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BRIEF REPORT

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Increased endogenous carbon monoxide production in severe sepsis

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Abstract *Objective:* A comparison was made between the endogenous carbon monoxide (CO) production in mechanically ventilated critically ill adult patients with, and those without, severe sepsis. *Design:* Prospective comparative study.
Setting: Medical ICU in a community hospital. *Patients:* Twenty-four patients with severe sepsis of various etiologies and five control patients with varying diagnoses.
Intervention: CO concentration was determined with an infrared CO analyzer on exhaled breath collected at the outlet of the ventilator. Endogenous CO production was estimated by the lung CO excretion rate measured at steady state.
Measurements and main results: Endogenous CO production was higher in the sepsis group during the first 3 days of treatment in comparison to the control group (10.9±5 (SD) µl/kg per h on day 1, 7.8±4.9 µl/kg per h on day 2 and 6.9±4.7 µl/kg per h on day 3 versus 2.1±0.5 µl/kg per h; $p<0.01$ for each comparison). Survivors of sepsis had a significantly higher endogenous CO production on day 1 compared to non-survivors (14.7±5.3 versus 8.5±3.3 µl/kg per h; $p=0.02$).
Conclusion: Endogenous CO production was significantly higher in mechanically ventilated patients suffering from severe sepsis. Further studies are required in order to determine the mechanism(s) and the functional significance of this increase.

Keywords Carbon monoxide · Sepsis · Breath test · Oxidative stress · Critical care · Heme oxygenase

Introduction

Previous studies have demonstrated that carbon monoxide (CO) is endogenously produced in humans, originating mainly from heme catabolism [1, 2]. Heme oxygenase is the rate-limiting enzyme of the heme degradation. One inducible (HO-1) and two constitutive (HO-2 and HO-3) isoforms have been described. The HO-1 isoform is induced within cells mainly during oxidative stress [3]. Synthesized CO diffuses out of cells, enters the blood to form carboxyhemoglobin and is transported to the lungs where it is excreted in the ambient air [4].

Severe sepsis in intensive care units (ICUs) is a frequently occurring condition often leading to multiple organ failure and death. A recent study has demonstrated an increased carboxyhemoglobin concentration in trauma patients with sepsis, which may reflect an increase in endogenous CO production [5]. However, to our knowledge, the endogenous CO production in sepsis has not been investigated so far.

Material and methods

Population

The study was performed on 29 mechanically ventilated patients studied from February to June, 1999. The clinical data of these patients are listed in Table 1.

Twenty-four patients were admitted to our department with, or developed during their ICU stay, severe sepsis (16 patients) or

Table 1 Demographic data of the study population (*COPD* chronic obstructive pulmonary disease, *SAPS* Simplified Acute Physiology Score, *LOD* Logistic Organ Dysfunction)

	Sepsis group (n=24)	Control group (n=5)
Age	66.5±14.3	76.4±10.6
Sex ratio (F/M)	10/14	3/2
Smokers (%)	6 (25)	1 (20)
Comorbidities	Liver cirrhosis (1) COPD (3) Alcoholism (3) Hypertension (7) Diabetes mellitus (2)	Heart failure (1) COPD (1) Alcoholism (2) Hypertension (3)
SAPS II score	50.4±13.8	44.8±7.5
LOD score	8.6±4.7	6.4±3.2
Ventilation (days)	16.5±14*	5.4±3.9
ICU stay (days)	19.7±15.2	8.4±4.9
Mortality (%)	11 (45.8)	2 (40)

* indicates $p<0.05$ between sepsis group and control group

septic shock (8 patients) according to the ACCP/SCCM consensus conference [6]. Septic patients were consecutively enrolled in the study. Sepsis was present at admission in 20 patients and occurred during the ICU stay in 4 others. The etiologies of sepsis were community-acquired pneumonia (14 patients), nosocomial pneumonia (4), peritonitis (3), pyelonephritis (1), meningitis (1) and Boerhave syndrome (1). Five patients with varying diagnoses (stroke, post-anoxic encephalopathy, pulmonary embolism, acute exacerbation of chronic obstructive pulmonary disease and cardiogenic pulmonary edema) were also included in our study and formed the control group. None of these patients developed sepsis during their ICU stay.

