, 0.7109) and for CAT activity in boys (y, $-3.5624x^2 + 21.866x + 61.095$; R^2, 0.6429) and in girls (y, $-2.2983x^2 + 6.5481x + 114.77$; R^2, 0.7425). Polynomial regression curves show a tendency for girls to having a higher enzyme activity, although because of the few individuals at each time point, statistical significance has not been achieved. In addition, enzyme activity is higher when corticoid administration in performed on days 2 to 4 before birth, and it is lower when administered between 0 and 2 days or > 5 days before birth takes place. To establish a correlation between antenatal corticosteroid administration and oxidative stress and antioxidant enzyme activities, we performed a logistic regression analysis. A statistically significant correlation between enzyme activities for SOD ($p < 0.05$), CAT ($p < 0.05$), GSR ($p < 0.05$), GST ($p < 0.05$), and oxidative stress (GSH/GSSG ratio; $p < 0.01$) was established.

Discussion

Generalization of the use of antenatal corticosteroids (ACs) has significantly reduced mortality and the incidence of severe complications in preterm infants of younger than 34 weeks of gestation (4, 11, 12, 28). Accordingly, ELGANs who received ACs in our study spent fewer days on oxygen, on mechanical ventilation, and had significantly less-

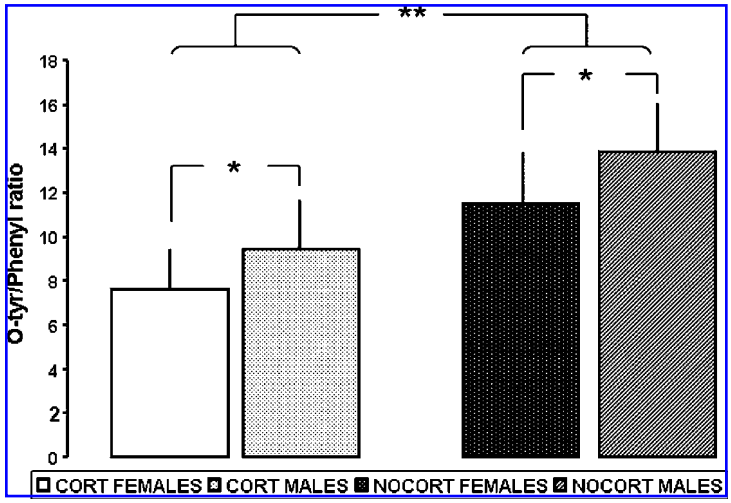

FIG. 5. *Ortho*-tyrosine/phenylalanine ×1,000 ratio (*o*-tyr/phenyl) in urine collected 24 h after birth in extremely low-gestational-age neonates receiving (CORT group) or not (NOCORT group) a full course of antenatal steroids. *O*-tyr/Phenyl ratio was significantly higher in patients of the NOCORT group than in the CORT group ($p < 0.01$). Within the same study group, male infants excreted significantly ($p < 0.05$) more *ortho*-tyrosine than did female infants.

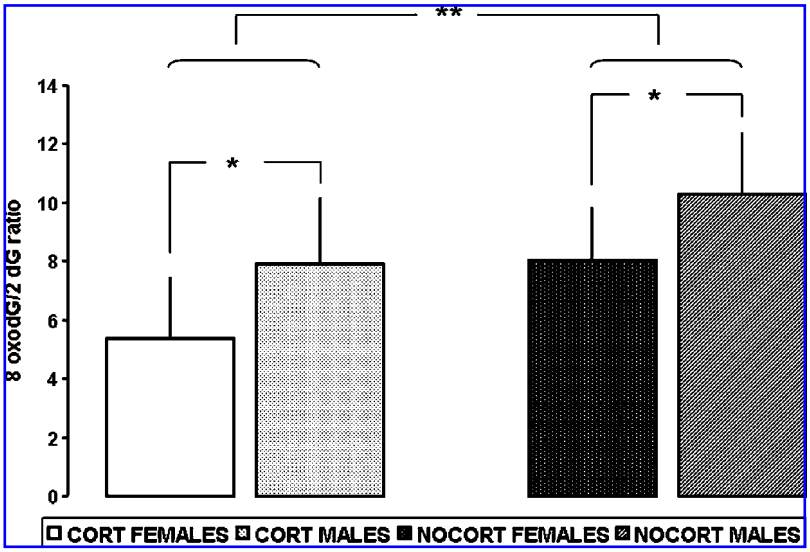

FIG. 6. 8-Hydroxy-2′-deoxyguanosine/2′-deoxiguanosine ×100 ratio (8 oxodG/2 dG) in urine collected 24 h after birth in extremely low-gestational-age neonates receiving (CORT group) or not (NOCORT group) a full course of antenatal steroids. The 8 oxodG/2 dG ratio was significantly higher in patients of the NOCORT group than in those of the CORT group ($p < 0.01$). Within the same study group, male infants excreted significantly ($p < 0.05$) more oxidized bases of DNA than did female infants.

