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Forum Review

Carbon Monoxide in Sepsis

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ABSTRACT

Despite modern practices in critical care medicine, sepsis or systemic inflammatory response syndrome remains a leading cause of morbidity and mortality in the intensive care unit. Thus, the need to identify new therapeutic tools for the treatment of sepsis is urgent. In this context, carbon monoxide has become a promising therapeutic molecule that can potentially prevent uncontrolled inflammation in sepsis. In humans, carbon monoxide arises endogenously from the degradation of heme by heme oxygenase enzymes. Both endogenously synthesized and exogenously applied carbon monoxide can exert antiinflammatory and antiapoptotic effects in cells and tissues. Based on these properties, carbon monoxide, when applied at low concentration, conferred protection in a variety of cellular and rodent models of sepsis, and furthermore reduced morbidity and mortality *in vivo*. Therefore, application of carbon monoxide may have a major impact on the future of sepsis treatment. This review summarizes evidence for salutary effects of carbon monoxide in sepsis of various organs, including lung, heart, kidney, liver, and intestine, and discusses the potential translation of the data into human clinical trials. *Antioxid. Redox Signal.* 9, 2013–2026.

INTRODUCTION

Sepsis, or systemic inflammatory response syndrome (SIRS), has attracted extensive investigation, as it remains a leading cause of mortality in intensive care units. This condition arises as a consequence of systemic responses to inflammation caused by acquired bacterial, fungal, parasitic, or viral infections, and may lead to organ failure and tissue damage. The definition of sepsis is currently under debate, because despite clinical evidence, positive blood cultures are often lacking. To consider this patient population, the term *sepsis syndrome* has been proposed, which includes clinical evidence of infection, fever or hypothermia, tachypnea, tachycardia, and impaired organ system function or perfusion (7). Existing therapeutic approaches have failed to reduce dramatically the incidence of this inflammatory disease. More than 500,000 new cases occur in the United States each year, with an average mortality rate of ~20–35% (7, 65, 98). Therefore, a clear urgency exists for the development of new therapeutic strategies.

Carbon monoxide (CO) has recently emerged as a potential therapy for sepsis. CO, a low-molecular-weight gas molecule, arises in nature as the product of the combustion of organic matter, such as from the burning of fossil fuels or tobacco (133). Environmental CO represents a major air pollutant, which is generally regarded as an inhalation hazard. The possibility that CO could be used clinically, which remains in experimental stages, arose from observations of dramatic tissue protection from the application of low concentrations of this gas in animal models of inflammation, sepsis, oxidative stress, and ischemia/reperfusion injury (105, 106). Such a proposition of using CO gas as an inhalation therapeutic has been challenged by the well-known reputation of this gas as a poison at high inspired concentrations. The binding of CO to hemoglobin (Hb) inhibits O_2 transport and delivery to tissues. Thus, at elevated concentrations, CO acts as an asphyxiant, which causes tissue hypoxia, associated with a number of clinical symptoms, including dizziness, loss of consciousness, and death, with prolonged or excessive exposure (106, 133). Symptoms of CO poi-

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soning in humans begin to appear at carboxyhemoglobin (CO-Hb) levels of 20%, whereas loss of consciousness (coma) leading to death occurs in the range of 50–80% CO-Hb (133).

Nevertheless, multiple studies have demonstrated cellular and tissue protection afforded by CO in rodent models of sepsis, which includes a dramatic increase of survival in these animals. Based on these observations, low-concentration CO therapy appears at the verge of entering clinical trials. One human study has been published, and the authors are aware of at least five ongoing investigations using inhaled CO in humans. This overview summarizes the beneficial effects of CO in systemic inflammation and sepsis, with an emphasis on animal studies with clinical relevance. The molecular and cellular signaling pathways potentially activated by CO have been reviewed extensively elsewhere (52, 105).