Endogenous carbon monoxide production

All patients were ventilated with either a Servo 900 C (Siemens, Germany) or a Cesar ventilator (Taema, Antony, France). Synchronized intermittent mandatory ventilation was used in every case. The protocol was approved by the local ethics committee. Exhaled CO was determined with an infrared CO analyzer (CO 2000, Sérès, La Duranne, France) with a sensitivity of 0.1 ppm and a sampling rate set at 1 l/min. The response time of the analyzer (time from the initial deflection to 90% of the plateau value when using a calibration gas at 10.3 ppm) was measured 5 times, which gave a result of 71±2 s. The analyzer was calibrated weekly with a 10.3 ppm calibration gas (Air Liquide, France). Expired breath was collected into a 6l plastic bag that did not react with CO. Two bags were successively placed at the expiratory outlet of the ventilator. Once full, they were rapidly connected to the CO analyzer. Due to the analyzer's slow response time, the latter of two consecutive measurements was used for analysis.

In a preliminary study, we have demonstrated that exhaled CO concentration was stable for a minimum of 4 h (and up to 24 h) on condition that the ventilation setting had remained unchanged for at least 7 h [7]. Carbon monoxide was also present in the inhaled gas at a concentration (0.25 ppm) independent of the inhaled oxygen fraction [7]. When a steady state was reached, lung CO excretion (calculated as follows: VCO(µl/kg/h)=(eCO(ppm)-0.25).expiratory flow(l/h)/body weight(kg)) was used as an estimate of endogenous CO production [8].

Exhaled CO concentrations were determined at 8 a.m. when routine biological studies including blood gas analysis were performed. In septic patients, determination of endogenous CO production was required on two different days at least, with the first measurement performed during the first 48 h of treatment. Day 1 was considered as the first day of "adequate" treatment (i.e., antibiotic therapy, surgery if necessary). Measurements of lung CO excretion were performed on day 1 in 13 patients, on day 2 in 15 patients, on day 3 in 14 patients and between days 4 and 7 in 18 patients.

In the control group, exhaled CO measurements were often only available for 1 day due to a shorter period of ventilation and the exclusion of patients who subsequently developed sepsis. Nine measurements of exhaled CO (and subsequent determinations of CO production) were available from five control patients. Four had one measurement performed during the first 48 h of ventilation and one had a measurement carried out each day for five consecutive days.

Statistics

All results are expressed as mean ± standard deviation (SD) unless mentioned otherwise. Comparisons were performed with Fischer's test, paired or unpaired Student *t*-tests, Mann-Whitney's test or ANOVA with repeated measurements when appropriate. Significance was defined as a p value of less than 0.05.

Results

In the control group, the unchanged ventilatory setting preceding the exhaled CO measurements covered a time period of 28±20.6 h (range: 7–67 h). In the sepsis group, this period lasted for 13±6.7 h (range: 7–23 h) on day 1, 23.2±19 h (range: 7–56 h) on day 2, 29.4±21.2 h (range: 18–74 h) on day 3 and 29.4±21.2 h (range: 18–74 h) on days 4–7.

Carbon monoxide was present in the exhaled breath of each patient at a higher concentration compared to the inhaled gas: 0.54±0.09 ppm (range: 0.4–0.7 ppm) versus 0.25 ppm ($p<0.001$) in the control group (nine measurements); 1.13±0.6 ppm (range: 0.4–2.9 ppm) versus 0.25 ppm ($p<0.001$) in the sepsis group (60 measurements). On 44 occasions (39 in the sepsis group, 5 in the control group), a second measurement was made 1 h after the first one. There was no significant difference between these two successive measurements regarding the exhaled CO concentration (1±0.57 versus 0.98± 0.56 ppm; NS) and the endogenous CO production (5.76±4.87 versus 5.73±4.82 µl/kg per h; NS).

