Carbon monoxide in the treatment of sepsis

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Nakahira K, Choi AM. Carbon monoxide in the treatment of sepsis. Am J Physiol Lung Cell Mol Physiol 309: L1387–L1393, 2015.—Carbon monoxide (CO), a low-molecular-weight gas, is endogenously produced in the body as a product of heme degradation catalyzed by heme oxygenase (HO) enzymes. As the beneficial roles of HO system have been elucidated in vitro and in vivo, CO itself has also been reported as a potent cytoprotective molecule. Whereas CO represents a toxic inhalation hazard at high concentration, low-dose exogenous CO treatment (~250–500 parts per million) demonstrates protective functions including but not limited to the anti-inflammatory and anti-apoptotic effects in preclinical models of human diseases. Of note, CO exposure confers protection in animal models of sepsis by inhibiting inflammatory responses and also enhancing bacterial phagocytosis in leukocytes. These unique functions of CO including both dampening inflammation and promoting host defense mechanism are mediated by multiple pathways such as autophagy induction or biosynthesis of specialized proresolving lipid mediators. We suggest that CO gas may represent a novel therapy for patients with sepsis.

CO as a Potential Cytoprotective Molecule

CO is a ubiquitous environmental product of organic combustion process, including the burning of wood, coal, gas, and tobacco (72). Indoor ambient levels of CO range are from 0.5 to 5 ppm. CO is also physiologically produced in cells and tissues as the byproduct of heme catalytic activity of heme oxygenase (HO) enzymes (55, 60). The rate of CO generation in the human body may substantially change, when cells or tissues increase the expression of inducible HO enzyme (i.e., HO-1) in response to various oxidative stresses (55, 60). HO-1 is the rate-limiting enzyme in the degradation of heme to generate CO, iron, and biliverdin-IXα, which is converted to bilirubin-IXα (55, 60). Beneficial roles of the HO enzyme system have been studied in various disease models (18, 51, 55, 59, 60). With the ever-increasing amount of experimental evidence demonstrating the beneficial roles of HO, much interest has been generated by both basic and clinical/translational investigators whether direct administration of such end-products may serve as a therapeutic approach in human diseases. Despite the notorious pharmacological effect of CO (i.e., the high affinity for hemoglobin and the resultant displacement of oxygen) (17, 52), many studies have demonstrated the protective roles of inhaled CO, when administered at low concentration, in various disease models including hyperoxia-induced acute lung injury (ALI), mechanical ventilation-induced ALI, ischemia/reperfusion (I/R) injury, vascular injury, graft rejection (43, 55, 60). Low-dose CO inhalation also improves the survival of septic rodents induced by cecal ligation and puncture (CLP), endotoxin, Enterococcus faecalis, or Escherichia coli (30, 42, 43, 74). Similar protective effects have also been observed in septic mice subjected to administration of chemical CO-donor compounds termed carbon monoxide releasing molecules (9, 13, 43). Of note, the protective roles of CO on sepsis is not limited to rodent models but also observed in large animal models such as swine and nonhuman primates subjected to LPS or Streptococcus pneumoniae administration (12, 15, 39).

