Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 2 (4): 177-181
July-August, 2002

Implications of Nitric Oxide and Carbon Monoxide in Neonatal Sepsis

[1]Shadia El-Sallab, [1]Hesham Abdel-Hady, [2]Ekbal Abu-Hashim and [1]Mohamed Matter

Nitric oxide (NO) and carbon monoxide (CO) are proposed to regulate physiologic vascular tone, blood pressure, and tissue perfusion. To investigate their role in the pathogenesis of neonatal sepsis, plasma levels of NO and CO in 45 neonates with clinical evidence of sepsis plus positive blood culture as well as in 20 healthy neonates were assessed. Significantly higher plasma levels of NO and CO in sepsis group compared with control group were found (56.4 ± 21.1 vs. 33.4 ± 12.1 μmol L^{-1}, $p < 0.0001$; 44.0 ± 12.0 vs 23.7 ± 8.5 nmol L^{-1}, $p < 0.0001$, respectively). This increase in NO and CO levels occurred independent of gestational age, onset of sepsis, and gram stain of the isolated organism. Moreover, there were significantly higher plasma levels of NO and CO in septic infants who required inotropic support compared with those who did not require this support ($p<0.0001$). Plasma CO was higher in non-survivors compared with survivors ($p=0.003$). Significantly positive correlation between plasma NO and CO was observed. In conclusion, plasma NO and CO was increased in neonatal sepsis. This increase is independent of gestational age, onset of sepsis and gram stain of the isolated organism. Assay of plasma CO and NO may help to assess the severity of sepsis and progression to septic shock. The role of nitric oxide synthase (NOS) and heme oxygenase (HO) inhibitors in the management of neonatal sepsis should be investigated in further trials.

Key words: Nitric oxide, carbon monoxide, plasma, sepsis, newborn

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Shadia El-Sallab
Department of Pediatrics,
Neonatal Intensive Care Unit,
Faculty of Medicine,
Mansoura University,
El-Mansoura, Egypt

ANSInet
Asian Network for Scientific Information

[1]Department of Pediatrics, Neonatal Intensive Care Unit,
[2]Department of Clinical Pathology, Faculty of Medicine,
Mansoura University, El-Mansoura, Egypt

Introduction

Neonatal sepsis is a life-threatening condition and it is one of the main causes of morbidity and mortality in newborns (Klein and Remington, 1990). Several lines of evidence indicate that endogenously produced mediators are involved in the pathogenesis of sepsis (Bone, 1991). Both NO and CO are proposed to regulate physiologic vascular tone, blood pressure, and tissue perfusion (Moncada *et al.*, 1991; Morita *et al.*, 1995). NO is an omnipresent physiologic agent formed from the conversion of l-arginine and oxygen to NO and citrulline by a family of 3 NOS enzymes (Cannon *et al.*, 1990). The constitutive forms of NOS present in endothelial and neuronal cells produce basal levels of NO that mediate homeostatic functions, such as resting vascular tone, neurotransmission, and inhibition of neutrophil and platelet adhesion. Infectious and inflammatory insults lead to NO production by the inducible form of the enzyme (iNOS) found in macrophages, monocytes, neutrophils, and Kupffer cells. Nitric oxide (NO) has a role in host defense by killing pathogens. Excessive levels, however, lead to pathologic loss of vascular tone, a central feature of sepsis-induced lethality (Lowenstein *et al.*, 1994; Wheeler and Bernard, 1999; Terregino *et al.*, 2000).

CO has been implicated to be a new endogenously produced mediator similar to NO. It is produced almost exclusively from heme catabolism by microsomal HO enzyme (Maines, 1997). Similar to NOS system, three isoforms of HO have been characterized, which are encoded by distinct genes: whereas the isoforms HO-2 and HO-3 are constitutively expressed, the isoform HO-1 is highly inducible (Choi and Alam, 1996; Bauer *et al.*, 1998). Only few reports are available in the literature on the role of NO and CO in neonatal sepsis (Shi *et al.*, 1993; Shi *et al.*, 2000b).

The purpose of this study was following:

a To assess the plasma levels of NO and CO in neonatal sepsis.
b To determine whether changes in NO and CO levels are dependent on gestational age, onset of sepsis, or gram stain of the organism.
c To determine whether these levels are associated with progression to severe sepsis, septic shock and death.
d To determine potential relationship between these mediators.

