

Lipid peroxidation in cord blood at birth

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OBJECTIVE: The purpose of this study was to determine oxygen free radical activity in the neonate at birth and relate it to umbilical cord blood acid-base status.

STUDY DESIGN: A series of 110 singleton deliveries had determination of two lipoperoxides in umbilical cord blood: malondialdehyde and organic hydroperoxide. Umbilical pH, P_{O_2} , P_{CO_2} , bicarbonate, and base excess were also measured.

RESULTS: There was a significant association between lipoperoxides and cord blood pH and base excess. A significant difference existed in the levels of umbilical artery lipoperoxides between nonacidemic and acidemic fetus, as defined by an umbilical arterial pH <7.20.

CONCLUSION: There is a positive association between lipoperoxide production and acid-base balance at delivery. (AM J OBSTET GYNECOL 1996;174:62-5.)

Key words: Cord blood, lipid peroxidation, malondialdehyde, organic hydroperoxide

Severe metabolic acidosis resulting from intrapartum or postpartum ischemia and hypoxemia may result in harmful effects such as periventricular hemorrhage and impaired myocardial function.^{1, 2} Persistent intrauterine asphyxia may result in ischemia to the organs, leading to permanent damage, especially the brain. The baby is at risk of newborn encephalopathy and, more severely, cerebral palsy.³

Tissue hypoxia or ischemia leads to enhancement in the production of hypoxanthine from adenosine monophosphate and a decrease in its ribosylphosphorylation to inosine monophosphate through the salvage pathway.^{4, 5} In asphyxiated babies a high level of cord blood hypoxanthine has been detected, and cord plasma hypoxanthine can be used as a marker of hypoxia.^{6, 7} Catabolism of hypoxanthine by xanthine oxidase in endothelial cells produces oxygen free radicals. Brain injury in a hypoxic infant may be induced by oxygen free radicals derived from hypoxanthine.^{8, 9}

Oxygen free radicals are highly reactive, the reactive species include the superoxide radical and the hydroxyl radical.¹⁰ Electron spin resonance spectroscopy, incorporating the technique of chemical spin traps, provides a direct method of measurement.¹¹ Chemiluminescence with luminol enhancement is a simple and sensitive method.¹² Oxidative activity of oxygen radicals in biologic systems can be determined by measuring their oxidative

products.¹³ One common approach is to measure the peroxidation product of membrane polyunsaturated fatty acids, malondialdehyde, by the thiobarbituric acid reaction.¹⁴ The malondialdehyde–thiobarbituric acid adduct can be readily extracted and measured by spectrophotometry or fluorometry.^{15, 16} Preceding their fragmentation into malondialdehyde, the lipid hydroperoxides formed after hydrogen abstraction of the peroxy radicals in the peroxidation process can also be measured collectively as organic hydroperoxides.^{17, 18} For measurement of oxygen radical the malondialdehyde and organic hydroperoxides assays lack specificity.¹⁹ However, they do provide a useful test of lipid peroxidation and an estimate of the oxidative activity of oxygen free radicals in biologic systems.^{12, 20}

In this study our purpose was to investigate the relationship between oxygen free radical activity and asphyxia during labor. We determined malondialdehyde and organic hydroperoxides in cord blood as a measure of oxygen radical activity. Asphyxia will be assessed by acid-base studies of cord blood.^{21, 23}

Material and methods

This study included 110 women with singleton pregnancies at 37 to 42 weeks' gestation delivered at Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, between December 1993 and May 1994. The study was approved by The Chinese University of Hong Kong ethics committee.

Immediately after delivery a segment of umbilical cord was isolated with clamps and blood was drawn from both the umbilical artery and umbilical vein into 10 ml pre-heparinized plastic syringes. Blood analysis was performed on the whole blood samples within 5 minutes of delivery with a Ciba Corning 288 (Medfield, Mass.) blood

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gas system. Measurements included pH, P_{CO_2} , P_{O_2} , bicarbonate content, and base excess. The bicarbonate was calculated by the machine. Acidemia was defined as an umbilical artery blood pH of ≤ 7.20 . The plasma in both artery and venous blood samples was obtained by refrigerated centrifugation at 1000g for 10 minutes. Before malondialdehyde and organic hydroperoxide analysis the plasma samples were stored at -20°C for not more than 2 weeks, during which the malondialdehyde and organic hydroperoxide levels were found to be stable.