Dampening inflammation. One of the potent protective functions of CO in sepsis models is the anti-inflammatory effect. In 2000, Otterebein et al. (48) reported that CO inhalation (250 ppm) suppresses proinflammatory cytokine production such as TNF-α and increased anti-inflammatory cytokine production...
such as IL-10 in endotoxemia. Furthermore, CO inhibits pro-inflammatory signaling pathways activated by other pathogen-associated molecular patterns including Toll-like receptor (TLR) 2-, TLR5-, and TLR9-ligands (46). These reports suggest that the anti-inflammatory property of CO is effective on a wide range of microbial components. The general mechanisms of anti-inflammatory effects of CO include activation of p38 MAPK, suppression of translocation of TLRs, and enhanced PPAR-γ signaling (43, 55, 57, 58). A recent report has also demonstrated another novel target of CO to dampen inflammation (25). Inflammasomes are cytosolic protein complexes that promote the cleavage of caspase-1 and secretion of IL-1β and IL-18 and are critically involved in the pathogenesis of various inflammatory diseases (20, 29). The levels of circulating IL-18 are increased in patients with acute respiratory distress syndrome (ARDS) and sepsis and are associated with the patients’ mortality in medical intensive care unit (14). CO negatively regulates NLRP3-dependent inflammasome activation and IL-18 secretion (25). Thus the negative regulation of CO on IL-18 secretion may represent one key mechanism to ameliorate the pathogenesis of sepsis. Although a number of studies show the anti-inflammatory effect of CO on immune cells such as macrophages, it should be noted that CO also inhibits production of chemokine such as monocyte chemotactic protein 1 in type I alveolar epithelial cells (4). Thus CO can efficiently dampen systemic inflammatory responses by regulating immune responses of various types of cells.

Eliminating microbes. Although regulating inflammation is an important arm to prevent multiorgan dysfunctions in sepsis, proper immune responses are critical host responses to eliminate microbes during infection (1, 54, 68). In this regard, CO can also regulate a key host defense mechanism in sepsis: promoting phagocytosis. CO promotes uptake of bacteria such as E. coli or E. faecalis by macrophages through activating autophagy (30) or redistribution of TLR4 on the cell surface (50). CO can also promote bacterial killing in macrophages by directly manipulating the metabolic pathways of microbes. Bacteria exposed with CO increase generation and secretion of ATP, in turn stimulating macrophage phagocytosis through activation of P2X7 receptor (74). Finally, recent reports demonstrate that CO promotes both bacterial killing and anti-inflammatory process mediated by biosynthesis of lipid mediators, critical molecules for maintaining tissue homeostasis. Emerging evidence indicates that cells/host can promote resolution of acute inflammation by activation of biosynthesis of specialized proresolving lipid mediators (SPMs) such as arachidonic acid-derived lipoxins or docosahexaenoic acid-derived D-series or E-series resolvins (RvDs or RvEs) (32, 61, 62). These SPMs terminate inflammation by inhibiting neutrophil infiltration to the inflaming sites and also enhancing macrophage phagocytosis, microbial clearance, and efflux to lymph nodes (32, 61, 62). The exogenous administration of SPMs such as resolvins improves the survival of septic mice subjected to bacterial infection (7, 67), and CO indeed upregulates phagocytosis by increasing biosynthesis of SPMs such as lipoxins and RvE in vivo and in vitro (8, 12, 63). Thus, unlike other immunomodulators used in previous clinical trials (e.g., targeting TNF-α or endothoxin), CO can exert its potent bacterial killing function and anti-inflammatory function through multiple mechanisms.

Regulating cell death and organ dysfunction. In addition to immune responses, cell death also critically contributes to the pathogenesis of sepsis (1, 68). Released damage-associated molecular patterns (DAMPs) from dead cells of damaged tissues during sepsis can promote further immune responses (1, 27, 45). CO inhibits cell death in various cell types including epithelial cells or endothelial cells by regulating mitochondrial functions (31, 73). Mitochondrial dysfunction has been observed in septic patients (6, 19, 64, 70, 75, 78), and recent studies suggest that mitochondrial dysfunction might be associated with critically ill patients’ outcome (28, 45, 47). For example, the levels of circulating cell-free mitochondrial DNA (mtDNA), a representative mitochondrial DAMP (45), are increased in patients with of sepsis (47). The elevated mtDNA levels predict an increased risk of death that persisted over up to 3.5 years of follow-up (47). Thus the inhibition of mitochondrial DAMPs release may likely to be a critical step to prevent sepsis-induced mortality. CO inhibits leakage of mtDNA from mitochondria by regulating mitochondrial membrane potential and mitochondrial reactive oxygen species generation (25). In addition, CO inhibits cell death in lung epithelial cells or kidney mesangial cells through upregulation of autophagy (26, 31). While autophagy is known as a cellular-protective mechanism, autophagy can also induce cell death in some oxidative stress models such as influenza A virus infection or sea water exposure (33, 44, 69). Although low-dose CO exposure may induce cell death, it should be noted that CO promotes resolution of acute inflammation in immune cells such as neutrophils and T cells by inducing cell death (8, 66). This pro-cell death function by CO may rather consequently inhibit tissue injuries triggered by inflammation. However, care may need to be taken for the cases with lung injuries in which autophagic cell death is involved. Finally, CO also exerts its protective effects in various organs or tissues including lung (12, 15, 30, 31, 34, 39), liver (30, 39, 81), heart (39), kidney (11, 26, 30, 39), and intestine (34, 41, 71) - important target organs of sepsis (Fig. 1). CO treatment can also improve the coagulation status with decreased disseminated intravascular coagulopathy (DIC) in a swine model of endotoxin challenge (39). Given the high mortality of sepsis with acute organ dysfunction and DIC, the prevention of organ/tissue dysfunction is an important strategy for treatment of sepsis (1, 54). Thus the protective effect of CO on multiple organs will be advantageous as an intervention for sepsis.

