0031-3998/94/3605-0619$03.00/0
PEDIATRIC RESEARCH

Vol. 36, No. 5, 1994
Printed in U.S.A.

Superoxide Anion Release by Polymorphonuclear Leukocytes in Whole Blood of Newborns and Mothers during the Peripartal Period

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ABSTRACT

Superoxide anion ($\cdot O_2^-$) production was investigated in whole blood of mothers in the peripartal period and in neonates. Blood samples from 14 mothers undergoing vaginal delivery (VD) were tested at the beginning of labor, during labor, after delivery, and 4 d after delivery. Nine mothers undergoing elective cesarean section (ECS) were tested before anesthesia, after extraction of the fetus, and 4 d later. Seventy-two healthy, full-term newborn infants were examined at birth and on the fourth day of life. Red cell glutathione peroxidase, catalase, glutathione reductase, and superoxide dismutase activities were also measured at birth and on the fourth day of life in 26 of the 72 neonates. Higher $\cdot O_2^-$ levels were detected in mothers undergoing VD compared with ECS ($p < 0.05$). A significant decrease was detected in zymosan-stimulated $\cdot O_2^-$ production between cord and fourth-day blood samples in both VD- and ECS-delivered infants ($p < 0.01$). Zymosan-stimulated samples showed higher values after VD than ECS, both in cord blood ($p < 0.004$) and on the fourth day of life ($p < 0.006$). A positive correlation was found between $\cdot O_2^-$ release in zymosan-stimulated cord blood and that found in the mothers at the beginning of labor ($r = 0.654$; $p < 0.01$), during labor ($r = 0.721$; $p = 0.008$), and after delivery ($r = 0.832$; $p = 0.0008$). A positive correlation was also found between $\cdot O_2^-$ release and glutathione peroxidase on the fourth day ($r = 0.709$, $p = 0.014$). The results of the present investigation demonstrate the role of peripartal events in modulating free radical release by polymorphonuclear leukocytes during the perinatal period. (*Pediatr Res* **36: 619–622, 1994**)

Abbreviations
PMN, polymorphonuclear leukocyte
$\cdot O_2^-$, superoxide anion
VD, vaginal delivery
ECS, elective cesarean section
GSH-Px, glutathione peroxidase
CAT, catalase
SOD, superoxide dismutase
US, unstimulated
ZS, zymosan-stimulated

Phagocyte function has been widely investigated in the newborn infant, and some impairment of oxidative burst activity has been demonstrated (1–3). Although contrasting results have been reported, there is presently little doubt that the most significant deficiencies in the phagocyte function in full-term and premature infants are related to chemoattractants, and that disturbances in free radical production due to reduced respiratory burst activity are slight, at least in nonstressed PMN (3, 4).

The current study was undertaken to detect whether the free radical release by PMN in whole blood of mothers and newborns could depend on the effect of plasma factors in relation to different modes of delivery.

Received March 22, 1993; accepted May 30, 1994.
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METHODS

Newborns. Seventy-two healthy, full-term newborn infants with Apgar scores greater than 9 at 5 min were examined. Their birth weights and gestational ages were 3321 ± 472 g and 39 ± 1.42 wk (mean ± SD), respectively. Thirty-nine were born by VD and 33 by ECS without labor. Twenty-three of them were born to the mothers tested as reported below. Blood samples were obtained at birth, through the umbilical vein from separated placentas immediately after cord clamping, and on the fourth day of life from the peripheral vein of infants in whom blood collection was required for metabolic screening or routine analyses.

Mothers. A total of 23 healthy mothers aged 25 ± 5 y were investigated. Blood samples were taken from 14 of them undergoing VD at the beginning of labor, during

labor, immediately after delivery, and 4 d after delivery. Nine mothers who underwent ECS without labor due to contracted pelvis, breech presentation, or previous cesarean section were tested before anesthesia, immediately after extraction of the fetus, and 4 d after delivery. The ECS group was comparable to the VD group in all other respects. All their neonates were evaluated at birth (cord blood) and on fourth day of life (peripheral vein). All mothers were tested for vaginal colonization of group B *Streptococcus*, *Candida*, *Enterococcus*, and *Klebsiella*, and neonates born to mothers with a positive culture, urinary tract infections, or fever were excluded from the study. Anesthesia for cesarean section was administered via premedication with 0.5 mg of atropine given 30 min before delivery, a 3-min preoxygenation, induction of general anesthesia with 5 mg of thiopentone per kg body weight followed by intubation (after 1 mg/kg succinylcholine), and ventilation with a 1:1 ratio of N_2O/O_2.

