Plasma Carbon Monoxide Levels In Pediatric Sepsis Syndrome

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Citation

Abstract
Aim: To determine whether carbon monoxide (CO) plays a role in pediatric sepsis syndrome.

Methods: A prospective, observational study of 12 patients aged 3 months to 8 years with the sepsis syndrome were performed. Plasma CO and nitric oxide (NO) levels were measured at the time of hospital admission and compared with those 30 age-matched control subjects.

Results: The plasma CO levels were found to be significantly increased in the patients with the sepsis syndrome as compared with those in the control subjects (P<0.05). The septic patients with shock had significantly higher plasma CO levels than those without shock (P<0.05). Moreover, the increased plasma CO levels were statistically related to the plasma NO levels (P<0.05).

Conclusions: The present study suggests that CO, in addition to NO, might play an important role in the pathogenesis of pediatric sepsis syndrome.

INTRODUCTION
In spite of widespread investigation and medical advances, the mortality of sepsis syndrome remains high. Although the role of a number of cytokines and vasoactive mediators, such as tumor necrosis factor, interleukin-1, interleukin-6, in mediating the pathophysiology of this condition is becoming clear, the pathogenesis about how the sepsis syndrome leads to septic shock, multiorgan dysfunction syndrome and lethality is still not entirely understood.

One of the characterized features of the sepsis syndrome is profound hypotension. A few studies have suggested that the hypotension may be due to the overproduced nitric oxide (NO), which induces excessive vascular relaxation and hence a fall in blood pressure through the way of activating guanylyl cyclase and increasing cellular levels of cyclic guanosine monophosphate (cGMP)(2,3,4). Very recently, biomedical interest in endogenous carbon monoxide (CO) has grown rapidly since CO has been proposed to function as a widespread transduction substance for the regulation of cell function and communication similar to NO (5,6).

CO, which forms endogenously from heme catabolism by heme oxygenase (HO),has been suggested to be possible to serve as a new endogenous mediator contributing to the pathophysiology of sepsis syndrome (7). We, therefore, wished to determine whether we could find evidence of increased production of CO in a group of pediatric patients with the sepsis syndrome by measuring their plasma CO levels.

PATIENTS AND METHODS

PATIENTS
This study was conducted in the Department of Pediatrics, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, China.

Children between 3 months and 8 years with a clinical diagnosis of sepsis were eligible for study entry when they
met the Bone criteria for sepsis syndrome modified for the pediatric population \((\alpha)\), which were shown in Table 1. Blood samples were taken at the time of hospital admission. Informed parental consent was obtained. Relevant data were collected to determine disease severity by the mean pediatric risk of mortality (PRISM) score \((\beta)\). The diagnosis of septic shock was made when the patients had hypotension or poor capillary refill in addition to the sepsis syndrome \((\gamma)\).

Thirty age-matched healthy children served as the control subjects.

Table 1. Eligibility criteria for pediatric patients with sepsis syndrome

<table>
<thead>
<tr>
<th>Condition</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>from 3 months to 13 years</td>
</tr>
<tr>
<td>Temperature</td>
<td>&lt;36.5°C or &gt;38.5°C</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>&gt;90% percentile for age</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>&gt;90% percentile for age</td>
</tr>
<tr>
<td>WBC</td>
<td>&gt;10.0 x 10⁹ or &lt;0.4 x 10⁹</td>
</tr>
<tr>
<td>Evidence of organ dysfunction/hypoperfusion</td>
<td></td>
</tr>
<tr>
<td>Decreased or fluctuating level of consciousness</td>
<td></td>
</tr>
<tr>
<td>Decreased systolic blood pressure</td>
<td>(&lt;5% for age)</td>
</tr>
<tr>
<td>Delayed capillary refill</td>
<td>(&gt;3 seconds)</td>
</tr>
<tr>
<td>Apnea</td>
<td>(&gt;15 seconds)</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>(pH&lt;7.28)</td>
</tr>
<tr>
<td>Decreased urine output</td>
<td>(&lt;0.5 ml/kg/hour for &gt;1 hour)</td>
</tr>
</tbody>
</table>