Materials and Methods

Materials: Study included 45 newborn infants with sepsis. They were consecutively admitted in the Neonatal Intensive Care Unit, Mansoura University Children's Hospital in the period from February 2001 to July 2001. Infants were eligible for enrollment if they had clinical evidence of sepsis plus positive blood culture. Infants with obvious major congenital abnormalities, metabolic disease, hyperbilirubinemia, birth asphyxia or receiving corticosteroids were excluded.

Twenty normal healthy newborns of matched gestational age, postnatal age and sex served as a control group, none of them had evidence of disease that might alter plasma NO and CO concentrations. Informed parental consent was obtained before enrollment.

Complete obstetric histories were obtained and examinations were performed at the time of admission. The neonatal clinical course was followed prospectively and data were recorded on predetermined preform sheets. The following laboratory investigations were done: complete blood count, c-reactive protein, blood culture, plasma NO and CO estimation.

Sample collection: Plasma samples were obtained using ethylene diamine tetra acetate (EDTA) as an anticoagulant. Centrifuged at 1000 Xg within 30 minutes of collection. Samples were stored at -20 °C until analysis was done.

Plasma NO assay: Nitrite/nitrate (NO_2^- / NO_3^-) has been confirmed to be a good indicator for NO production (Evans *et al.*, 1993). The plasma concentrations of NO were assayed by enzyme immunoassay kit (R&D System inc., USA). This technique determines the total NO based on the enzymatic conversion of nitrates to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction.

In brief, samples were two-fold diluted into reaction buffer. Twenty-five μl of NADH were added to 50 μl of nitrate standard or samples and incubated for 30 minutes at 37 °C. Fifty μl of Griess Reagent I (sulfanilamide) were added and then 50 μl of Griess Reagent II (N-1-naphthyl ethylenediamine in 2 N hydrochloric acid) were added and then incubated for 10 minutes at room temperature. The optical density was determined using a microplate reader set at 540 nm. Plasma NO concentrations were determined from a linear standard curve obtained from standards of known concentration. The detection limit for assay for NO was 1.0 μmol L^{-1}, the concentration range of the standard curve was 1.0 to 500.0 μmol L^{-1} (Hegesh and Shiloah, 1982).

Plasma CO assay: Carbon monoxide concentration was measured using simple, sensitive spectrophotometric method (Chalmers, 1991). CO is trapped with hemoglobin (Hb) to form carboxyhemoglobin (COHb) and subsequently estimated by dilution reduction. One ml of Hb solution (0.25 ml of fresh-packed erythrocytes in 50 ml of 0.24 mol L^{-1} ammonia solution) was mixed with 0.25~1ml of a sample or an equivalent amount of water, which was used as a blank to measure the endogenous CO present in the Hb solution. Then 0.1 ml of 20 % sodium dithionite solution was added to both the test sample and water-containing blank solution, vortex-mixed, and let stand for 10 minutes. The absorbance at 541 and 555 nm against a reference corvette containing water was read and the ratio of 541 to 555 reading was measured. Then % COHb was calculated from a standard curve derived by mixing 100 % HbO_2 and 100 % COHb in different proportions. Carbon dioxide concentration in x ml of the sample was determined as follows (Shi *et al.*, 2000a; Shi *et al.*, 2002):

$$CO\ (nmol\ L^{-1}) = \frac{COHb\,\% \times Hb\ (mg/L) \times 4000}{100 \times 64456}$$

Where
COHb = Carboxyhemoglobin, Hb = Hemoglobin

The detection limit of the assay was 3.5 nmol L^{-1}. The concentration range of the standard curve was 3.5 to 500 nmol L^{-1}.

Statistical analysis: The data were described as mean ± SD. The independent samples t-test was used for group comparisons of continuous variables and Chi square test for categorical variables. To test for associations Pearson's correlation coefficient were used. Statistical significance was determined at $p < 0.05$ and all p-values reported were of the two-sided type. The statistical analyses were completed on SPSS for windows (SPSS Version 10.0.1, Inc, Chicago, IL).

