Free Radical Research, December 2007; 41(12): 1358–1363

informa
healthcare

Changes of nitric oxide, carbon monoxide and oxidative stress in term infants at birth

CARLO DANI[1], LUCIA GIANNINI[2], GIOVANNA BERTINI[1], SIMONE PRATESI[1], IURI CORSINI[1], MARIANGELA LONGINI[3], GIUSEPPE BUONOCORE[3], EMANUELA MASINI[2], & FIRMINO F. RUBALTELLI[1]

[1]*Department of Surgical and Medical Critical Care, Section of Neonatology,* [2]*Department of Clinical and Preclinical Pharmacology, Careggi University Hospital of Florence, Viale Morgagni, 85, 50134 Florence, Italy, and* [3]*Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Viale M. Bracci, 16, 53100 Siena, Italy*

Accepted by Professor Giovanni Mann

(Received 1 August 2007; in revised form 4 October 2007)

Abstract

The higher risk of respiratory problem in infants delivered by elective caesarean section in comparison with vaginally born infants may be favoured by lower level of nitric oxide (NO) and carbon monoxide (CO) and higher oxidative stress in infants born by caesarean section. We studied healthy term infants born by vaginal delivery or by elective caesarean section. Nitric oxide, CO, guanosine 3–5 cyclic monophosphate, total hydroperoxide and advanced oxidation protein products (AOPP) were measured at birth and 48–72 h of life. Nitric oxide, CO and cGMP were lower at birth and at 48–72 h of life in infants born by elective caesarean delivery. Total hydroperoxide and AOPP levels were similar in the two groups and increased from birth to 48–72 h of life. In conclusion, nitric oxide and CO concentrations were higher in term infants vaginally born than in infants born by elective caesarean section and decreased from birth to 48–72 h of life. The mode of delivery did not affect the oxidative stress which increases from birth to 48–72 h of life.

Keywords: *Nitric oxide, carbon monoxide, oxidative stress, infant, vaginal delivery, caesarean section*

Introduction

It is widely accepted that term infants delivered by elective caesarean section are at increased risk for respiratory problems in comparison with term infants delivered vaginally [1–3]. This likely occurs because elective caesarean section can exert negative effects on physiological mechanisms which regulate the transition from foetal to neonatal circulation and the beginning of neonatal breathing, such as the decrease of pulmonary vascular resistance and development of lung volumes.

After birth, with initiation of lung ventilation and increase in alveolar oxygen tension, pulmonary vascular resistance decreases and pulmonary blood flow increases 8–10-fold [4–7]. Recent findings suggest that this decrease is regulated by both mechanical and metabolic factors. Among the metabolic factors, reactive oxygen species and nitric oxide (NO) have been demonstrated to play a major role in the regulation of pulmonary vascular tone [8,9]. In fact, inhibition of NO synthesis increases pulmonary vascular resistance in late-gestation foetal lambs [4], whereas inhaled NO has the opposite effect [10]. Also the production of guanosine 3–5 cyclic monophosphate (cGMP), the intracellular second messenger of NO, influences the tone of pulmonary vessels

Correspondence: Carlo Dani, MD, Division of Neonatology, Careggi University Hospital, Viale Morgagni 85, I-50134 Firenze, Italy. Tel: +39 055 794 7428. Fax: +39 055 794 7428. E-mail: cdani@unifi.it

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760701732822

during the first weeks of life [11–13]. Moreover, carbon monoxide (CO) has been found to induce vasodilation, increasing intracellular levels of cGMP in vascular smooth muscle cells similarly to NO [14,15]. Increasing evidence in the literature also suggest a regulatory interaction in vascular smooth muscle cells between NO and CO [16], particularly during hypoxia [17], when vascular production of CO is reported to be a compensatory response to the suppression of NO production [18].

In addition, it has been demonstrated that other mediators are important in endothelium-dependent vasodilation. In particular, endogenously produced hydrogen superoxide (H_2O_2) can induce vasorelaxation of mice small mesenteric arteries [19] and bovine pulmonary arteries [20]. Thus, also oxidative stress might be involved in the regulation of transition from foetal to neonatal circulation and NO synthesis.