CO as a Therapeutic in Human Diseases

Although published works from the bench demonstrate CO as a potent “antiseptic” molecule, there are also important data from bedsides showing that the pathological conditions may increase production of CO in patients (22, 56). Exhaled CO (eCO) has been studied in patients as an inflammatory marker of diseases (3, 22, 56, 80) and may reflect increased production of systemic CO derived from HO induction in stressed pathophysiological conditions. The origin of eCO reflects a systemic elimination process of CO from the pulmonary circulation to the alveole (22, 56). The levels of eCO are increased in patients who have systemic inflammatory responses or local inflammation (56). Of note, the levels of eCO are higher in mechanically ventilated septic patients, compared with ventilated patients without exacerbation. The potential therapeutic use of CO in sepsis is consistent with recent studies showing that CO as an inhalation therapy can improve respiratory function in patients (10, 220.33.1 on March 30, 2017). CO administration is capable of improving respiratory function in patients with sepsis. This improvement in respiratory function is accompanied by a decrease in inflammatory markers, such as IL-6 and TNF-α, as well as an increase in anti-inflammatory markers, such as IL-10. These observations suggest that CO has the potential to improve respiratory function and modulate the inflammatory response in patients with sepsis.
sepsis (79). More importantly, elevated eCO values on the first day of treatment are correlated with the probability of patient survival (79), suggesting the potentially critical roles of CO in septic patients. The first CO study in human was performed to investigate the effect of CO inhalation on inflammation during human endotoxemia (38). Each volunteer inhaled synthetic air (as placebo) and 500 ppm CO for 1 h in random order and received LPS intravenously after the inhalation. CO-Hb was increased from 1.2 to 7% by CO inhalation (38). In this study, the CO inhalation did not show significant effects on production of proinflammatory cytokine or anti-inflammatory cytokine. However, in addition to the limited scope of the study, a single short duration of CO exposure (1 h) may not have been sufficient to gain the benefits of CO in human subjects. Subsequently, ex-smoking patients with stable chronic obstructive pulmonary disease were subjected to CO inhalation with 100–125 ppm for 2 h/day for 4 days (5). After the exposure CO-Hb was increased to 4.5% in the patients. Importantly, patients with CO inhalation showed decrease number of eosinophils in sputum and improvement of methacholine responsiveness (5). In a recent completed trial of Inhaled Carbon Monoxide to Treat Idiopathic Pulmonary Fibrosis (NCT01214187), inhaled CO at 100–200 ppm was administered two times weekly for 2 h per dose to complete 12 wk of treatment (Table 1). Although the full analysis of this completed trial is pending and additional studies are needed in the future to assess the efficacy of CO on idiopathic pulmonary fibrosis, this trial has importantly demonstrated that inhaled CO at 100–200 ppm can be administered safely to patients with underlying lung dysfunction. Finally, a new clinical trial of CO, Safety Study of Inhaled Carbon Monoxide to Treat Acute Respiratory Distress Syndrome (ARDS) (NCT02425579), has started recruiting participants (Table 1). The purpose of the trial is to assess the safety of inhaled CO in intubated patients with sepsis-induced ARDS. Since sepsis is still one of the leading causes of ARDS, this new clinical trial may provide the critical information how CO can be efficiently and safely delivered to septic patients.