Synthesis of CO

The endogenous production of CO in humans has been recognized for more than half a century (118, 119). Under physiologic conditions, humans produce ~0.4 ml (18 μmol) CO per hour (18), which increases to 3.6 ml/h (143 μmol/h) under pathophysiologic circumstances (19). About 85% of endogenous CO synthesis arises from heme degradation, catalyzed by the heme oxygenase (HO) enzymes (127), the majority of which is ascribed to systemic turnover of Hb. In cells and tissues not specialized in Hb turnover. CO also can arise from the degradation of heme derived from the turnover of cellular hemoproteins, including cytochromes. A minor part of systemic CO production (~15%) may arise from nonheme sources such as lipid peroxidation, photooxidation of organic molecules, or metabolism of halogenated hydrocarbons (61, 134, 136).

HO activity constitutes the rate-limiting step in the heme degradative pathway leading to the production of CO, iron, and bilirubin-IXα, the latter of which arises from the subsequent reduction of biliverdin-IXα by NAD(P)H: biliverdin reductase (BVR) (129) (Fig. 1). Two major isoforms of HO (HO-1, HO-2) have been characterized. HO-2 is a constitutive isoform expressed highly in testis, liver, brain, and endothelial cells of the cardiovascular system, among other tissues (63). The inducible form of heme oxygenase (HO-1), a major cellular stress protein, responds to transcriptional regulation by multiple forms of chemical and physical stress, such as xenobiotics, nitric oxide, hyperoxia, ischemia/reperfusion, shock, proinflammatory cytokines, and bacterial endotoxins. HO-1 represents a general marker of oxidative or pro-inflammatory stress, and its elevated expression has been observed consistently in states of experimental endotoxemia and sepsis (13, 37, 40–43, 50, 62, 105, 113).

The protective function of HO-1 has been attributed to several possible mechanisms. HO-1 may provide an antioxidative function by removing heme, which, when accumulating in excess of its utilization for hemoprotein synthesis, may exert prooxidant effects involving its iron center. Furthermore, all three HO-reaction products (*i.e.*, iron, biliverdin, and CO) have been extensively discussed as potentially contributing to HO-mediated cytoprotection. CO, when applied exogenously, appears to mimic protective effects of HO-1 upregulation, and, as discussed later, confers cell and tissue protection by modulating intracellular signaling pathways, culminating in antioxidative, antiinflammatory, antiproliferative, and vasodilatory effects (105, 106).

Dose and mode of application

The CO concentration that has been applied experimentally in animal studies ranges from 10 to 1,000 parts per million (ppm). Most investigators have applied CO by inhalation of 250 ppm, corresponding to an inspiratory fraction of 0.025%. CO diffuses from the alveolar space into the capillaries and