On these bases, we hypothesized that the lack of labour in healthy infants born by elective caesarean section might be followed by lower plasma levels of NO, CO, cGMP and lower oxidative stress in comparison with vaginally born healthy infants, thus favouring their increased risk for the development of respiratory disorders. To evaluate this hypothesis and investigate possible relationships between plasma levels of NO, cGMP, CO and oxidative stress, we carried out the present prospective study in which healthy term infants born by elective caesarean section or vaginally delivered were enrolled.

Materials and methods

Patients

We prospectively studied healthy infants with gestational age ≥ 37 weeks and birth weight appropriate for gestational age, born after uncomplicated pregnancy by vaginal delivery or elective caesarean section after epidural anaesthesia. Infants were enrolled only if they had an uneventful delivery with a 5 min Apgar score of 8–10, were clinically well and had no respiratory distress. In order to obtain a proper patient enrolment and allow for data collection, we studied infants who were born by elective caesarean section early in the morning and the first following infant who was born by vaginal delivery.

For each newborn gestational age, birth weight, sex, type of delivery, blood cord pH and Apgar score at 5 min were recorded.

In both groups, 1 mL blood samples were obtained from cord vein at birth and from a heel prick at 48–72 h of life together with that required for metabolic screenings. A small amount of blood (100 μL) was collected for CO measurement, while the remaining was centrifuged and plasma was separated in two aliquots. In the first aliquot oxidative assays were carried out within 2 h from blood withdrawal to avoid the effects of storage. The second aliquot was frozen at -20°C for the determination of NOx and cGMP.

Moreover, total bilirubin was measured in blood samples by reflectance spectrophotometry (Microbilimeter™, Ginevri, Rome, Italy) in cord blood and in venous blood at 48–72 h of life.

Measurement of NO, cGMP, and CO

Evaluation of NO production was performed in plasma by determining the nitrite (NO_2^-) amount, the stable end products of NO metabolism, spectrophotometrically by the Griess reaction [21]. Briefly, samples were supplemented with 276 mU nitrate reductase and 40 μm $NADPH^+$ (to convert nitrates to nitrites) and then allowed to react with the Griess reagent (aqueous solution of 1% sulphanylamide and 0.1% naphthylethylendiamine dihydrochloride in 2.5% H_3PO_4) to form a stable cromophore absorbing at 546 nm wavelength. The values were obtained by comparison with reference concentrations of sodium nitrite and expressed as nmoles of NOx per ml of plasma.

The concentrations of cGMP were determined in plasma added with 10^{-4} 3′-isobutyl-1-methylxanthine (IBMX) by a competitive enzyme immunoassay kit (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's specifications. Values were expressed as fmol of cGMP per ml of plasma.

For carbon monoxide blood determinations we prepared the reaction mixture for liberating haemoglobin-bound CO from blood as previously described [22]. The following were added to 5 ml of distilled water: 50 mg of saponin, 1 g of $K_3[Fe(CN)_6]$ and 1 ml of potassium phosphate buffer, 1 mol/l, pH 6. The volume was adjusted to 10 ml. The solution was prepared freshly each day and kept at ambient temperature, out of direct sunlight. Reactions were performed in septum-sealed glass vials with screw caps (12×32 mm, 2 ml, Supelco, Bellefonte, PA). Twenty five mictolitres of reaction mixture was dispensed onto the bottom of each vial with 5 μl of blood samples. We determined the amount of CO liberated into the reaction vial headspace with an ultra-trace level gas chromatograph capable of detecting ppb concentrations of CO in samples (ta3000 Gas Analyzer, Trace Analytical, SAES Getters Milan, Italy). The headspace gas components of interest were separated chromatographically within an isothermal mandrel-heating column oven at a temperature of 100°C with CO-free air carrier gas (N_2, 70 cc/min). Species eluted from the chromatographic column passed immediately into the detector. Carbon monoxide was quantitated in the detector by means of the A_{254} of Hg generated from the reaction of CO with HgO at 225°C [23]. A CO standard curve was prepared immediately prior to sample analysis. Standard gas was obtained from a commercial source

(Rivoira, Milan, Italy). Values were expressed as ppm of CO determined in 5 μl of blood.

The measurement of NO and CO in blood sample derived from systematic circulation is commonly considered in literature a valid estimation of their endogenous production within the pulmonary circulation because the stimuli which increase the endothelial NO synthase (eNOS) and inducible heme oxygenase (HO-1) expression and activity are the same in the systematic and pulmonary vessels. Thus, it is reasonable to recognize that an increase of NO and CO plasma level imply their consensual contemporary increase in the pulmonary circulation.