In comparison to the control group, the exhaled CO concentration was greater in the sepsis group on day 1 (1.53±0.42 ppm versus 0.54±0.09 ppm; $p<0.001$), on day 2 (1.2±0.5 ppm versus 0.54±0.09 ppm; $p<0.01$) and on day 3 (1.09±0.45 ppm versus 0.54±0.09 ppm; $p<0.01$). In the sepsis group, the endogenous CO production decreased over time but remained significantly higher compared to the control group during the first 3 days of treatment (Fig. 1).

On the first day of treatment, endogenous CO production was significantly lower in patients with sepsis who did not survive (8.5±3.3 versus 14.7±5.3 µl/kg per h; $p=0.02$). Ventilatory (FIO_2, minute ventilation, PEEP)

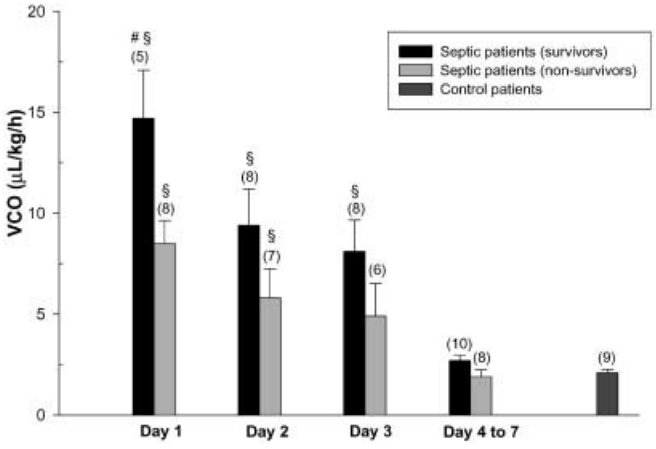

Fig. 1 Endogenous carbon monoxide (VCO) production during treatment of sepsis. Results are expressed as mean ± SEM. The difference between (a) survivors and non-survivors of sepsis is indicated by # ($p<0.05$) and (b) septic and non-septic patients is indicated by § ($p<0.01$). The number of measurements are marked above the bars

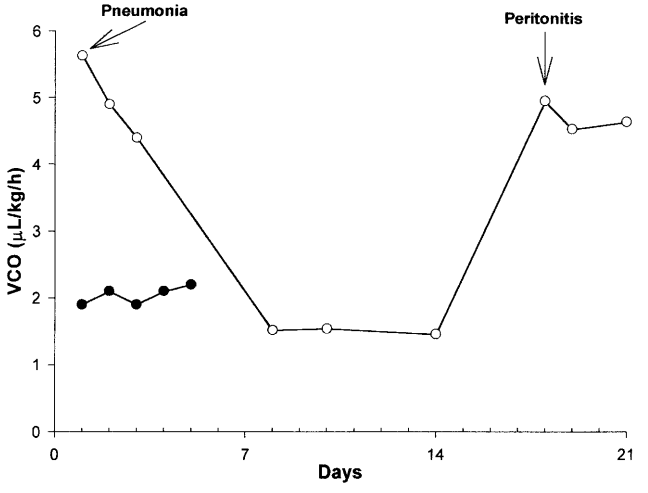

Fig. 2 Comparative evolution of the endogenous carbon monoxide production (VCO) in a control patient (*closed circle*) suffering from stroke and a septic patient (*open circle*) who experienced two successive severe infections

and hemodynamic parameters (mean arterial pressure, heart rate) did not differ significantly on day 1 between the patients who survived or not during their ICU stay (data not shown).

Serial measurements were obtained in one control patient (who suffered from stroke) and in one cirrhotic patient who developed a nosocomial infection (spontaneous bacterial peritonitis) 2 weeks after an initial community-acquired pneumonia. Endogenous CO production was increased during both infectious episodes, whereas it remained constant in the control patient during the study period (Fig. 2).

Discussion

In this study we have demonstrated for the first time that the exhaled CO concentration and the endogenous CO production were increased in mechanically ventilated patients suffering from severe sepsis or septic shock of various etiologies. In addition, both parameters decreased with treatment of the disease during its initial course.