Results

Demographic data and perinatal characteristics of the studied groups showed that no differences were found between septic and control groups regarding gestational age, birth weight, postnatal age, sex, type of delivery and place of delivery, but results of premature rupture of membranes were statistically significant at $p < 0.0001$ (Table 1).

Eighteen infants (40 %) had early onset sepsis and 27 (60 %) had late onset sepsis, 15 (33 %) had gram-positive sepsis and 30 (67 %) had gram-negative sepsis. Klebsiella pneumonia was the commonest organism (54%) isolated from blood cultures followed by *Staphylococcus epidermidis* (20 %), *Staphylococcus aureus* (9 %), *Eschereschia coli* (9 %), group B *Streptococci* (4 %) and

Table 1: Demographic data and clinical characteristics of the studied population

Parameters	Septic group (n = 45)	Control group (n = 20)
Gestational age (weeks)	36.3±4.0	37.6± 2.2
Birth-weight (Kg)	02.31±0.80	02.56± 0.94
Postnatal age (days)	11.4±7.7	12.2± 4.3
Male sex (%) #	28.0 (62.2%)	08.0 (40.0%)
Cesarean section #	24.0 (53.3%)	11.0 (55.0%)
Inborn #	26.0 (57.8%)	15.0 (75.0%)
Premature rupture of membranes*	18.0 (40.0%)	00.0 (0%)

Values expressed as mean ± SD and as number (%) for categorical variables # *: $p < 0.0001$

Table 2: Plasma nitric oxide (NO) and carbon monoxide (CO) levels in septic and control groups

Groups	N	Nitric oxide (μmol L^{-1})	Carbon monoxide (μmol L^{-1})
Septic	45	56.4±21.1	44.0±12.0
Control	20	33.4±12.1*	23.7±8.5*
Septic full-term infants	27	57.4±19.8	41.3±11.9
Septic preterm infants	18	56.4±23.4	48.0±11.4

*: $p < 0.0001$

Table 3: Plasma nitric oxide (NO) and carbon monoxide (CO) levels in relation to the onset of sepsis and gram stain of the organism

Groups	N	Nitric oxide (μmol L^{-1})	Carbon monoxide (μmol L^{-1})
Early onset sepsis	18	51.6±18.1	40.5±10.2
Late onset sepsis	27	59.5±22.7	46.3±12.7
Gram-positive sepsis	15	58.7±22.7	46.2±11.7
Gram-negative sepsis	30	55.2±20.5	42.9±12.2

Table 4: Plasma nitric oxide (NO) and carbon monoxide (CO) levels in relation to inotropic support requirements and outcome

Groups	N	Nitric oxide (μmol L^{-1})	Carbon monoxide (μmol L^{-1})
Inotropic support required	24	54.1±23.2	46.6±12.9
No inotropic support required	21	31.3±12.2**	35.1±13.2*
Survivors	16	60.5±20.3	33.1±14.4
Non-Survivors	29	54.1±21.5	43.6±12.5*

Values expressed as mean ± SD N = no. of patients
*: $p < 0.05$, **: $p < 0.001$

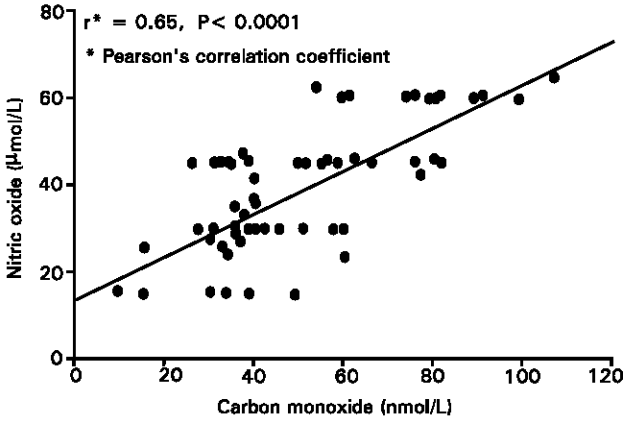

Fig. 1: Correlation between plasma nitric oxide and carbon monoxide levels in the studied population (n = 65)

Pseudomonas auerogenosa (4 %). Twenty-four patients (53 %) required inotropic support and twenty-three patients (51 %) succumbed.