Oxidative stress assay

Total hydroperoxide (TH) concentration represents a measure of overall oxidative stress, given that it is the intermediate oxidative products of lipids, peptides and amino acids. Its production was measured with a d-ROMs Kit (Diacron srl, Italy) by the method described by Buonocore et al. [24]. This method makes it possible to estimate the total amount of hydroperoxide present in a 10 μL sample by using a spectrophotometric procedure. Hydroperoxidic groups were attacked by the iron, decompartmentalized from transport protein in 1 mL of acetate buffer at a pH 4.8, to catalyse reactive oxygen metabolite formation by Fenton's reaction. The peroxy and alkoxy radicals produced, whose quantities were directly proportional to peroxides present in the bronchial aspirate, were trapped chemically by 10 μL of chromogen (N,N-dyethyl.para-phenyldiamine) in an electron-transfer process leading to the formation of the radical cation of this chromogen. The purple colour resulting from this reaction over time was then monitored in an UV-VIS spectrophotometer (Perkin Elmer λ 16, Norwalk, CN) at 505 nm. The results were expressed in conventional units, (Carr units: the value of 1 Carr unit is equal to a concentration of 0.08 mg/dL of hydrogen peroxide).

Simultaneous determination of the advanced oxidation protein products (AOPP) provides information regarding another aspect of protein involvement in free radical reactions, namely oxidized proteins that have lost their oxidant properties [25]. We measured AOPP by the method of Witko-Sarsat et al. [25], using spectrophotometry on a microplate reader. The AOPP were calibrated with chloramine-T solutions that absorb at 340 nm in the presence of potassium iodide. In test wells, 200 μL of bronchial aspirate sample diluted 1:5 in PBS were distributed on a 96-well microtiter plate and 20 μL of acetic acid were added. In standard wells, 10 μL of 1.16 m potassium iodide were added to 200 μL of chloramine-T solution (0–100 μmol/L) followed by 20 μL of acetic acid. The absorbency of the reaction mixture was immediately read at 340 nm on the microplate reader against a blank containing 200 μL of PBS, 10 μL of potassium iodide and 20 μL of acetic acid. Because the absorbency of chloramine-T at 340 nm is linear up to 100 μmol/L, AOPP concentrations were expressed as μmol/L chloramine-T equivalents.

Data analysis

Clinical characteristics of the two groups were described by median values and ranges, laboratory results by mean values and standard deviations. Statistical analysis was performed using the Student *t*-test for parametric continuous variables and the Fisher's exact test for categorical variables. A $p < 0.05$ was considered statistically significant.

Simple regression analysis was used to assess the correlation between NO, CO, cGMP, TH and AOPP.

Results

Our study ran for 3 months, April–June 2004. One hundred infants were eligible in the study, but only 24 were enrolled. Among these, 11 infants were born by vaginal delivery and 13 by elective caesarean section, which were performed for previous caesarean section in 10 cases and dystocia in three cases.

The median gestational age [38.9 (37–41) vs 39.1 (38–41) weeks], birth weight [3160 (2440–4000) vs 3470 (2520–4250) g], Apgar score at the 5th min [9.1 (9–10) vs 8.9 (8–9)] and blood cord pH [7.28 (7.20–7.32) vs 7.26 (7.22–7.34)] were similar in infants born by vaginal route or elective caesarean section. Seven infants in both the groups were males. The median age at second blood sampling was 59.3 (52.1–66.5) and 61.9 (57.5–66.4) h, respectively.

Total bilirubin plasma concentrations increased, although not significantly, from birth to 48–72 hof life in both the groups (vaginal delivery: 26 ± 34 and 46 ± 22 μmol/L; caesarean section: 27 ± 31 and 51 ± 31 μmol/L), without differences between the groups.