Lung excretion is considered as a quasi-exclusive way of CO elimination from the body. This pulmonary excretion depends on many variables, such as alveolar ventilation, CO lung diffusing capacity, lung capillary oxygen partial pressure, carboxyhemoglobin concentration and endogenous CO production [4]. When a steady state exists (i.e., when the CO body content remains constant), the pulmonary CO excretion is considered as a valid estimate of endogenous CO production since CO catabolism is negligible [4, 8].

In a preliminary experiment, we have observed that a steady state was achieved after a 7h period of unchanged ventilatory setting, in mechanically ventilated and hemodynamically stable critically ill adult patients [7]. We have extended our previously described method of determination of endogenous CO production in catecholamine-dependent patients (data not shown). The most important point in measuring endogenous CO production is to make sure that steady state has effectively been reached. Based on our experience, we recommend a second measurement to be performed 1–2 h after the first one. In the present study, we applied the same methodology to determine the exhaled CO concentration and the endogenous CO production in patients suffering from severe sepsis or septic shock compared to control patients.

For a measurement of endogenous CO to be valid, lung CO excretion should be determined after steady state has been reached [4, 8]. Exposure to exogenous CO or pulmonary diseases is likely to affect the time required to achieve a steady state. Since our protocol allowed measurement of lung CO excretion during steady state, determination of endogenous CO production can be considered as valid in our patients, whether or not they were smokers or suffered from a pulmonary disease (such as COPD or pulmonary embolus).

Moncure et al. [5] have recently observed an increase in carboxyhemoglobin concentration in trauma patients with sepsis. We have extended their clinical experience by demonstrating for the first time that endogenous CO production in critically ill patients with severe sepsis is increased. In the study of Moncure et al.[5] there was a wide overlap of the carboxyhemoglobin concentration between the groups of trauma patients with or without sepsis. This was probably the consequence of the carboxyhemoglobin concentration being dependent on multiple factors, as shown earlier by Coburn et al. [4]. We did not observe such an overlap regarding endogenous CO production between septic and control patients.

Our study, however, was not designed to evaluate the potential sources of the increased endogenous CO production. Oxidation of various organic compounds can lead to endogenous CO production [2]. Nevertheless, it has previously been reported that endogenously produced CO mainly originates from heme degradation, which is dependent on heme oxygenase, the step-limiting enzyme of heme catabolism [3]. The expression of the heme oxygenase 1 isoform can be upregulated in various tissues by numerous stimuli including proinflammatory cytokines, endotoxin or oxidative stress [3]. A widespread overexpression of heme oxygenase 1 has recently been described in a mouse model of sepsis [9]. We speculate that the increased endogenous CO production in human sepsis mainly reflects an increased heme turnover secondary to an upregulation of the expression of heme oxygenase 1. Further studies are required to identify the potential sources of increased endogenous CO production in patients with sepsis.

The small size of our population is clearly a limitation of the study. However, despite the low power of comparisons, the differences observed regarding exhaled CO and endogenous CO production between the two study groups were important. The control group consisted of five mechanically ventilated patients with or without an abnormal chest X-ray. The small size of the control group can be explained by the exclusion of patients who developed sepsis or in whom a stable exhaled CO concentration was not achieved due to the short period of ventilation (including mainly postoperative patients or patients with a drug overdose). Studies on a large population are, therefore, required to confirm our preliminary results.

Carbon monoxide has long been regarded as a waste product of heme metabolism. Anti-inflammatory properties, however, such as the inhibition of proinflammatory cytokines production have recently been attributed to CO [10]. In the present study, survivors of sepsis initially had a higher endogenous CO production. Our sepsis population, however, was too small to determine whether the level of endogenous CO production was an independent predictor of survival in severe sepsis.

In summary, we have demonstrated for the first time that the endogenous CO production in critically ill patients with severe sepsis or septic shock is increased during the initial course of the disease. However, the mechanism(s), the functional significance and the usefulness of monitoring the exhaled CO remain to be determined.

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