The plasma malondialdehyde levels were estimated as reactive substances by a thiobarbituric acid adduction method described by Richard et al., with modification.¹⁶ The primary reagents were thiobarbituric acid/perchloric acid (2:1, vol/vol) mix, a calibration solution of 1,1,3,3-tetraethoxypropane (10 $\mu\text{mol/L}$) and butylated hydroxytoluene. Each analytic run included the assay of a reagent blank, tetraethoxypropane working standard solutions, plasma specimens, and quality control samples. The continuation of lipid peroxidation during the reaction was prevented by addition of 10 $\mu\text{mol/L}$ butylated hydroxytoluene (20 gm/L). In each reaction tube 100 $\mu\text{mol/L}$ plasma and 750 $\mu\text{mol/L}$ thiobarbituric acid-perchloric acid mix were combined, vortex mixed, tightly capped and placed in a 95°C water bath. After 60 minutes the samples were chilled in ice to stop the reaction. Reagent blank, standard blank, and assay blank were left at room temperature. Two milliliters of *n*-butanol was added to each tube to extract the thiobarbituric acid-malondialdehyde complex by vigorous vortex mixing and centrifugation. The thiobarbituric acid-malondialdehyde adduct in the butanol extract was determined by fluorometry at 532 nm excitation and 553 nm emission. The coefficient of variation was 1.7% within assays and 8.2% between assays.

The method used for determination of organic hydroperoxides was the enzymatic technique described by Heath and Tappel,¹⁷ with modification. Briefly, 50 $\mu\text{mol/L}$ plasma and 350 $\mu\text{mol/L}$ Tris-ethylenediamine-tetraacetic acid, pH 7.6, were incubated with 5 $\mu\text{mol/L}$ catalase (17,500 kU/L) at room temperature for 10 minutes. Then 25 $\mu\text{mol/L}$ reduced nicotinamide adenine dinucleotide phosphate (2 mmol/L), 5 $\mu\text{mol/L}$ glutathione peroxidase (2500 kU/L), and 50 $\mu\text{mol/L}$ glutathione (4.25 mmol/L) were added. After further incubation at 33°C for 10 minutes the absorbance of the reaction mixture at 340 nm was measured on a Response spectrophotometer (Gilford Instruments Laboratory, Oberlin, Ohio). A 2.5 μl aliquot of glutathione reductase (100 kU/L) was added immediately, and the reaction mixture was allowed to proceed for another 10 minutes at 33°C . Its absorbance at 340 nm was again measured to determine the net amount of reduced nicotinamide adenine dinucleotide phosphate oxidized. Values were expressed by comparison with a standard of butyl hydroperoxide (0 to 600 $\mu\text{mol/L}$). The coefficient of varia-

tion within assays was 5.8% and between assays 11.4%.

Statistical analysis was performed with SPSS (Statistical Package for Social Science, Windows 6.1 version). A *p* value <0.05 was considered significant.

Results

Maternal age ranged from 18 to 41 years (mean \pm SD, 29 ± 5 years). Gestational age ranged from 37 to 42 weeks (mean \pm SD, 39 ± 2 weeks). Ninety-five patients were admitted in spontaneous labor and the other 15 had labor induced. Twenty-two patients were delivered by cesarean section for either failure to progress ($n = 14$) or fetal distress ($n = 4$) or both ($n = 4$). Twenty-five deliveries were by vacuum extraction, 7 by forceps, and the remaining 56 were spontaneous deliveries.

A significant positive correlation (Pearson) existed between arterial and venous levels of malondialdehyde, organic hydroperoxides, pH, P_{CO_2} , bicarbonate, and base excess (Table I). The mean arterial levels were higher than the venous levels for malondialdehyde, P_{CO_2} , and bicarbonate but lower for pH, P_{O_2} , and base excess. There was no significant difference between the mean organic hydroperoxides levels in artery and vein. Both arterial malondialdehyde and organic hydroperoxides had a significant negative correlation with cord blood pH, whereas arterial malondialdehyde alone showed significant correlation with P_{CO_2} and P_{O_2} (Table II). The arterial-venous difference of malondialdehyde correlated significantly with pH, P_{CO_2} and P_{O_2} , whereas that of organic hydroperoxides did not (Table II).