At birth, plasma NOx (24.7 ± 2.9 vs 31.4 ± 2.9 μmol/l; $p < 0.0001$), cGMP (131.4 ± 18.4 vs 197.6 ± 25.1 fmol/l; $p < 0.0001$) and blood CO (6.3 ± 0.6 vs 8.5 ± 0.8 ppm; $p < 0.0001$) were lower in infants born by elective caesarean delivery than in infants born vaginally. At 48–72 h of life the concentration of NOx (22.1 ± 2.7 vs 29.9 ± 4.6 μmol/l; $p < 0.05$), cGMP (118.6 ± 19.6 vs 170.6 ± 14.5 fmol/l; $p < 0.0001$) and CO (4.3 ± 0.4 vs 6.3 ± 0.5 ppm; $p < 0.0001$) remained lower in infants born by elective caesarean delivery. Moreover, the concentrations of NOx, cGMP and CO decreased from birth to 48–72 h of life in both the groups, although the difference for cGMP was not statistically significant at 48–72 h (Table I).

There were no differences in TH and AOPP plasma levels in infants born by vaginal delivery or

Table I. Changes of NOx (nmol/ml), CO (ppm), cGMP (fmol/l), AOPP (μmol/l) and TH (Carr units) at birth and 48–72 h of life in healthy term infants born by vaginal delivery or elective caesarean section.

	Birth	48–72 h of life	*p*
NOx:			
Vaginal delivery	31.4 ± 2.9	29.9 ± 4.6	<0.05
Caesarean section	24.7 ± 2.9	22.1 ± 2.7	<0.05
p	<0.0001	<0.05	
CO:			
Vaginal delivery	8.5 ± 0.8	6.3 ± 0.5	<0.05
Caesarean section	6.3 ± 0.6	4.3 ± 0.4	<0.05
p	<0.0001	<0.0001	
cGMP:			
Vaginal delivery	197.6 ± 25.1	170.6 ± 14.5	<0.05
Caesarean section	131.4 ± 18.4	118.6 ± 19.6	>0.05
p	<0.0001	<0.0001	
TH:			
Vaginal delivery	110.0 ± 35.1	165.3 ± 50.9	<0.001
Caesarean section	94.6 ± 29.3	172.9 ± 60.4	<0.0001
p	>0.05	>0.05	
AOPP:			
Vaginal delivery	84.3 ± 37.5	182.0 ± 36.7	<0.0001
Caesarean section	86.2 ± 23.9	188.3 ± 49.1	<0.0001
p	>0.05	>0.05	

elective caesarean section both at birth (TH: 110.0 ± 35.1 vs 94.6 ± 29.3 Carr units; AOPP: 84.3 ± 37.5 vs 86.2 ± 23.9 μmol/l) and at 48–72 h of life (TH: 172.9 ± 60.4 vs 165.3 ± 50.9 Carr units; AOPP: 182.0 ± 36.7 vs 188.3 ± 49.1 μmol/l). Similar statistically significant increase of TH and AOPP occurred in both the groups from birth to 48–72 h of life. (Table I)

Our results indicate a positive relationship between NOx and CO ($r = 0.427$, $p = 0.001$), NOx and cGMP ($r = 0.816$, $p = 0.001$), and CO and cGMP ($r = 0.391$, $p = 0.03$), while NOx and TH (0.540, $p = 0.003$), NOx and AOPP ($r = 0.405$, $p = 0.036$), CO and TH ($r = 0.503$, $p = 0.008$), and CO and AOPP ($r = 0.601$, $p = 0.001$) are inversely correlated.

Discussion

In our study, we found that NOx, CO and cGMP decreased from birth to 48–72 h of life in both vaginally born or born by elective caesarean section healthy term infants. In addition, we observed that NOx, CO and cGMP were lower in infants born by caesarean section.

We speculate that this occurs because during parturition foetuses experience many stresses, such as hypoxia, ischemia-reperfusion and tissue shear stress, which can stimulate strongly the expression of genes encoding inducible NO synthase (iNOS) and inducible enzyme heme oxygenase-1 (HO-1) [26–30]. Therefore, we suggest that an increase in activity of iNOS and HO-1 occurs at delivery and promotes the synthesis of NO, CO and, in turn, cGMP. After delivery inducing stimuli disappear and the production of NO and CO progressively decreases and then at 48–72 h of life their values become significantly lower than at birth. Certainly, stimuli promoting the activity of iNOS and HO-1 are stronger in infants born by vaginal route after labour than in infants born by caesarean section and this might be the reason why in these latter the concentrations of NOx, CO and cGMP were found persistently lower.