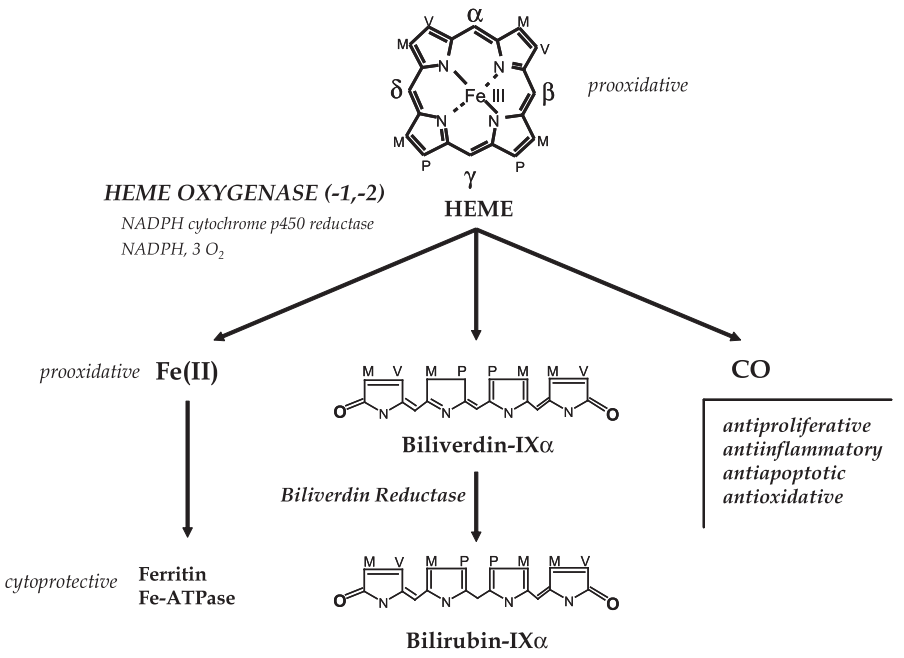

FIG. 1. Heme metabolism and synthesis of carbon monoxide (CO). Heme oxygenase activity is the rate-limiting step in the degradation of heme. Free iron, biliverdin, and CO are generated in equimolar amounts. All three products of heme oxygenase metabolism have been demonstrated to display cyto- and tissue-protective characteristics.

Table 1. Resulting Carboxyhemoglobin (CO-Hb) Fraction in Response to Low-Dose CO Inhalation in Humans

CO (ppm)	*CO-Hb (%) 1-h exposure*	*CO-Hb (%) 2-h exposure*	*CO-Hb (%) 3-h exposure*
0	0.8–2.3	0.8–2.3	0.8–2.3
10	1	1.3	1.4
25	1.5	2.2	3.6
50	1.6–2.0	4	6–7
100	3	7.5	11–13
200	5–8	9–14	12–24
500	9–15	30	45
1,000	23	50	60

subsequently binds Hb for transport to the systemic tissues. The extent of CO-Hb formation depends on the dose and the time of application (Table 1). As an alternative to inhalation for delivery of CO, prodrugs (*i.e.*, transition metal carbonyls) that release CO in dose- and time-dependent fashion have recently been developed and are referred to as carbon monoxide–releasing molecules (CORMs) (28, 79, 80). Water-soluble forms of CORMs allow intravenous administration. Interestingly, and in contrast to inhaled CO, CORMs appear to deliver CO directly to the tissues without significant formation of CO-Hb (78). A few experimental studies have applied CO by oral intake of methylene chloride, which is metabolized by cytochrome p450 enzymes in the liver to release CO.

EFFECTS OF CO IN SEPSIS

The synthesis of CO in humans is affected by systemic inflammation, such as in SIRS, sepsis, or septic shock. For instance, HO-1 expression and consequently CO production are upregulated in aortic smooth muscle cells and polymorphonuclear cells of septic patients (102). The levels of CO measured on the exhaled breath were higher in septic patients as compared with control subjects. Most interestingly, survivors showed higher levels of exhaled CO in this study than did nonsurvivors (141). Pediatric patients illustrated similar findings. CO-Hb levels in the blood of septic children were elevated and more pronounced in septic shock (116). It has been postulated that increased CO synthesis contributes to systemic vasodilation in sepsis (102).

Given the hypothesis that upregulation of endogenous CO synthesis may act as a rescue mechanism to defeat septic conditions, one might speculate that CO application should improve outcome and survival. Many studies involving animal models of sepsis demonstrated a protective effect of CO administration associated with prolonged survival and reduced mortality (Table 2).

One of the most-investigated sepsis models uses gram-negative bacterial lipopolysaccharide (LPS), a constituent of the bacterial cell wall. When administered to rodents or human cells, LPS evokes an inflammatory response similar to that of the whole bacterium. LPS activates macrophages, lymphocytes, polymorphonuclear leukocytes, epithelial cells, and complement in different models. Macrophages are the key inflammatory cells involved in LPS-induced sepsis. LPS binds to their CD14 cell-surface protein and toll-like receptor-2/4, activating signaling pathways, resulting in the release of proinflammatory cytokines and programmed cell death (apoptosis) (16, 33, 34, 99). As described later, CO can exert multiple effects on these mechanisms.