**CO ASSAY**

CO concentration was measured using the simple, sensitive spectrophotometric method described by Chalmers \((\iota)\). CO is trapped with hemoglobin (Hb) and subsequently estimated by dithionite reduction. One ml of Hb solution (0.25 ml of fresh-packed erythrocytes in 50 ml of ammonia solution) was mixed with 0.25 ml 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. 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 10 min. The absorbance at 541 and 555 nm against a reference cuvette containing water was read and the ratio \((R)\) of the 541 to 555 readings was measured. Then the %HbCO was calculated from a standard curve derived by mixing 100%HbO2 and 100%HbCO in different proportions:

\[
% \text{HbCO} = \frac{(R - 0.83)}{0.00383}
\]

Then the CO concentration in x ml of the sample was given:

\[
\text{HbCO} = \frac{\% \text{HbCO} \times \text{Hb} (\text{mg/L}) \times 4000}{\text{CO} (\mu\text{g/L})}
\]

100 \times \text{Hb} \times 64456

**NO ASSAY**

Nitrite/nitrate (NO\(_2^-\)/NO\(_3^-\)) concentration has been confirmed to be a good indicator for NO production. The classic method described by Hegesh and Shiloah was used, and modified slightly in our laboratory \((\iota, \nu)\). Briefly, the measured samples were first deproteinated (30% ZnSO\(_4\), 0.05 ml/ml of sample), then passed through a cadmium reduction column (100 x 8 mm) prewashed with HCl (0.1 mol/L) and ammonium hydroxide buffer (0.1 mol/L). After application of the sample, the column was eluted with ammonium chloride buffer (0.05 mol/L). A 5 ml fraction of the effluent was collected, and then, sulfanilamide solution (29.0 mmol/L) was added to it. The tube was mixed, and 3 min later, N-(1-naphthyl)-ethylenediamine dihydrochloride was added. Absorbance was measured after 20 min at 540 nm. Serial dilutions of sodium nitrite were used to prepare a standard curve.

**STATISTICAL ANALYSIS**

All data were presented as mean \(\pm\) deviation or median (range) for descriptive purposes. The Kruskal-Wallis test, and the Spearman rank correlation co-efficient were used. Significance was accepted at \(P<0.05\).

**RESULTS**

Between January 1996 and December 1998, a total of 12 patients were eligible and enrolled for admission to the study. The median age of the study subjects was 1.5 years (range from 3 months to 8 years), with a male/female ratio of 1.4:1. The median age of the control subjects was 2.5 years (range from 3 month to 12 years), with a male/female ratio of 1.5:1. There were no significant differences between the septic and control subjects in age and sex \((P>0.05)\).

Table 2 shows the clinical data of the patients. Five patients
(41.7%) had culture-positive bacterial sepsis, and all patients except one had received antibiotics before the culture samples were taken. The mean PRISM score was 7.75 with the range from 0 to 28. Three patients developed septic shock.

Table 2. The clinical data of the patients with sepsis syndrome

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Major diagnosis</th>
<th>Leukocyte count (JÁ10⁶)</th>
<th>PRISM score</th>
<th>Septic shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3yr</td>
<td>F</td>
<td>Bacillus</td>
<td>21.8</td>
<td>28</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>3yr</td>
<td>F</td>
<td>Culture negative</td>
<td>12.6</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>2yr</td>
<td>M</td>
<td>Culture negative</td>
<td>18.9</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>1yr</td>
<td>M</td>
<td>Culture negative</td>
<td>4.0</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>3yr</td>
<td>F</td>
<td>Culture negative</td>
<td>19.2</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>4yr</td>
<td>M</td>
<td>Culture negative</td>
<td>15.6</td>
<td>12</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Figure 2

Table 3. Changes of CO and NO in pediatric patients with sepsis syndrome

As Table 3 shows, significant increased plasma CO and NO levels were found in the septic patients as compared with the control subjects (P<0.05 respectively). Moreover, plasma CO levels were statistically higher in the 3 septic patients with shock than the 9 septic patients without shock (3.94±1.19 vs 2.21±0.62 µg/L, P<0.05). Similarly, plasma NO levels were significantly higher in the patients with septic shock than those without septic shock (65±1.10 vs 28.61±6.60°mol/L, P<0.05). In addition, the increased plasma CO levels were significantly related to the NO levels (rs=0.98, P<0.05).