On the other hand, it has been demonstrated in piglet [31], sheep [32] and rabbit pulmonary arteries [33] that NO-induced vasorelaxation increases with postnatal age; therefore, although in our infants NOx decreases at 2–3 days of life, its level probably remains sufficient to promote the physiologic decrease of pulmonary vascular resistance.

NOx plasma level and CO blood content were significantly and positively correlated. This finding confirms in a clinical setting the experimental evidence of a regulatory interaction in vascular smooth muscle cells between NO and CO via HO-1 [17], with HO-1 being up-regulated by NO during hypoxia [16]. It is interesting that analysing the temporal pattern that characterizes the interaction between the NO- and CO-generating system it was found that the increase of iNOS protein precedes HO-1 induction [26]. Moreover, it has been demonstrated *in vitro* that CO and NO produced vasorelaxation in neonatal vessels, although the vasorelaxant potency of CO is markedly less than that of NO [34]. Thus, NO and CO, acting as modulators of pulmonary vasculature tone, might play a physiologic role in the transition from foetal to neonatal circulation lowering the pulmonary vascular resistances. Accordingly, the lower level of NOx and CO observed in our infants born by elective caesarean section could represent one of the causes for the increased risk of respiratory disorders (through the inadequate decrease of pulmonary vascular resistance) which these infants exhibit in comparison with vaginally delivered infants [1–3].

We wondered if maternal NO and CO blood content might influence infants' blood concentrations. It is reasonable that the high avidity of haemoglobin for NO and CO contributes to avoid their diffusion across the placenta, but, in any case, it has recently been demonstrated that human placenta represents a significant barrier to gas transfer [35].

To the best of our knowledge, the possible relationship between oxidative stress and NO and CO production has never been studied before in term infants. We found that oxidative stress was similar in infants born by vaginal delivery or elective caesarean section, and increased from birth to 48–72 h of life in both the groups. These data confirm previous reports demonstrating that the route of delivery does

not affect the oxidative stress in newborns at birth, probably due to the fact that the antioxidant system works more efficiently in newborns vaginally delivered to overcome the higher oxidative stress [36,37]. We suggest that oxidative stress increased at 48–72 h of life likely as a consequence of the beginning of respiration and increase of oxygen arterial partial pressure which strongly favours the development of oxidative processes.

We found that oxidative stress was inversely related to NOx and CO blood concentration. The negative correlation between the concentration of NOx and the concentrations of measured markers of oxidative stress, TH and AOPP, can be explained by different mechanisms which might occur in the newborns: 1) an antioxidant effects of NO [38–40]; 2) reactive oxygen species, such as superoxide anions, can inactivate NO forming peroxynirite [41]; 3) the stimuli inducing NO synthesis could also directly or indirectly decrease oxidative stress through other unknown mechanisms.

In contrast, the inverse correlation between the concentration of CO and TH and AOPP cannot be explained by reactive oxygen species CO inactivation, because it is not a free radical and is not expected to react with superoxide [34]. In fact, the antioxidant enzyme superoxide dismutase has no effect on CO-induced vasorelaxation [34]. Hence, we speculate that the decreases of CO might be secondary to 1) the decrease of NO which is a powerful inducer of HO-1 expression [16,17,26,42]; 2) an antioxidant effects of CO by mechanisms other than bilirubin formation [43]; similarly to NO, also the stimuli inducing CO synthesis could also directly or indirectly decrease oxidative stress.

We concluded that NOx and CO concentrations are higher in infants vaginally born than in infants born by elective caesarean section and decreases from birth to 48–72 h of life. These changes may be due to the progressive exhaustion of stimuli promoting the expression and the activity of iNOS and HO-1 at birth, which are stronger after vaginal delivery. However, NOx decline may be induced also by NO oxidation and promotes itself the decrease of CO through the down-regulation of HO-1. Moreover, we observed that the mode of delivery does not affect the oxidative stress which increases from birth to 48–72 h of life likely for the beginning of breathing and the intensification of oxidative processes following the increase of oxygen partial arterial pressure.

Acknowledgements

This work was supported by a grant from 'Ente Cassa di Risparmio di Firenze', 2004 (E.M.).