Anti-inflammatory effects of CO in sepsis

Cytokine release. At the cellular level, the inflammatory response is mediated mainly by macrophages. Proinflammatory cytokines such as TNF-α and interleukins (IL-1β, IL-6, IL-8) released from macrophages carry out direct effects on the organs or activate secondary mediators such as prostaglandin E_2, thromboxane A_2, platelet-activating factor-1, bradykinin, angiotensin, vasoactive intestinal peptide, and complement-derived products. These mediators exert overlapping effects on endothelial cell function, vascular function, coagulation, hemodynamics, and the cardiovascular mechanism. At

Table 2. Effect of Carbon Monoxide (CO) Treatment in Rodent Models of Sepsis and Systemic Inflammatory Response Syndrome (SIRS)

Organ	*Model*	*Species*	*CO dose (ppm)*	*Effect*	*Year*	*Ref.*
SIRS/sepsis	Hemorrhagic shock	Mouse	250	Cellular hypoxia ↓ Inflammation ↓, IL-10 ↑ Organ injury ↓	2005	145
	Sepsis	Rat	10–250	Inflammatory mediators ↓ Survival ↑	2004	108
	Sepsis	Mouse	250	Inflammatory mediators ↓ Survival ↑	2003	77
	Sepsis	Mouse	250	TNF-α ↓, IL-10 ↑	2000	88
	SIRS	Mouse	250	Inflammatory response ↓ Liver perfusion ↑ Liver cell injury ↓	2005	87

present, it is thought that antiinflammatory mechanisms can compensate for proinflammatory mechanisms in an ideal case. This compensation can take place in an organ, before sepsis occurs or during recovery from sepsis. Antiinflammatory mediators such as IL-10 and IL-11 limit the inflammatory process. If the balance between pro- and antiinflammatory responses is compromised because of an overwhelmed immune system or severe infection, the sepsis further propagates to shock and multiple organ dysfunction syndrome (MODS).

CO exerts direct effects on immune competent cells (Fig. 2). For instance, CO inhibits the activation of monocytes, macrophages, and leukocytes *in vitro* as well as *in vivo* (30, 107, 108). Furthermore, the ability of T cells to proliferate is suppressed by the application of CO (91). CO has also been shown to reduce the adhesion and migration of leukocytes in rats (74).

In vitro, CO prevented the LPS-induced production of proinflammatory cytokines such as TNF-α, IL1-β and macrophage inflammatory protein-1β (MIP-1β) in cultured macrophages (88). The inhibitory effects of CO on TNF-α production were also demonstrated with the application of CORM (*e.g.*, CORM-2) (112). This protection mimicked that which could be achieved by the artificial overexpression of HO-1 in this model, suggesting a link between CO and the cytoprotective effect of HO-1 induction. CO treatment also promoted the increased production of the antiinflammatory cytokine IL-10 during LPS challenge (88). Most interestingly, the effect of IL-10 itself appeared to be mediated by HO activity and specifically required CO to exert its antiinflammatory properties (57).

These general antiinflammatory effects of CO were also demonstrated *in vivo*. In murine models of endotoxemia, CO preconditioning reduced the production of serum TNF-α, IL-1β, and IL-6, and reduced organ injury and prolonged survival after LPS challenge (77, 87, 88). Conversely, CO dose-dependently increased LPS-inducible IL-10 production (88). In a pig study, CO reduced the development of disseminated intravascular coagulation and completely suppressed serum levels of the proinflammatory IL-1β in response to LPS, while augmenting the antiinflammatory cytokine IL-10 after LPS challenge (69). Whereas CO increases IL-10 levels in various models, IL-10 was not absolutely essential for the antiinflammatory effects of CO. In IL-10$^{-/-}$ mice, CO inhibited TNF-α levels within the first hour of LPS treatment to a similar extent as in wild-type mice (88).