**The Future of CO Therapy in Sepsis**

The first report of protective roles of inhaled CO was demonstrated in rodents with hyperoxia-induced ALI in 1999 (49). Low-dose CO exposure (250 ppm) reduced the volume of pleural effusion and the protein concentration and neutrophil recruitment in the airways (49). Furthermore, this study also demonstrated that CO inhalation alone is enough to protect lung injuries regardless of endogenous CO production in the...
### Table 1. List of ongoing clinical trials using CO inhalation in human diseases

<table>
<thead>
<tr>
<th>Target Disease</th>
<th>Phase</th>
<th>Purpose</th>
<th>Assigned Interventions</th>
<th>Primary Outcome Measures</th>
<th>Second Outcome Measures</th>
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<tr>
<td>Acute Respiratory Distress Syndrome (ARDS) (NCT02425579)</td>
<td>1</td>
<td>• To assess the safety of inhaled CO in intubated patients with sepsis-induced ARDS</td>
<td>• Inhaled CO at 100 or 200 ppm&lt;br&gt;• Algorithm-specified dose of inhaled CO to achieve CO-Hb of 6–8%</td>
<td>• Number of administration-associated adverse events&lt;br&gt;[time frame: 60 days]</td>
<td>• Mean daily SOFA score&lt;br&gt;• PaO2/FiO2 ratio and oxygenation index&lt;br&gt;• ALI score&lt;br&gt;• Vasopressor-free days&lt;br&gt;• ICU-free days&lt;br&gt;• Hospital-free days&lt;br&gt;• Plasma biomarkers of inflammation, lung epithelial injury, endothelial injury, markers of change in other end-organ function&lt;br&gt;• Skeletal muscle biomarkers of mitochondrial biogenesis</td>
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<td>Intestinal Paralysis After Colon Surgery (NCT01050712)</td>
<td>2</td>
<td>• To determine whether inhaling very low doses of CO before and after colon surgery will shorten the duration of normal POI and/or prevent the development of POI complications in patients undergoing colon surgery</td>
<td>• Concentration of CO to be inhaled by patients will be determined in a safety trial performed in healthy volunteers prior to the commencement of this trial&lt;br&gt;• Patients will receive CO for 1 h prior to colon resection and for 1 h after colon resection</td>
<td>• Duration of POI (radiological)&lt;br&gt;• Incidence of pathological POI&lt;br&gt;• Duration of POI (clinical)</td>
<td>• To compare blood to right atrial tissue biochemical markers of mitochondrial biogenesis&lt;br&gt;• Biochemical markers in both right atrial tissue and blood will be measured and compared to see whether the more easily obtained blood markers accurately describe changes expected in the heart</td>
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<tr>
<td>Aortic Valve Surgery Patients (NCT01727167)</td>
<td>1 and 2</td>
<td>• To test whether inhalation of CO will increase the numbers of mitochondria in heart muscle</td>
<td>• 200 ppm of CO for 1 h on the day prior to surgery</td>
<td>• Biochemical markers for mitochondrial biogenesis (blood and right atrial tissue)&lt;br&gt;• Right atrial biochemical markers will be measured one time only, intra-operatively&lt;br&gt;• Blood biochemical markers will be measured before CO exposure and at intervals up to one week postoperatively</td>
<td>• To compare blood to right atrial tissue biochemical markers of mitochondrial biogenesis&lt;br&gt;• Biochemical markers in both right atrial tissue and blood will be measured and compared to see whether the more easily obtained blood markers accurately describe changes expected in the heart</td>
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<tr>
<td>Pulmonary Arterial Hypertension (NCT01523548)</td>
<td>1 and 2</td>
<td>• To examine the potential of CO to decrease elevated blood pressure in the pulmonary artery</td>