References

[1] Hook B, Kiwi R, Amini SB, Fanaroff A, Hack M. Neonatal morbidity after elective repeat cesarean section and trial of labor. Pediatrics 1997;100:348–353.
[2] Wax JR, Herson V, Carignan E, Mather J, Ingardia CJ. Contribution of elective delivery to severe respiratory distress at term. Am J Perinatol 2002;19:81–86.
[3] Madar J, Richmond S, Hey E. Surfactant-deficient respiratory distress after elective delivery at term. Acta Paediatr 1999;88: 1244–1248.
[4] Abman SH, Chatfield BA, Hall SL, McMurtry IF. Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. Am J Physiol Heart Circ Physiol 1990;259:H1921–H1927.
[5] Dawes GS, Mott JC, Widicombe JG, Wyatt DG. Changes in the lungs of the newborn lamb. J Physiol 1953;121:141–162.
[6] Iwamoto HS, Teitel D, Rudolph A. Effect of birth-related events on blood flow distribution. Pediatr Res 1987;22:634–640.
[7] Rudolph AM. Fetal and neonatal pulmonary circulation. Annu Rev Physiol 1979;41:383–395.
[8] Fineman JR, Soifer SJ, Heymann MA. Regulation of pulmonary vascular tone in the perinatal period. Annu Rev Physiol 1995;57:115–134.
[9] Endo A, Izumi H, Ayusawa M, Minato M, Takahashi S, Harada K. Spontaneous labor increases nitric oxide synthesis during the early neonatal period. Pediatr Int 2001;43:340–342.
[10] Kinsella JP, McQueston JA, Rosenberg AA, Abman SH. Hemodynamic effects of exogenous nitric oxide in ovine transitional pulmonary circulation. Am J Physiol Heart Circ Physiol 1992;263:H875–H880.
[11] Levy M, Souil E, Sabry S, Favatier F, Vaugelade P, Mercier JC, Dall'Ava-Santucci J, Dinh-Xuan AT. Maturational changes of endothelial vasoactive factors and pulmonary vascular tone at birth. Eur Respir J 2000;15:158–165.
[12] Perreault T, DeMarte J. Maturational changes in endothelium-derived relaxations in newborn piglet pulmonary circulation. Am J Physiol Heart Circ Physiol 1993;264:H302–H309.
[13] Shaul PW, Farrar MA, Magness RR. Pulmonary endothelial nitric oxide production is developmentally regulated in the fetus and newborn. Am J Physiol Heart Circ Physiol 1993;265:H1056–H1063.
[14] Utz J, Ullrich V. Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. Biochem Pharmacol 1991;41:1195–2001.
[15] Vedernikov YP, Graser T, Vanin AF. Similar endothelium-independent arterial relaxation by carbon monoxide and nitric oxide. Biomed Biochim Acta 1989;48:601–603.
[16] Carraway MS, Ghio AJ, Carter JD, Piantadosi CA. Expression of heme oxygenase-1 in the lung in chronic hypoxia. Am J Physiol Lung Cell Mol Physiol 2000;278:L806–L812.
[17] Hartsfield CL, Alam J, Cook JL, Choi AM. Regulation of heme oxygenase-1 gene expression in vascular smooth muscle cells by nitric oxide. Am J Physiol 1997;273:L980–L988.
[18] Ramos KS, Lin H, McGrath JJ. Modulation of cyclic guanosine monophosphate levels in cultured aortic smooth muscle cells by carbon monoxide. Biochem Pharmacol 1989; 38:1368–1370.
[19] Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 2000;106:1521–1530.
[20] Burke TM, Wolin MS. Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. Am J Physiol 1987;252:H721–H732.

[21] Salvemini D, Mollace V, Pistelli A, Anggard E, Vane J. Metabolism of glyceryl trinitrate to nitric oxide by endothelial cells and smooth muscle cells and its induction by Escherichia coli lipopolysaccharide. Proc Natl Acad Sci USA 1992;89: 982–986.

[22] Vreman HJ, Kwong LK, Stevenson DK. Carbon monoxide in blood: an improved microliter blood-sample collection system with rapid analysis by gas chromatography. Clin Chem 1984; 30:1382–1386.

[23] Robbins RC, Borg KM, Robinson E. Carbon monoxide in the atmosphere. J Air Pollut Control Assoc 1968;18:106–110.

[24] Buonocore G, Perrone S, Longini M, Terzuoli L, Bracci R. Total hydroperoxide and advanced oxidation protein products in preterm hypoxic babies. Pediatr Res 2000;47:221–224.