Granulocyte–macrophage colony-stimulating factor (GM-CSF), a glycoprotein, promotes the proliferation and differentiation of hematopoietic progenitor cells into neutrophils and macrophages. In addition to this function, GM-CSF also plays a critical role in antigen- and complement-mediated immunoreactions during sepsis. For instance, GM-CSF enhances the secretion of proinflammatory cytokines including TNF-α, IL-1, interferon (IFN)-γ, as well as inflammatory mediators (*e.g.*, superoxide anion, E-series prostaglandins, leukotrienes, arachidonic acid, plasminogen activator, and other colony-stimulating factors (103). RAW 264.7 macrophages treated with LPS produced an increased amount of GM-CSF, which could be attenuated by CO pretreatment. Nuclear factor kappa B (NF-κB), a transcription factor that regulates proinflammatory cytokine release, can mediate the expression of the GM-CSF gene in certain cell types. CO inhibited the LPS-induced activation of NF-κB in RAW 264.7 cells, by preventing the phosphorylation and degradation of the inhibitory subunit Iκ-Bα (107).

Mitogen-activated protein kinases (MAPK). In macrophages, LPS binds to the CD14 cell-surface protein and toll-like receptor 2/4. The complex activates tyrosine kinases and the major mitogen-activated protein kinases (MAPKs) (16, 33, 34, 99). In RAW 264.7 macrophages, LPS activated the p38 MAPK, extracellular-regulated kinase-1/2 (ERK1/2), and c-Jun NH_2-terminal kinase (JNK) pathways. Exogenously administered CO (250 ppm) inhibited the production of cytokine release in RAW 264.7 cells in response to LPS treatment and concurrently increased p38 MAPK activation. Of the MAP kinase kinases (MKKs: MKK3, MKK4, and MKK6) that activate p38 MAPK, CO enhanced the LPS-mediated stimulation of MKK3 in RAW 264.7 and epithelial cells (21, 88, 90, 100).

In contrast, the mechanism for the CO-dependent downregulation of IL-6 production in macrophages involved the JNK pathway (see Fig. 2). JNK regulates several transcription fac-

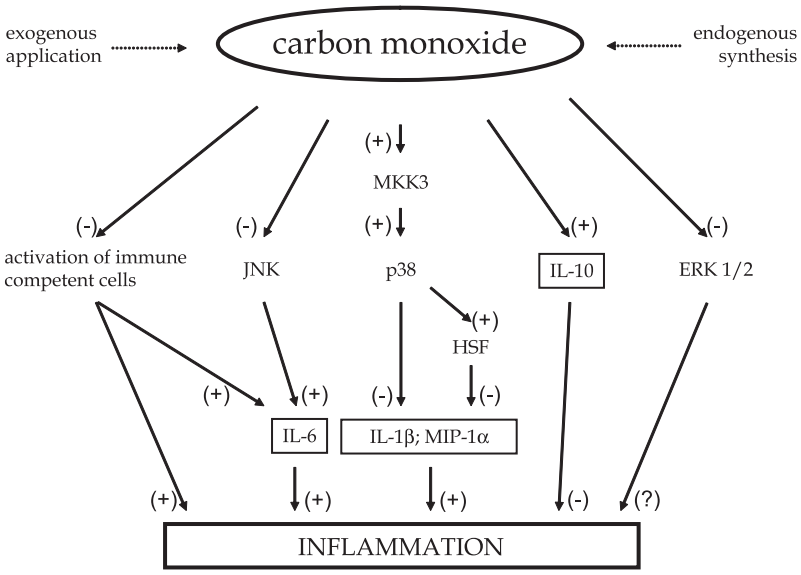

FIG. 2. Carbon monoxide–mediated signal-transduction pathways affecting inflammation. A simplified scheme of the signal-transduction pathways initiated by CO. p38 remains the most-investigated MAPK responsible for CO signaling and appears to play the major role in mediating antiinflammatory effects of CO.