DISCUSSIONS

It is not until nineties that CO has been appreciated as a new messenger which might be part of a complex cascade of mediators participating in the pathogenesis of sepsis syndrome, although endogenous production of CO has been confirmed in mammals for over 30 years (13). CO shares many of the chemical and biological properties of NO. HO is the enzyme that generates CO and biliverdin, which is subsequently reduced to bilirubin, during the course of heme metabolism. Two distinct constitutive forms of HO have been identified: One of the isoforms is inducible (HO-1) by heme and other agents such as stress, endotoxin, hypoxia or/and ischemia, whereas the second form is not inducible (HO-2), which may serve as a potential neurotransmitter in the brain (14). A portion of the endogenous CO is exhaled and a portion is present as carboxyhemoglobin, which has been proposed to bind to the heme moiety of cytosolic guanylyl cyclase and/or Fe-S center of some enzymes in order to exert physiologic effects (15). Moreover, like NO, CO has been shown to induce vasodilation in the isolated perfused heart, relax cultured aortic smooth muscle cells, and inhibit platelet aggregation (16).

The endogenously produced CO has been indicated to modulate blood vessel tone apparently due to activation of guanylyl cyclase, resulting in an significantly increased production of cGMP (16). A study showed prominent immunocytochemical staining for HO-2 in the vascular endothelium under basal condition (18). Various studies have also demonstrated that CO can be produced by arteries in vivo, and administration of HO inhibitors such as zinc protoporphyrin (ZnPP) result in an increase in arterial pressure in rats (19). In our previous experiments, plasma CO levels were found to be significantly increased after endotoxin administration in rabbits, and injection of ZnPP, an inhibitor of HO, was shown to abrogate the LPS-induced hypotension and metabolic derangements markedly. Moreover, administration of hemin, an HO inducer, to healthy rabbits revealed the hypotension and metabolic derangements similar to the animals given endotoxin (20). Recently, a dramatic increase in HO-1 mRNA was found in aortic tissue from rats receiving lipopolysaccharide (LPS) compared with rats receiving vehicle, and this increase in message was associated with a significant increase in HO enzyme activity (21). The increase in HO-1 protein was present in vascular smooth muscle cells and endothelial cells of both large and small blood vessels. Taken together these studies suggest that CO generated by HO-2, may contribute to the regulation of vascular tone under basal conditions. However, the marked increase in HO-1 activity stimulated by LPS would suggest that HO-1-generated CO may contribute to the reduction in vascular tone during endotoxin shock. In 1994, Hunter et al. were able to demonstrate that carboxyhemoglobin was a marker of stress in the critically
ill surgical patients (23). Just in 1999, Moncure et al. showed that significantly elevated carboxyhemoglobin levels were found during stress, sepsis, and shock states (23). Our present study provided the first evidence that the plasma CO levels were significantly increased in pediatric patients with the sepsis syndrome, and that the increased plasma CO levels were statistically remarkable in the septic patients with shock as compared with those without shock.

The present study also indicated that the increased plasma CO levels were related to the elevated NO levels in the children with the sepsis syndrome. The importance of NO in the pathogenesis of sepsis and septic shock has been emphasized in recent years (24–26). There is much parallelism between the biological actions and functions of CO and NO, and their regulation is intimately linked. NO has been shown to induce HO-1 gene expression and CO production in vascular smooth muscle cells (27), whereas the induction of HO has been revealed to inhibit NO synthase mRNA expression and attenuate hypotension in endotoxin-challenged rats (28). Thus, in addition to NO, CO may be another endogenously produced mediator which plays an important role in the pathogenesis of sepsis and septic shock.

Nevertheless, controversy still remains. Otterbein et al. has proposed that further induction of HO-1 by Hb may protect against the oxidative damage of endotoxiaemia by generating bilirubin which has antioxidant properties, and the administration of HO inhibitors at doses that decrease HO enzyme activity below basal levels such as ZnPP at 50–100 μmol/kg can make rats more susceptible to LPS-induced death (29), in the other hand, a recent study by Gaine et al. suggested that the induction of HO-1 with Hb could depress vasoreactivity in rat aorta (30). Hence, the clinical meaning of CO and the possible usage of drugs of modulating HO in the course of the sepsis syndrome need further investigating.

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