Plasma NO and CO levels were significantly higher ($p < 0.0001$) in septic infants compared to controls (Table 2). Elevation of plasma NO >41.7 μmol L^{-1}, and CO >30.1 nmol L^{-1} were significantly associated with sepsis. There were no statistically significant differences between plasma NO and CO levels in septic full-term and septic preterm infants (Table 2). There was a significant positive correlation between plasma NO and CO levels ($r = 0.65$, $p < 0.0001$) (Fig. 1).

Non-significant difference was observed in plasma levels of NO and CO between infants with early onset sepsis and late onset sepsis and between infants with gram-positive and gram-negative sepsis (Table 3).

Plasma NO and CO were significantly higher ($p < 0.0001$) in septic infants requiring inotropic support (Table 4). Plasma CO was significantly higher whereas, plasma NO was not significantly different in those who died compared with survivors (Table 4).

Discussion

Some important findings have been emerged from this work, first, plasma NO and CO were significantly elevated in newborn infants with sepsis, with a significant positive correlation between plasma NO and CO and with no significant difference between full-term and preterm infants (Table 2). Second, no significant differences were found in plasma levels of NO and CO between early or late onset sepsis or gram-positive or gram negative sepsis (Table 3). Third, plasma NO and CO were significantly higher in septic newborns requiring inotropic support compared with those not requiring inotropic support and plasma CO levels were significantly higher in those who died compared to survivors (Table 4).

Plasma NO was reported to be elevated in adult patients with severe sepsis or shock (Ochoa *et al.*, 1991; Battafarano and Dunn, 1992), in septic children hospitalized in intensive care settings (Wong *et al.*, 1995; Spack *et al.*, 1997), and in septic full-term infants (Shi *et al.*, 2000b). On the other hand, some researchers did not find elevation of NO metabolites in patients with sepsis (Terregino *et al.*, 2000). However, this study was performed on patients with presumed sepsis on arrival to the emergency department and before the development of septic complications such as altered perfusion or shock. Some sources suggest that constitutive NOS may be down regulated in the early stages of inflammation, and that the time for increased production by the iNOS may take from 4 to 12 hours (Curran *et al.*, 1991; Shieh *et al.*, 2000).

In the present work plasma CO levels were significantly related to NO production. Similar findings were reported by Shi *et al.* (2000b). It was reported that NO is an important stimulator of HO and so it increases CO production (Foresti and Motterlini, 1999). Moreover, this study shows the first evidence that septic preterm infants have similar changes in plasma NO levels as septic full-term infants. Similar to these findings, Shi *et al.* (1993), found no differences in plasma NO levels between gram-positive sepsis and gram-negative sepsis.

NO plays an important role in the mediation of the main cardiovascular features of septic shock (Kilbourn and Griffith, 1992). Excessive formation of NO is associated with profound vasodilatation, hypotension, hyporeactivity to catecholamines and inadequate tissue perfusion (Teale and Atkinson, 1992; Bhagat *et al.*, 1999). Moreover, a broad range of evidence has suggested that much of the depressant effect of proinflammatory cytokines and septic serum on myocardial tissue is mediated through NO-dependent-mechanisms (Kumar *et al.*, 1999). Current results confirms the findings of previous studies that highly elevated NO concentration were related to the occurrence of septic shock and organ failure (Shi *et al.*, 1993; Wong *et al.*, 1995; Spack *et al.*, 1997, Duke *et al.*, 1997).

These findings demonstrate that high level of plasma NO is a dangerous sign of septic shock, and would support the concept that overproduction of NO should be controlled. Inhibition of excessively produced NO may eventually prevent the detrimental hemodynamic effects associated with septic shock (Wolkow, 1998). There has been much recent enthusiasm regarding the possibility of using inhibitors of NOS for the treatment of septic shock (Grover *et al.*, 1999). On the other hand, NO is a physiologically important molecule at low concentrations that is

produced by the constitutive enzyme and that maintains blood vessel vasodilator tone. Complete inhibition of NO production during sepsis is detrimental (Grandel *et al.*, 2001). A more selective iNOS inhibitor, ONO-1714, was found to improve blood pressure without impairing tissue perfusion in animal studies (Mitaka *et al.*, 2001). Evaluation of specific inhibitors of iNOS represent one potentially fruitful area for further investigation (Chang, 2001).