tors including activator protein-1 (AP-1). The ability of CO to inhibit LPS-induced IL-6 production was abolished when the AP-1 DNA binding site was mutated in the IL-6 promoter. Mutation of NF-κB and CCAAT/enhancer-binding protein (C/EBP) binding sites, however, had no effect on the inhibitory effect of CO on IL-6 gene transcription (77). In pulmonary epithelial cells, CO inhibited the IL-17-stimulated inflammatory response (*i.e*., IL-6 production) *via* the ERK1/2-dependent pathway without altering p38 MAPK or JNK (86). In rat pulmonary artery endothelial cells treated with TNF-α, which rapidly increased MAPK activities, CO treatment specifically reduced ERK1/2 activation, and increased p38 MAPK activation (115). Taken together, CO exerts differential effects on p38 MAPK, JNK, and ERK1/2, dependent on stimulus and cell type (see Fig. 2). These antiinflammatory effects of CO *in vitro* were substantiated *in vivo*, in experiments in which mice received injections of LPS with or without CO pretreatment (250 ppm). CO dose-dependently inhibited LPS-inducible serum TNF-α levels and increased LPS-inducible IL-10 production. The responsiveness of TNF-α to LPS treatment, as well as the inhibitory effects of CO, appeared downregulated in MKK3$^{-/-}$ mice compared with wild-type mice, suggesting that the MKK3/p38 MAPK pathway plays a major role in CO-mediated antiinflammatory signaling (88).

Downstream of p38 MAPK, heat-shock proteins appear to play an important contributory role in the antiinflammatory effects of CO. Kim *et al*. (54) demonstrated *in vitro* that CO signaling depends on the p38 MAPK-dependent upregulation of heat-shock protein 70 (54). Antiinflammatory effects of CO, with respect to modulation of pro- or antiinflammatory cytokines, were diminished in heat-shock factor knockout mice, indicating a potential role for the heat-shock response in CO-dependent cytoprotection. Caveolin-1, a structural protein responsible for forming the caveolae within the cell membrane, potentially acts as a downstream mediator for CO effects. CO, by activating p38 MAPK, upregulated caveolin-1 in smooth muscle cells (53). Genetic depletion of caveolin-1 abolished the antiproliferative effects of CO in this cell type. We recently observed that caveolin-1 may also contribute to the antiinflammatory effects of CO in macrophages (X. Wang and A. M. K. Choi, unpublished data).

Anti-/prooxidative effects of CO in sepsis

Reactive oxygen species (ROS) play an important signaling role in inflammation and sepsis. Although CO itself is inert with respect to direct participation in redox reactions, CO application exerts apparent anti- or prooxidative effects *in vitro*, dependent on model system (Fig. 3). Whether CO inhibits ROS to limit the inflammatory response or whether CO requires ROS production to act as an antiinflammatory is unclear. We recently demonstrated that CO inhibits the intracellular trafficking of toll-like receptor-2, -4, -5, and -9 to lipid rafts, by an ROS-dependent mechanism. In this study, the inhibition of NADPH oxidase–dependent ROS production by CO was observed to be essential to limit the inflammatory response (82). The mechanism for this downregulation of membrane-dependent ROS production involved inhibition of NADPH oxidase activity, through either direct binding or inhibition of subunit synthesis and assembly. In a recent study, CO application to macrophages led to a brief burst of mitochondrial ROS production, which was essential for antiinflammatory signaling, leading to the expression of peroxisome proliferator–activated receptor-gamma (PPARγ) (10). Both these potential mechanisms are supported by a recent study in smooth muscle cells showing that CORM increased mitochondrial oxidant production, but at the same time inhibited cytosolic ROS production by inhibiting NADPH oxidase activity at plasma membrane (126).

Anti-apoptotic effects of CO

In sepsis, the systemic and local inflammation leads regularly to apoptosis in various tissues. CO has previously been shown to confer protection against apoptosis in several *in vitro* models (Fig. 4). Exogenous CO inhibited TNF-α–initiated apoptosis in mouse fibroblasts (96) and endothelial cells (12). This protection against apoptosis involved p38 MAPK and NF-κB activation in endothelial cells (11). Further studies also implicated the p38 MAPK–dependent upregulation of heat-shock factor-1, and heat-shock protein-70 expression in the cytoprotective effect of CO (54). In contrast, in the case of Fas ligand (FasL)-induced apoptosis, CO promoted, rather than inhibited apoptosis in Jurkat T cells. This was associated with the downstream activation of caspases and the inhibition of antiapoptotic Bcl family members (*i.e*., Bcl-2). In this cell type, the proapoptotic effect of CO was associated with downregulation of ERK1/2 activation (121).