Sixteen cord blood samples were classified as acidemic and 94 as nonacidemic (Table III). Arterial plasma levels of malondialdehyde and organic hydroperoxides were significantly higher in the acidemia group (Student *t* test, $p < 0.05$). There was no significant difference in the arterial-venous difference of malondialdehyde and organic hydroperoxides between the acidemia and nonacidemia groups.

Comment

In 1969 McCord and Fridovich²⁴ discovered an enzyme specific for the catalytic removal of the superoxide radical, which was found to be distributed throughout all mammalian cells. This has led to extensive work relating free radical species to a wide range of cellular damage in biologic systems. Free radicals are now implicated in vast spectrum of disease processes.

Free radicals are produced primarily by univalent, biochemical redox reactions involving oxygen. Because of their high reactivity it is often difficult to detect free radicals in vivo, particularly in biologic systems. Polyunsaturated fatty acids, proteins, deoxyribonucleic acid, and carbohydrates are all susceptible to free radical attack, particularly by the hydroxyl radical. Loss of cell membrane unsaturated fatty acids, formation of lipid perox-

Table I. Correlation analysis between umbilical arterial and venous levels of malondialdehyde, organic hydroperoxide levels, and blood gas in 110 term deliveries

| | Artery (mean \pm SD) | Vein (mean \pm SD) | Correlation coefficient (significance) | Arterial-venous difference (significance) |
|---|------------------------|----------------------|--|---|
| Malondialdehyde ($\mu\text{mol/L}$) | 1.487 \pm 0.119 | 1.463 \pm 0.112 | 0.831 ($p < 0.001$) | $p < 0.05$ |
| Organic hydroperoxide ($\mu\text{mol/L}$) | 0.256 \pm 0.037 | 0.254 \pm 0.032 | 0.658 ($p < 0.05$) | NS |
| pH | 7.27 \pm 0.07 | 7.30 \pm 0.07 | 0.914 ($p < 0.001$) | $p < 0.001$ |
| Pco ₂ (kPa) | 6.68 \pm 1.20 | 5.95 \pm 1.10 | 0.828 ($p < 0.001$) | $p < 0.001$ |
| Po ₂ (kPa) | 2.69 \pm 0.75 | 3.61 \pm 0.83 | 0.682 ($p < 0.001$) | $p < 0.001$ |
| Bicarbonate (mmol/L) | 22.82 \pm 2.86 | 21.98 \pm 2.82 | 0.847 ($p < 0.001$) | $p < 0.001$ |
| Base excess (mmol/L) | -4.41 \pm 3.55 | -3.95 \pm 3.53 | 0.739 ($p < 0.05$) | $p < 0.05$ |

NS, Not significant ($p > 0.05$).**Table II.** Correlation analysis: A, Umbilical arterial lipid peroxides and acid-base status at birth ($n = 110$); B, umbilical arterial-venous difference of lipid peroxides and acid-base status (correlation coefficient and significance)

| | pH | Pco ₂ | Po ₂ | Bicarbonate | Base excess |
|--|------------------------|-----------------------|------------------------|-----------------------|-----------------------|
| A | | | | | |
| Malondialdehyde | -0.383 ($p < 0.001$) | 0.398 ($p < 0.001$) | -0.281 ($p < 0.05$) | -0.001 (NS) | -0.216 ($p < 0.05$) |
| Organic hydroperoxide | -0.225 ($p < 0.05$) | 0.073 (NS) | -0.123 (NS) | -0.223 ($p < 0.05$) | -0.268 ($p < 0.05$) |
| B | | | | | |
| Malondialdehyde (arterial-venous difference) | -0.241 ($p < 0.05$) | 0.330 ($p < 0.001$) | -0.331 ($p < 0.001$) | 0.088 (NS) | -0.082 (NS) |
| Organic hydroperoxide (arterial-venous difference) | 0.027 (NS) | 0.013 (NS) | -0.083 (NS) | 0.072 (NS) | 0.024 (NS) |