Controls. Ten healthy, fertile, nonpregnant women were studied as the adult control group for the mothers and as the reference group for newborns. With the aim of evaluating the role of anesthesia, 11 fertile, nonpregnant women undergoing general (eight cases) or epidural (three cases) anesthesia for routine surgery for indications such as uterine fibroma, lipoma, and cysts, excluding cancer, were also tested before anesthesia and at the end of surgery to evaluate the possible inhibitory effect of anesthesia on $\cdot O_2^-$ production.

The study was approved by the Human Ethics Committee of the Medical Faculty of the University of Siena. Informed consent was obtained from the parents of the newborn infants and from the adult subjects.

$\cdot O_2^-$ generation. Blood samples from mothers and neonates were collected in heparinized tubes (10 U/mL) and were tested within 10 min. $\cdot O_2^-$ generation was measured spectrophotometrically by the method of SOD-inhibitable reduction of ferricytochrome *c*, with and without opsonized zymosan stimulation of PMN in whole blood, according to the method of Bellavite *et al.* (5). Samples with and without zymosan were incubated at 37°C for 20 min. $\cdot O_2^-$ production was expressed as nmol/10^6 PMN. Care was taken to avoid methodologic variations throughout the experiment. All assays were performed in duplicate.

Antioxidant enzyme activities. In a subpopulation of 26 newborn infants (12 born by ECS, 14 by VD), red cell GSH-Px, CAT, glutathione reductase, and SOD activities were measured in duplicate in cord blood and in samples taken on the fourth day. The preparation of hemolysate from washed red cells and the assays of GSH-Px, CAT, and glutathione reductase were performed according to the methods summarized by Beutler (6). SOD was measured by the partially modified method of Beauchamp and Fridovich (7).

Statistics. The data are presented as means ± 1 SD. The analysis for within-group and between-group differences was determined by the two-tailed *t* test for paired and grouped data, respectively. Simple linear regression analysis was used to calculate statistical significance between $\cdot O_2^-$ and antioxidant enzyme activities levels.

RESULTS

$\cdot O_2^-$ release in all samples taken as a whole (mothers, newborn infants, and controls; Tables 1 and 2). Significantly lower values were observed in US *versus* ZS blood samples ($p < 0.001$).

$\cdot O_2^-$ release in mothers (Table 1). In US samples, no significant differences were found between mothers undergoing VD and ECS, whereas these values were significantly lower in both groups compared with the controls. ZS samples showed significantly greater $\cdot O_2^-$ release in mothers who underwent VD compared with those delivered by ECS. In comparison with the controls, values were significantly higher in the peripartal period in VD and lower in ECS. Four d after delivery, both groups showed significantly lower levels than the controls.

$\cdot O_2^-$ release in total population of newborn infants. A comparison of results found in cord blood and on the fourth day of life showed a significant decrease in both US (3.2 ± 2.44 *versus* 0.68 ± 0.7 nmol $\cdot O_2^-/10^6$ PMN, $p < 0.001$) and ZS (14.3 ± 11.6 *versus* 3.6 ± 2.8 nmol $\cdot O_2^-/10^6$ PMN, $p < 0.001$) samples.

$\cdot O_2^-$ release in neonates in relation to mode of delivery (Table 2). Neonates born by VD showed statistically significant, higher $\cdot O_2^-$ values compared with those born by ECS. In US samples, spontaneously delivered infants showed higher $\cdot O_2^-$ levels than those born by ECS only in cord blood. After zymosan stimulation, neonates born by VD had higher $\cdot O_2^-$ levels than those born by ECS in both cord blood and on the fourth day of life. The comparison of results found in cord blood and on the fourth day of life showed a significant decrease in $\cdot O_2^-$ levels in both groups of neonates.