In this study, plasma NO levels were not significantly different between survivors and non-survivors (Table 4). This finding is similar to that of Duke *et al.* (1997) who reported that the serum concentrations of NO were weakly but positively associated with illness severity but there was no association with mortality. These results are in contradistinction to Shi *et al.* (1993) who reported that higher plasma NO levels were related to severity of illness, pediatric risk of mortality (PRISM) scores, and occurrence of septic shock.

There are very few reports regarding the levels of CO during sepsis in humans. Very recently, Shi *et al.* (2000b) found that plasma CO levels in newborns with sepsis were higher than in healthy neonates. However, this study included only 7 full-term newborn infants with sepsis. Earlier, in an animal study, Shi *et al.* (1997) demonstrated that in newborn rat endotoxicosis, CO levels in the circulation as well as in the liver, kidney and lung were found to be significantly increased and Moncure *et al.* (1999) reported significantly elevated COHb levels during septic shock. Here reported the first evidence that septic preterm infants have similar changes in plasma CO levels as septic full-term infants.

An interesting finding is that no differences between plasma CO levels in neonates with early onset and those with late onset sepsis and that the elevation of CO levels were independent of the gram stain of the organisms isolated (Table 3). This may imply that both gram-negative and positive organisms are capable of activating HO system and increasing CO production in a fashion similar to activation of iNOS and production of NO. Recent studies have suggested that CO generated by HO-2 might contribute to the regulation of vascular tone under basal condition and that the relatively large amount of CO generated by markedly increased HO-1 activity could contribute to the reduction of vascular tone during sepsis. This action is mediated via activation of guanylate cyclase and the production of cyclic guanosine monophosphate "cGMP" (Morita *et al.*, 1995). It has been hypothesized that both NO and CO release could contribute to the abnormal vasodilatation characteristic of sepsis. Thus, both stress-inducible vasodilator systems, NOS and HO, can contribute to the critical balance of vasoconstrictor and dilator forces regulating microvascular perfusion in septic shock (Shi *et al.*, 2000b).

Administration of HO inhibitors, zinc protoporphyrin (ZnPP), resulted in an increase in arterial blood pressure in animals (Johnson *et al.*, 1995). ZnPP was shown to abrogate the endotoxin-induced hypotension and metabolic derangement (Shi *et al.*, 2000b). On the other hand, Downard *et al.* (1997) suggested that HO-dependent CO production might be a hepatic adaptive response to sepsis. Pannen *et al.* (1998) found that endogenous CO has a protective role in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. Moreover, Otterbein *et al.* (1995) have indicated that induction of HO-1 might protect against oxidative damage of endotoxemia, and administration of HO inhibitors such as ZnPP to decrease HO activity below basal levels could make rats more susceptible to endotoxin-induced death. Thus we believe that the clinical meaning of CO and the use of HO inhibitors in neonatal sepsis worth further investigation.

The higher plasma CO levels in non-survivors compared to survivors indicate the prognostic value of plasma CO levels (Table 4). This is a unique finding reported for the first time. If such finding is validated, measurement of plasma CO may add to standard clinical evaluation and management. This test may help in the triage of septic newborns to a certain level of care (e.g. SCBU or NICU) and may help targeting certain patients for new interventions such as HO inhibitors.

In conclusion, plasma NO and CO levels are elevated in newborn infants with sepsis. This elevation is independent of gestational age, gram stain of the isolated organism, and whether it is early or late onset sepsis. The elevation in CO production is related to increased NO production. Assay of plasma CO and NO may have some clinical values to assess the severity of sepsis and progression to septic shock. The role of NOS and HO inhibitors in the management of neonatal sepsis should be investigated in further trials. Plasma levels of NO and CO can be used to decide which patient should be a candidate for these trials.

References

Battafarano, R. and D. Dunn, 1992. Role of nitric oxide during sepsis. Crit. Care Med., 20: 1504-1505.

Bauer, I., G. Wanner, H. Rensing, C. Alte, E. Miescher, B. Wolf, B. Pannen, M. Clemens and M. Bauer, 1998. Expression pattern of heme oxygenase isoenzyme 1 and 2 in normal and stress-exposed rat liver. Hepatology, 27: 829-838.