severe complications (see Table 2). ACs accelerate lung maturation, speeding the thinning of the double capillary loop to form the thin gas-exchanging walls of alveoli, and enhancing the production of surfactant by the type II pneumocytes (19, 21, 38). However, initiation of breathing, together with the use of oxygen and mechanical ventilation, will cause oxidative stress, inflammation, and alterations in surfactant turnover (3, 9, 40). Of note is that the effect of ACs on lung antioxidant enzyme expression in premature infants is unclear. In this regard, studies on antioxidant enzyme expression in the fetal lung of different species of mammals revealed that it generally increases toward term; however, results have led to different results, depending on the animal model used (17, 34, 46). Thus, Arima *et al*. (5) found that antenatal dexamethasone administered to pregnant rats did not modify the pattern of maturation of antioxidant enzymes measured in the offsprings' lungs at days 19 and 21 of gestation and day 1 and 3 after birth, but significantly enhanced endothelial and inducible NO synthases expression. Walther *et al*. (52) found that MnSOD, CAT, and GPX activity significantly increased in premature lambs after just one dose of betamethasone given to the mother, whereas Cu/ZnSOD activity increased only after several doses were given to the pregnant ewe. Increased AEA was accompanied by reduced levels of hydroperoxide and carbonyl proteins in lung tissue, reflecting a reduction in the production of reactive oxygen species and oxidative damage to proteins. In the human, the regulation of antioxidant enzymes in response to hyperoxia or proinflammatory cytokines is complex and age dependent. Asikainen *et al*. (8) found an increased MnSOD, Cu/ZnSOD, and CAT mRNA expression toward adulthood, whereas GPX did not substantially change from fetal to adult life. MnSOD, considered the cornerstone of lung defense against reactive oxygen species, is induced by hyperoxia and proinflammatory cytokines such a TNF-α, interferon-γ, interleukin 1 (IL-1), or interleukin 6 (IL-6) (8). In our study, neonates in the group receiving prenatal corticosteroids had better clinical outcomes and needed less respiratory support in the form of supplemental oxygen, mechanical ventilation, or surfactant therapy (see Table 2). In addition, babies receiving ACs showed significantly less oxidative stress at birth in the umbilical cord and at day 1 after birth, as measured by the reduced-to-oxidized glutathione ratio (Fig. 4). The GSH/GSSG ratio is a reliable and comprehensive marker of oxidative stress, reflecting cytosolic oxidant status (48). Interestingly, a decreased GSH/GSSG ratio correlates with hyperoxia and reoxygenation damage to myocardium and kidney in asphyxiated human term neonates (47, 50). Thus, we found that the expression of the antioxidant enzymes SOD and CAT was significantly increased in preterm infants receiving ACs as compared with those who did not; however, no differences for GPX were found. In addition, ACs enhanced the glutathione redox-cycle enzymes activity, thus promoting resynthesis of reduced glutathione and stabilization of the intracellular redox status. Increased antioxidant activity is not always accompanied by oxidative damage, and it may reflect a physiologic response to an increased production of free radicals (*e.g*., superoxide anion) (33). To verify whether increased expression of antioxidant enzymes contributed to reducing biologic damage caused by oxidative stress, we determined the urinary elimination of *ortho*-tyrosine, indicative of oxidation of circulating phenylalanine in proteins, and 8-oxodG, reflecting oxidation of guanosine bases of DNA in the cell nucleus (44). In previous experimental studies, it was shown that both *ortho*-tyrosine/phenylalanine and 8-oxodG/2dG ratios correlated with increased production of reactive oxygen species on reoxygenation with various oxygen concentrations (44). Moreover, in healthy and stable premature babies, the use of human milk reduced both ratios, as indicative of its antioxidant properties as compared with artificial formula (32). In this regard, our analytic data showed that treatment with ACs significantly reduced elimination of biomarkers of oxidative damage caused by the hydroxyl radical in the urine (Figs. 5 and 6).