The comparison between neonates and adult nonpregnant women (controls) showed a significantly lower $\cdot O_2^-$

Table 1. *$\cdot O_2^-$ release (nmol/10^6 PMN) in US and ZS samples of maternal blood**

	US samples	ZS samples
VD		
Beginning of labor	3.01 ± 2.1[a]	40.7 ± 36.7[i]
During labor	2.64 ± 1.4[b]	32.0 ± 28.6[l]
After delivery	2.58 ± 1.8[c]	42.9 ± 20.6[m]
4th d	1.82 ± 0.9[d]	9.2 ± 7.4[n]
ECS		
Before anesthesia	3.19 ± 1.2[e]	16.8 ± 5.2[y]
After extraction	3.02 ± 1.2[f]	18.6 ± 2.2[q]
4th d	1.22 ± 0.7[g]	8.9 ± 6.2[r]
Anesthesia control group		
Before anesthesia	4.11 ± 2.32[t]	27.62 ± 14.87[v]
After anesthesia	2.56 ± 3.24[u]	14.22 ± 8.79[z]
Controls	4.60 ± 1.60[h]	25.00 ± 6.8[s]

* The data are presented as means ± 1 SD. The analysis for within-group and between-group differences was determined by the two-tailed *t* test for paired and grouped data, respectively. a *vs* i, b *vs* l, c *vs* m, d *vs* n, e *vs* y, f *vs* q, g *vs* r, h *vs* s: $p < 0.001$; a *vs* d, i *vs* m, l *vs* m, m *vs* n, v *vs* z: $p < 0.01$; i *vs* y, m *vs* q, t *vs* u: $p < 0.05$; a, b, c, d, e, f, g, *vs* h: $p < 0.02$; y, q, r, *vs* s: $p < 0.009$.

Table 2. *$\cdot O_2^-$ release (nmol/10^6 PMN) in US and ZS blood samples of newborn infants**

	US samples	ZS samples
VD		
Cord blood	3.96 ± 2.1[a]	19.2 ± 13.7[e]
4th d	0.74 ± 0.5[b]	4.7 ± 2.3[f]
ECS		
Cord blood	2.12 ± 1.1[c]	8.1 ± 3.8[g]
4th d	0.44 ± 0.4[d]	2.1 ± 2.3[h]
Controls	4.60 ± 1.6[i]	25.0 ± 6.8[l]

* The data are presented as means ± 1 SD. The analysis for within-group and between-group differences was determined by the two-tailed *t* test for paired and grouped data, respectively. a *vs* b, c *vs* d, e *vs* f, g *vs* h: $p < 0.01$; a *vs* e, i *vs* l, b *vs* i: $p < 0.002$; e *vs* g: $p < 0.004$; f *vs* h: $p < 0.006$; a *vs* c: $p < 0.05$; b, c, d *vs* i: $p < 0.01$; f, g, h *vs* l: $p < 0.001$.

release in US samples of both groups of neonates on the fourth day of life. Neonates born by ECS showed lower $\cdot O_2^-$ levels than controls in ZS samples both in cord blood and on the fourth day. Neonates born by VD presented lower $\cdot O_2^-$ levels than controls only on the fourth day of life.

$\cdot O_2^-$ release in newborns in relation to $\cdot O_2^-$ release in their mothers. The comparison between the ZS $\cdot O_2^-$ release in mothers delivering spontaneously and that in cord blood of their respective neonates demonstrated a significant positive correlation. This correlation becomes more significant as labor progresses (at the beginning of labor, $r = 0.654$ and $p < 0.01$; during labor, $r = 0.721$ and $p = 0.008$; and immediately after delivery, $r = 0.832$ and $p = 0.0008$).

$\cdot O_2^-$ release in nonpregnant women undergoing general or epidural anesthesia. A lower $\cdot O_2^-$ production was observed after anesthesia than before anesthesia (Table 1).

Antioxidant red cell enzyme activities at birth and on the fourth day of life in a subpopulation of 26 newborn infants (Table 3). Significantly higher GSH-Px levels were observed on the fourth day of life than in cord blood. No significant correlation was observed between $\cdot O_2^-$ release and red cell antioxidant enzyme activities (GSH-Px, SOD, and CAT) in cord blood. A significant positive correlation was found between $\cdot O_2^-$ release and GSH-Px on the fourth day ($r = 0.709$, $p = 0.014$), whereas a significant negative correlation was seen between $\cdot O_2^-$ release and CAT activity ($r = -0.631$, $p = 0.036$). No other significant correlation was found between red cell enzyme activities and $\cdot O_2^-$ release.

Table 3. *Antioxidant red cell enzyme activities at birth and on the fourth day of life in a subpopulation of 26 newborn infants**

Enzyme activities	Cord blood	4th d blood
SOD (IU/μg Hb)	1.38 ± 0.15	1.31 ± 0.13
GSH-Px (IU/g Hb)	11.86 ± 1.54[a]	14.95 ± 3.42[b]
Glutathione reductase (IU/g Hb)	5.12 ± 1.53	5.98 ± 1.62
CAT (IU/g Hb)	10.96 ± 1.71	11.10 ± 1.96

* The data are presented as means ± 1 SD. a *vs* b: $p < 0.02$ (paired data).