Bhagat, K., A. Hingorani, M. Palacios, J. Charles and P. Vallance, 1999. Cytokine- induced venodilataion in humans *in vivo*: eNOS masquerading as iNOS. Cardiovasc. Res., 41: 754-764.

Bone, R., 1991. The pathogenesis of sepsis. Ann. Inter. Med., 115: 457-469.

Cannon, G., R. Tompkins, J. Gelfand, H. Michie, G. Stanford, J. Van der Meer, S. Enders, G. Lonnemann, J. Corsetti and B. Chernow, 1990. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. J. Infec. Dis., 161: 79-84.

Chalmers, A., 1991. Simple, sensitive measurement of carbon monoxide in plasma. Clin. Chem., 37: 1442-1445.

Chang, D., 2001. Selective inducible nitric oxide synthase inhibition in septic shock. Crit. Care Med., 29: 135-139.

Choi, A. and J. Alam, 1996. Heme oxygenase-1: Function, regulation and implication of a novel stress- inducible protein in oxidant-induced lung injury. Am. J. Respir. Cell Mol. Biol., 15: 9-19.

Curran, R., F. Ferrari, P. Kispert, J. Stadler, D. Stuehr, R. Simmons and T. Billar, 1991. Nitric oxide and nitric oxide-generating compounds inhibit hepatocyte protein synthesis. Federation of American Societies for Experimental Biology, 5: 2085- 2092.

Downard, P., M. Wilson, D. Spain, P. Matheson, Y. Siow and R. Garrison, 1997. Heme oxygenase-dependent carbon monoxide production is a hepatic adaptive response to sepsis. J. Surg. Res., 71: 7-12.

Duke, T., M. South and A. Stewart, 1997. Activation of the L-arginine nitric oxide pathway in severe sepsis. Arch. Dis. Child., 76: 203-209.

Evans, T., A. Carpenter, H. Kinderman and J. Cohen, 1993. Evidence of increased nitric oxide production in patients with sepsis syndrome. Circulatory Shock, 41: 77- 81.

Foresti, R. and R. Motterlini, 1999. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. Free Radic. Res., 31: 459-474.

Grandel, U., U. Sibelius, J. Schrickel, D. Schmidt, M. Buerke, L. Fink, E. Bournelis, M. Heep, K. Mayer, R. Bohle, W. Seeger and F. Grimminger, 2001. Biosynthesis of constitutive nitric oxide synthase-derived nitric oxide attenuates coronary vasoconstriction and myocardial depression in a model of septic heart failure induced by staphylococcus aureus alpha toxin. Crit. Care Med., 29: 1- 7.

Grover, R., D. Zaccardello, G. Colice, K. Guntupalli, D. Watson and J. Vincet, 1999. An open-lable dose escalation study of the nitric oxide synthase inhibitors N(G)-Methyl-1-arginine hydrochloride (546C88), in patients with septic shock. Galaxo Wellcome International Septic Shock Study Group. Crit. Care Med., 27: 913-922.

Hegesh, E. and J. Shiloah, 1982. Blood nitrate and infantile methemoglobinemia. Clin. Chem. Acta., 125: 107-115.

Johnson, R., M. Lavesa, B. Askari, N. Abraham and A. Nasjletti, 1995. A heme oxygenase product presumably carbon monoxide mediate a vasodepressor function in rats. Hypertension, 25: 166-169.

Kilbourn, R. and O. Griffith, 1992. Overproduction of nitric oxide in cytokine-mediated and septic shock. J. Natl. Cancer Inst., 84: 827-832.

Klein, J. and J. Remington, 1990. Current concepts of infection of the fetus and newborn infant. Ann. Trop. Ped., 11: 19-21.

Kumar, A., R. Brar, P. Wang, L. Dee, G. Skorupa, F. Knadour, R. Sculz and J. Parrillo, 1999. The role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. Am. J. Physiol., 276: 265-276.

Lowenstein, C., J. Dinerman and S. Synder, 1994. Nitric oxide: A physiological messenger. Ann. Emerg. Med., 120: 227-237.

Maines, M., 1997. The heme oxygenase system: A regulator of second messenger gases. Ann. Rev. Pharmacol. Toxicol., 37: 517- 554.