NS, Not significant ($p > 0.05$).**Table III.** Umbilical arterial malondialdehyde and organic hydroperoxide levels at birth in acidemia and nonacidemia

| | Acidemia ($n = 16$) | Nonacidemia ($n = 94$) | Significance |
|-----------------------|-----------------------|--------------------------|--------------|
| Malondialdehyde | 1.631 \pm 0.160 | 1.462 \pm 0.091 | $p < 0.05$ |
| Organic hydroperoxide | 0.282 \pm 0.190 | 0.251 \pm 0.038 | $p < 0.05$ |

ides, and oxygen uptake by lipid preparations are indicative of peroxidation. The highly reactive free radicals of oxygen also attack membrane phospholipids and act on unsaturated fatty acid to produce lipid peroxides. Peroxidation of fatty acids containing three or more double bonds will produce malondialdehyde. The presence of this oxidation by-product can be measured with thiobarbituric acid, which correlates with the extent of lipid peroxidation.

Fetal acidosis results when fetal oxygenation is insufficient; therefore measurement of umbilical cord blood acid-base balance is useful to determine whether the fetus has undergone hypoxia during labor.²¹ It serves as a measure of the welfare of the fetus and provides indications about the mechanisms responsible for fetal asphyxia. The fetus reacts at the onset of asphyxia with a remarkable series of responses, primarily a complexly regulated redistribution of blood flow that serves to limit the deleterious effects of oxygen limitation in vital or-

gans. This usually enables the fetus to survive hypoxia unless the insult is too profound or prolonged. The most common hypoxic stresses imposed on the fetus during labor are related to insufficiency of uterine or umbilical blood flow and occasionally to a decrease in uterine arterial oxygenation.

The values reported here for umbilical cord blood acid-base status in 110 term deliveries are in general agreement with those of other workers.²² The significant difference in arterial-venous levels of Po₂ and Pco₂ suggested good placental function in all the deliveries in this study. Fetal acid-base values from the umbilical artery reflect the condition of the fetus in utero because this blood flows from the fetus on its way back to the placenta and therefore reflects the metabolic status of the fetal tissues. The placenta is drained by the umbilical vein, and blood from this source reflects transplacental respiratory exchange with the mother. Umbilical arterial pH was lower than corresponding venous pH, whereas the

base excess was larger in cord artery than in vein.

In this study the umbilical arterial and venous levels of both malondialdehyde and organic hydroperoxides correlated well. The arterial-venous difference of malondialdehyde is statistically significant, but the difference is small. There was no arterial-venous differences for organic hydroperoxides. Reason for the failure of the placenta to clear the lipid peroxides is unclear, because good placental function was demonstrated by the blood gas results. It may be that the placenta was producing lipid peroxides, as were the fetal tissues during delivery. The placenta might also be more effective in the removal of malondialdehyde than its organic hydroperoxides precursors.

Significantly higher umbilical arterial malondialdehyde and organic hydroperoxides was found in acidemic cord blood samples than in nonacidemic samples. Arterial malondialdehyde and its arterial-venous difference has significant negative correlation with pH and P_{O_2} and positive correlation with P_{CO_2} . Such association did indicate the possibility of higher production of lipid peroxides during delivery in acidemia. Increased lipid peroxidation may be a direct result of enhanced oxidative activity of oxygen free radicals.

Abnormal fetal heart rate patterns correlate well with acid-base abnormalities. Cardiotocography may indicate signs of fetal distress, whereas fetal acid-base status remains undisturbed. Myocardial ischemia and reperfusion are known to induce oxygen free radical generation and result in increased levels of lipid peroxides.^{25, 26} Whether the increased malondialdehyde and organic hydroperoxide levels in our study patients were due to repeated myocardial ischemia and reperfusion events or were a manifestation of a more generalized impairment of the vasodilatory reserve is not known. The fetal electrocardiogram and cardiotocogram in these patients were recorded continuously during labor. A study of the relationships between fetal cardiac dynamic patterns, lipoperoxidation, umbilical cord artery pH, and cord plasma purine metabolites is now underway in these 110 births and cases collected subsequently.

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