DISCUSSION

The results of the present investigation demonstrate the role of peripartal events in modulating free radical release by the blood cells during the perinatal period. Because a highly significant difference has been found between cord blood and blood of 4-d-old neonates, it is feasible that intrauterine factors modulate $\cdot O_2^-$ release by PMN.

The hypothesis of an important role of plasma factors is confirmed by the data obtained in the mothers, which demonstrate that the mode of delivery is associated with differences in $\cdot O_2^-$ release.

Our results are in disagreement with previously reported data demonstrating that ZS oxygen consumption, hexosemonophosphate shunt activity, and quantitative nitroblue tetrazolium dye reduction were lower in cord blood PMN from infants delivered vaginally than in those delivered by ECS (8). However, the study by Frazier *et al.* (8) was carried out in isolated leukocytes, whereas our investigation was performed on whole blood. Because plasma factors may modulate phagocyte function, it is possible that the isolation of PMN substantially alters the conditions of phagocyte activation. The finding of lower values in ZS samples from spontaneously delivered infants compared with adult controls is in agreement with the findings of Ambruso *et al.* (9).

It is important to note that, although the values of $\cdot O_2^-$ production in US blood samples from mothers undergoing either VD or ECS did not differ, the $\cdot O_2^-$ values in the same subjects after zymosan were significantly higher in VD than ECS. The significant positive correlation between $\cdot O_2^-$ release after zymosan stimulation in cord blood and that found in the mother is of considerable interest. The *r* coefficient of this correlation increased progressively from the beginning of labor to the end of delivery.

The $\cdot O_2^-$ release in the maternal samples was also significantly higher after VD than after ECS. The difference between the two modes of delivery was also evident in the neonates. In particular, newborn infants delivered by ECS without labor showed significantly lower $\cdot O_2^-$ production after stimulation by zymosan and in basal conditions. Samples to which zymosan was added still showed significant differences between infants born by ECS and those born by VD, even after 4 d, when $\cdot O_2^-$ production was greatly reduced compared with that found in the cord blood.

The results of the determination of $\cdot O_2^-$ release during anesthesia, performed in women undergoing surgery for indications other than ECS, demonstrated lower $\cdot O_2^-$ release after anesthesia than that measured before anesthesia. Therefore, it cannot be ruled out that anesthesia or surgery plays a role in suppressing $\cdot O_2^-$ release. However, the differences that we observed in $\cdot O_2^-$ release between VD and ECS are independent of anesthesia, because the comparison was made between mothers during labor and mothers before anesthesia for ECS. Fur-

thermore, the importance of possible plasma factors is stressed by the significant correlation between $\cdot O_2^-$ release in mothers delivering vaginally and that in the cord blood samples of their neonates.

The differences in plasma hormone levels seen in ECS and VD might play a role in the differences in PMN activation (10). However, the results of previous investigations demonstrating variations in interleukin levels during labor (11, 12) and significant differences in IL-1 and IL-6 between cord blood of infants delivered by different modes (13) suggest that cytokine production during labor may be the most important factor. Because interleukins have a key role in the activation of the PMN burst (14), the agreement between the differences in IL-1 and IL-6 levels in cord blood and the differences in $\cdot O_2^-$ release suggest that interleukins may be one of the factors involved in the modulation of phagocyte function in the first hours of life.

Our recent observations demonstrated increased IL-1β and IL-6 during labor and immediately after delivery in mothers who had VD compared with mothers who had ECS. A significant positive correlation was found between values of IL-6 plasma concentrations and $\cdot O_2^-$ release (15).

The significant increase in GSH-Px on the fourth day of life and the positive correlation between $\cdot O_2^-$ release and erythrocyte GSH-Px in the same samples suggest that GSH-Px can increase in the red cell as a result of the toxic oxygen species deriving from the activated PMN oxidative burst (16–18). Therefore, the possibility that phagocyte activity may play a role in the increase in GSH-Px activity observed in the first days of life (19) cannot be ruled out.

The significance of negative correlation between $\cdot O_2^-$ release and CAT remains unknown.

Although the clinical significance of changes in $\cdot O_2^-$ release as a consequence of VD is unclear, the modifications reported in this study suggest that plasma factors modulate PMN activation in the peripartal period and during the first few days of life. In particular, it is evident that the mechanism of delivery *per se* constitutes a factor in the activation of PMN free radical release.

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