Mitaka, C., Y. Hirata, K. Yokoyama, K. Makita and T. Imai, 2001. A selective inhibitor for inducible nitric oxide synthase improves hypotension and lactic acidosis in canine endotoxic shock. Crit. Care Med., 29: 2156-2161.

Moncada, S., R. Palmer and E. Higgs, 1991. Nitric oxide: Physiology, pathophysiology, and pharmacology. Eur. J. Clin. Invest., 21: 361-374.

Moncure, M., C. Brathwaite, E. Samaha, R. Marburger and S. Ross, 1999. Carboxyhemoglobin elevation in trauma victims. J. Trauma, 46: 424-427.

Morita, T., M. Perrella, M. Lee and S. Kourembanas, 1995. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proc. Natl. Acad. Sci., USA, 92: 1475-1479.

Ochoa, J., A. Udekwu, T. Billar, R. Curran, F. Cerra, R. Simons and A. Peitzman, 1991. Nitrogen oxide levels in patients after trauma and during sepsis. Ann. Surg., 338: 1560- 1562.

Otterbein, L., S. Sylvester and A. Choi, 1995. Hemoglobin provides protection against lethal endotoxemia in rats: The role of heme oxygenase-1. Am. J. Respir. Cell Mol. Biol., 13: 595-601.

Pannen, B., N. Kohler, B. Hole, M. Bauer, M. Clemens and K. Geiger, 1998. Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. J. Clin. Invest.,102: 1220- 1228.

Shieh, P., M. Zhou, D. Ornan, I. Chaudry and P. Wang, 2000. Upregulation of inducible nitric oxide synthase and nitric oxide occurs later than the onset of the hyperdynamic response during sepsis. Shock, 13: 325- 329.

Shi, Y., H. Li, C. Shen, J. Wang, S. Qin, R., Liu and J. Pan, 1993. Plasma nitric oxide level in newborn infants with sepsis. J. Pediatr., 123: 435-438.

Shi, Y., H. Li, J. Pan, S. Qin, Z. Tao, D. Jiang and J. Shen, 1997. Evidence of increased endogenous carbon monoxide production in newborn rat endotoxicosis. Chin. Med. Sci. J., 12: 212-215.

Shi, Y., F. Pan, H. Li, J. Pan, S. Qin and C. Shen, 2000a. Role of carbon monoxide and nitric oxide in newborn infants with postasphyxial hypoxic-ischemic encephalopathy. Pediatrics, 106: 1447-1451.

Shi, Y., F. Pan, H. Li, J. Pan, S. Qin, D. Jiang and C. Shen, 2000b. Plasma carbon monoxide level in term newborn infant with sepsis. Biol. Neonate, 78: 230–232.

Shi, Y., H. Li and C. Shen, 2002. Role of carbon monoxide and nitric oxide in newborn infants with postasphyxial hypoxic-ischemic encephalopathy (letter to the editor). Pediatrics, 109: 715-716.

Spack, L., P. Havens and O. Griffith, 1997. Measurements of total plasma nitrite and nitrate in pediatric patients with the systemic inflammatory response syndrome. Crit. Care Med., 25: 1071-1078.

Teale, D. and A. Atkinson, 1992. Inhibition of nitric oxide synthesis improves survival in a murine model of sepsis that is not cured by antibiotic alone. J. Antimicrob. Chemother., 30: 839-842.

Terregino, C., B. Lopez, D. Karras, A. Killian and G. Arnold, 2000. Endogenous mediators in emergency department patients with presumed sepsis: Are levels associated with progression to severe sepsis and death. Ann. Emerg. Med., 35:26-34.

Wheeler, A. and G. Bernard, 1999. Treating patients with severe sepsis. New Engl. J. Med., 340: 207- 214.

Wolkow, P., 1998. Involvement and dual effects of nitric oxide in septic shock. Inflamm. Res., 47: 152-166.

Wong, H., J. Carcillo, G. Burckart, N. Shah and J. Janosky, 1995. Increased serum nitrite and nitrate concentrations in children with sepsis syndrome. Crit. Care Med., 23: 835- 842.

MS received 24th June, 2002; accepted 3rd July, 2002