Accumulated evidence indicates that the optimal response to antenatal steroids occurs when dosing is between 24 h and 7 days before delivery (11, 28, 37). In a recent study, McEvroy *et al*. (36) concluded that remotely treated (>14 days before

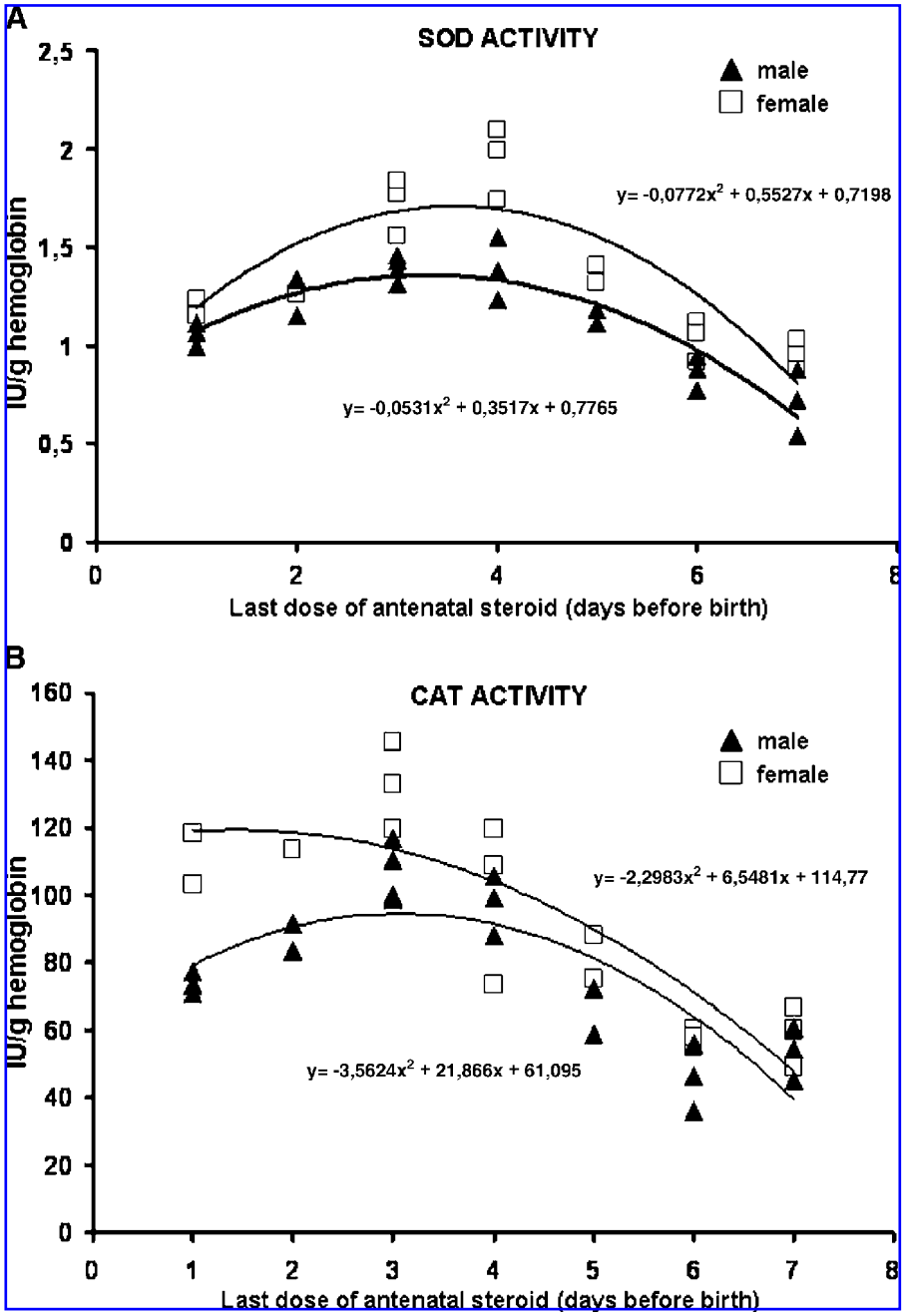

FIG. 7. Values for superoxide dismutase (SOD) (A) and catalase (CAT) (B) activities expressed in International Units per gram hemoglobin for male infants (*solid triangles*) and female infants (*open squares*) have been plotted on the day of gestation before birth in which the last dose of antenatal steroids was administered to the mother. Correlation between timing of the last dose of antenatal steroid administration, gender, and antioxidant enzyme (SOD and CAT) activities by using polynomial regression adjustment curves is shown. Regression coefficients are as follows: R^2 (SOD activity) boys, 0.8258; girls, 0.7109; R^2 (CAT activity) boys, 0.6429; girls, 0.7425.

birth) premature infants (32 weeks of gestation or less) had lower respiratory compliance, as compared with those whose mothers received a single course of steroids >7 days before delivery. Interestingly, 14 days seemed to be the limit for the dissipation of the effectiveness of antenatal steroids on lung compliance (36). A similar result was obtained by Ring *et al.* (42), showing a higher risk for ventilatory support and surfactant treatment in neonates older than 28 weeks of gestation. Of note, we found a correlation between prenatal timing of ACs administration and biologic response. Thus, the peak of AEA was reached when ACs were given between 2 to 4 days before delivery and decreased when they were given before or after this time.