NO-CO interactions

Nitric oxide (NO) and CO share similarities in signal-transduction targets and cellular effects. Like NO, CO activates soluble guanylate cyclase (sGC), stimulating the production of cyclic 3′:5′-guanosine monophosphate (cGMP), a second messenger (32, 75). Both monoxides can be produced by constitutively expressed enzymes (*i.e*., CO is synthesized by HO-2, and NO is synthesized by endothelial nitric oxide synthase, eNOS), and furthermore, their production can be increased through the upregulation of inducible enzymes [*i.e*., HO-1 and inducible ni-

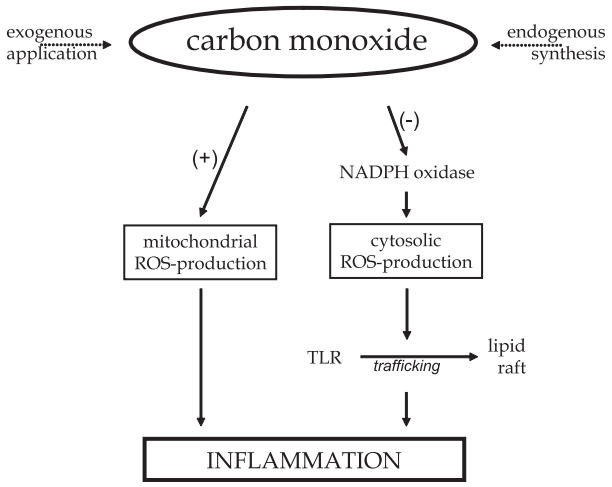

FIG. 3. Effects of carbon monoxide on ROS production. Both the increase of ROS in the mitochondria as well as the inhibition of (NADPH oxidase)-dependent ROS production potentially mediate antiinflammatory properties initiated by CO.

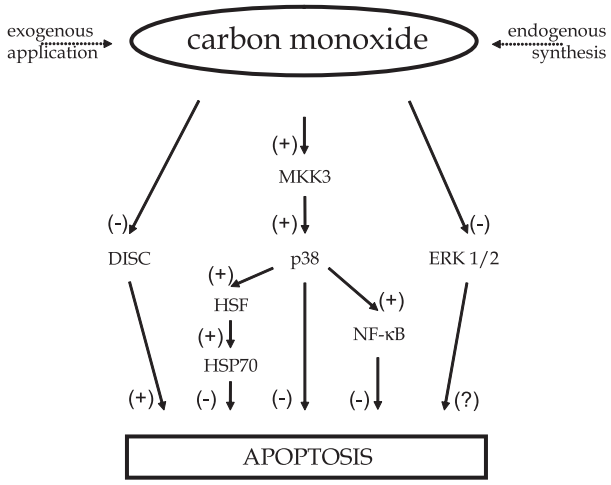

FIG. 4. Carbon monoxide–mediated signal-transduction pathways interfering with apoptosis. The antiapoptotic effects of CO, in which p38 MAPK appears to be of central importance. p38 MAPK regulated the heat-shock response as well as NF-κB to inhibit apoptosis.

tric oxide synthase, iNOS, respectively]. Furthermore, both NO/NOS and CO/HO systems may interact by several mechanisms (46, 61). For instance, NO induces HO-1 gene regulation in a variety of tissues and cell lines, which results in increased CO formation (29). This action of NO appears to be independent of the sGC/cGMP pathway, and most likely results from increased production of ROS (29). Conversely, NOS is a cytochrome p450-type hemoprotein and as such is susceptible to inhibition by CO. Application of CO has been shown to inhibit NOS activity and subsequently to reduce NO production. In addition, CO may interfere with NO binding to guanylate cyclase by competitive inhibition. HO may regulate NOS synthesis through its function in the control of intracellular heme levels. As part of the normal protein turnover, the heme derived from NOS likely constitutes a substrate for heme oxygenase activity (29).