<td>• 150 ppm × 3 h once weekly (week 1) 150 ppm × 3 h twice weekly (week 2) -150 ppm × 3 h three times a week (weeks 3–16)</td>
<td>• Evidence of a 20% decrease in pulmonary vascular resistance posttherapy when compared to pretherapy value</td>
<td>• Effect of 16-wk CO inhalation on other pulmonary and systemic hemodynamic parameters&lt;br&gt;• Effect of 16-wk CO inhalation on functional capacity assessed by 6-min walk test&lt;br&gt;• Effect of 16-wk CO inhalation on brain natriuretic peptide levels&lt;br&gt;• Effect of 16-wk CO inhalation on right ventricular echocardiographic parameters&lt;br&gt;• Effect of 16-wk CO inhalation on acute pulmonary vasoreactivity&lt;br&gt;• Total lung capacity&lt;br&gt;• Diffusing capacity for CO&lt;br&gt;• 6-min walk distance&lt;br&gt;• St. George’s Respiratory Questionnaire</td>
</tr>
<tr>
<td>Idiopathic Pulmonary Fibrosis (NCT01214187)</td>
<td>2</td>
<td>• To determine whether low-concentration inhaled carbon monoxide is effective in treating idiopathic pulmonary fibrosis</td>
<td>• The intervention will be inhaled CO at 100–200 ppm administered two times weekly for 2 h per dose to complete 12 wk of treatment</td>
<td>• Serum MMP7 level&lt;br&gt;[time frame: 3 mo]</td>
<td>• Effect of 16-wk CO inhalation on other pulmonary and systemic hemodynamic parameters&lt;br&gt;• Effect of 16-wk CO inhalation on functional capacity assessed by 6-min walk test&lt;br&gt;• Effect of 16-wk CO inhalation on brain natriuretic peptide levels&lt;br&gt;• Effect of 16-wk CO inhalation on right ventricular echocardiographic parameters&lt;br&gt;• Effect of 16-wk CO inhalation on acute pulmonary vasoreactivity&lt;br&gt;• Total lung capacity&lt;br&gt;• Diffusing capacity for CO&lt;br&gt;• 6-min walk distance&lt;br&gt;• St. George’s Respiratory Questionnaire</td>
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rodent models (49). In the past 15 years, CO inhalation has been successfully shown as a potential therapeutic in a number of animal models, and the inhalation of CO gas has now been tested in multiple early phase I clinical trials (Table 1). Although beneficial roles of low-dose CO inhalation are proven in rodent models, the studies of inhaled CO as the treatment for human diseases are at an early phase in the clinical development. Further studies are needed to establish the optimal delivery and dosing protocol for CO inhalation therapy in critically ill patients (e.g., differences in lung physiology and mechanics between rodents and humans should be kept in mind). As much as we are most committed and excited by the potential therapeutic applicability of CO in sepsis going forward, we must remain vigilant in designing the optimal treatment protocol and further works still lies ahead. Patients who have risk of neurotoxicity or carboxyhemoglobinemia may be excluded from CO inhalation therapy (the possible exclusion criteria are listed on https://clinicaltrials.gov/ct2/show/NCT02425579). Nonetheless, given a dreadful disease state such as sepsis with no effective therapeutic target and enormous unmet medical need, the potent protective molecule CO is hard to be overlooked but should be evaluated for the benefits of patients. We have entered an exciting era wherein Phase I CO trials have been completed in several disease states: there are challenges ahead of us to ensure that carefully planned Phase II trials we can rigorously test the tenet whether CO inhalation therapy will be effectively tested in clinical trials and in the future can represent a viable treatment among the available therapeutic armamentariums for patients suffering from sepsis.

REFERENCES

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