[25] Witko-Sarsat V, Freidlander M, Capeillere-Blandin C, Nguyen-Khoa T, Thu Nguyen A, Zingraff J, Jungers P, Descamps-Latsca B. Advanced oxidation protein product as a novel marker of oxidative stress in uremia. Kidney Int 1996;49:1304–1313.

[26] Motterlini R, Foresti R, Bassi R, Calabrese V, Clark JE, Green CJ. Endothelial heme oxygenase-1 induction by hypoxia. Modulation by inducible nitric-oxide synthase and S-nitrosothiols. J Biol Chem 2000;275:13613–13620.

[27] Sonin NV, Garcia-Pagan JC, Nakanishi K, Zhang JX, Clemens MG. Patterns of vasoregulatory gene expression in the liver response to ischemia/reperfusion and endotoxemia. Shock 1999;11:175–179.

[28] Searles CD. Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression. Am J Physiol Cell Physiol 2006;31:C813–C816.

[29] Morita T, Perrella MA, Lee ME, Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proc Natl Acad Sci USA 1995;92:1475–1479.

[30] Morita T, Mitsialis SA, Koike H, Liu Y, Kourembanas S. Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle cells. J Biol Chem 1997;272: 32804–32809.

[31] Liu SF, Hislop AA, Haworth SG, Barnes PJ. Developmental changes in endothelium-dependent pulmonary vasodilatation in pigs. Br J Pharmacol 1992;106:324–330.

[32] Steinhorn RH, Morin 3d FC, Gugino SF, Giese EC, Russell JA. Developmental differences in endothelium-dependent responses in isolated ovine pulmonary arteries and veins. Am J Physiol 1993;264:H2162–H2167.

[33] Morecroft I, MacLean MR. Developmental changes in endothelium-dependent vasodilation and the influence of superoxide anions in perinatal rabbit pulmonary arteries. Br J Pharmacol 1998;125:1585–1593.

[34] Villamor E, Perez-Vizcaino F, Cogolludo AL, Conde-Oviedo J, Zaragoza-Arnaez F, Lopez-Lopez JG, Tamargo J. Relaxant effects of carbon monoxide compared with nitric oxide in pulmonary and systemic vessels of newborn piglets. Pediatr Res 2000;48:546–553.

[35] Sangalli MR, Mclean AJ, Peek MJ, Rivory LP, Le Couteur DG. Carbon monoxide disposition and permeability-surface area product in the foetal circulation of the perfused term human placenta. Placenta 2003;24:8–11.

[36] Inanc F, Kilinc M, Kiran G, Guven A, Kurutas EB, Cikim IG, Akyol O. Relationship between oxidative stress in cord blood and route of delivery. Fetal Diagn Ther 2005;20:450–453.

[37] Fogel I, Pinchuk I, Kupferminc MJ, Lichtenberg D, Fainaru O. Oxidative stress in the fetal circulation does not depend on mode of delivery. Am J Obstet Gynecol 2005;193:241–246.

[38] Storme L, Zerimech F, Riou Y, Martin-Ponthieu A, Devisme L, Slomianny C, Klosowski S, Dewailly E, Cneude F, Zandecki M, Dupuis B, Lequien P. Inhaled nitric oxide neither alters oxidative stress parameters nor induces lung inflammation in premature lambs with moderate hyaline membrane disease. Biol Neonate 1998;73:172–181.

[39] Turanlahti M, Pesonen E, Lassus P, Andersson S. Nitric oxide and hyperoxia in oxidative lung. Acta Paediatr 2000;89:966–970.

[40] Hamon I, Fresson J, Nicolas MB, Buchweiller MC, Franck P, Hascoet JM. Early inhaled nitric oxide improves oxidative balance in very preterm infants. Pediatr Res 2005;57:637–643.

[41] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am J Physiol 1996;271:C1424–C1437.

[42] Foresti R, Motterlini R. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. Free Radic Res 1999;31:459–475.

[43] Dani C, Masini E, Bertini G, di Felice AM, Pezzati M, Ciofini S, Rossetti M, Gallori G, Ciuti R, Buonocore G, Paffetti P, Mannaioni PF, Rubaltelli FF. Role of heme oxygenase and bilirubin in oxidative stress in preterm infants. Pediatr Res 2004;56:873–877.