Finally, we also studied the correlation between gender and response to ACs administration. Very few studies in the literature have approached the importance of gender in the process of postnatal adaptation in the preterm infant. Although it is well known that mortality and morbidity is lower in girls than in boys of similar perinatal characteristics during the neonatal period, information regarding the possible causes is lacking. In a recent study, it was shown that antenatal betamethasone exposure produced gender-specific alterations in renal function and thus suggested that different mechanisms underlie the antenatal-steroid–induced elevations in blood pressure in male and female offspring (45). Recently, Deulofeut *et al.* (13) showed a gender-specific dif-

ference favoring girls in the beneficial effects produced by avoiding high Spo_2 and hyperoxia. This is in accordance with our findings revealing that female ELGANs had consistently higher AEA, as compared with male infants of similar gestational age and who had received ACs within the same time frame before birth and therefore were more capable of successfully confronting the consequences of increased production of reactive oxygen species.

Importantly, ACs could improve oxidative stress by enhancing antioxidant enzyme expression, accelerating overall maturation, or both simultaneously. In this regard, babies receiving ACs needed less respiratory aid in the form of mechanical ventilation, oxygen supplementation, or surfactant administration (see Table 2). However, data collected at birth in the umbilical cord and confirmed in the first day of life indicated an increased AEA and reduced oxidative stress, which is in favor of an increase in AEA associated with antenatal corticosteroid administration in extremely preterm neonates concomitant with an overall accelerated maturation, as reflected in the statistical analysis.

Our study has obvious limitations. Although mothers from both groups did not differ in nutritional status, education, or smoking and drinking habits (Table 1), a significantly greater number of ELGANs in the NONCORT group resulted from inadequately controlled pregnancies. Preterm delivery is frequently triggered by intrauterine infection or inflammation or decidual hemorrhage (or a combination of these) that could have inadvertently occurred, causing premature rupture of membranes and delivery (43). In addition, mothers in the NONCORT group had a significantly higher incidence of pregnancy-induced hypertension and *abruptio placentae*, both associated with preterm delivery and with increased oxidative stress, which could definitely be a potential confounding factor altering the analytic results of our study. Finally, the number of babies recruited has been limited, thus precluding more-robust statistical analysis. It is important to underscore that the possibility of recruiting ELGANs without antenatal corticosteroids has become extremely difficult, and to perform a randomized trial including a nonsteroid group would be totally unethical. However, we performed the recruitment (power calculation) based on the assumption that the use of ACs would produce an increase by 25% of the GSH/GSSG ratio, considered the most relevant marker of oxidative stress. Another factor to be considered before drawing definitive conclusions is the restricted value of AEA as markers of antioxidant defense system maturity (10, 48); however, the use of molecular biology tools in human infants is still very limited. The correlation between the AEA and GSH/GSSG ratio and markers of oxidative damage to proteins and DNA altogether is supportive of the influence of ACs on antioxidant enzyme maturation. Thus, we conclude that ACs in ELGANs are influenced by gender and thus are more effective in female than in male infants. Moreover, their effectiveness also is influenced by timing, and optimal response is achieved when they are given between 2 and 4 days before delivery. Further studies should be undertaken to delimit dosage and timing of administration of ACs in ELGANs to improve its effectiveness. Moreover, gender differences in response to fetal corticoid administration in the newborn and adult individuals are a promising field of research.

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Author Disclosure Statement

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Abbreviations Used

AC = antenatal corticosteroids
AEA = antioxidant enzyme activity
BPD = bronchopulmonary dysplasia
CAT = catalase
ELGAN = extremely low-gestational-age neonate
GPX = glutathione peroxidase
GSH = reduced glutathione
GSR = glutathione reductase
GSSG = oxidized glutathione
GST = glutathione *S*-transferase
ICH = intracranial hemorrhage
kPa = kilopascal
NEC = necrotizing enterocolitis
PDA = patent ductus arteriosus
RDS = respiratory distress syndrome
ROP = retinopathy of prematurity
SOD = superoxide dismutase

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