The potential relation between NO and CO in protection against sepsis was evaluated in a model of acute liver failure. Exposure of hepatocytes *in vitro* to CO resulted in a rapid upregulation of iNOS followed by subsequent induction of HO-1. The protective effect of CO against TNF-α–induced liver injury *in vitro* and *in vivo* required induction of iNOS and NO production, whereas the protective effect of NOS-derived NO required HO-1 activation. As a consequence, the increased HO-derived CO was proposed to drive an amplification cycle, leading to further iNOS activation. Loss of iNOS/NO function as in *inos*$^{-/-}$ mice, or wild-type mice treated with iNOS inhibitor L-Nil resulted in a loss of CO-mediated protection against TNF-α–induced liver failure, which could be compensated by chemical induction of HO-1 with cobalt protoporphyrin (CoPPIX). In *ho-1*$^{-/-}$ mice, regardless of the presence of iNOS/NO, the protection afforded by CO against liver injury was lost. These experiments illustrate a co-dependence of NO and CO generating systems in mediating cytoprotection, as illustrated in a model of liver injury (144).

EFFECTS OF CO IN SEPTIC ORGANS

As outlined in the following sections, the effects of CO therapy have been examined *in vivo* in several organ-specific models of sepsis, including lung, heart, liver, kidney, and intestine. An overview of the findings is presented in Tables 3 and 4.

CO and the septic lung

CO synthesis has been described for almost all tissues investigated. In the lung, CO is produced by alveolar macrophages, pneumocytes, endothelial cells, and fibroblasts (24, 59). Human studies showed increased CO-Hb concentrations in arterial versus venous blood, proving the CO synthesis under physiologic conditions in humans. Elevations in endogenous CO synthesis have been observed in lung disease. For instance, patients with hepatopulmonary syndrome or chronic obstructive lung disease increase their endogenous CO production, as demonstrated by elevated CO-Hb levels (6, 139).

Based on these findings, and because 80% of endogenously produced CO is exhaled in the lung, some investigators have proposed to use exhaled CO as a disease marker. Measurements of exhaled CO demonstrated increases in patients with asthma, cystic fibrosis, chronic obstructive pulmonary disease, or in critically ill patients (5, 44, 94, 130) that correlate with the progression of the disease and also respond to successful treatment (138). However, the utility of exhaled CO as a diagnostic parameter may be limited. Endogenous CO synthesis is induced by many inflammatory or oxidative reactions and, therefore, CO can certainly not be designated as a specific marker for a specific disease. Second, the variances of exhaled CO among patients with the same disease are still very high and are complicated by smoking behavior, which introduces CO (14, 51, 60, 140).

Exogenous application of CO by inhalation dilates bronchioles, inhibits the release of pro-inflammatory cytokines, reduces the consecutive lung edema, and diminishes the proliferation of vascular smooth muscle cells in the lung (68, 69, 86, 122). Acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS) are frequent complications of septicemia. The pathophysiology of ALI/ARDS after sepsis or SIRS involves oxidative stress as well as pro-inflammatory processes. *In vivo* models of hyperoxia demonstrated that 100% oxygen application for several days is a lethal condition for animals (76). In rodent models of ALI/ARDS induced by hyperoxia, the degree of lung injury and mortality could be significantly decreased by simultaneous CO inhalation (89, 90). In a ventilator-induced lung-injury model, in which rats received high-tidal-volume ventilation after LPS priming, lung injury was diminished when animals inhaled 250 ppm CO through the ventilator circuit (23). In a sepsis model, inhaled CO prevented lung edema induced by endotoxic shock (68). In terms of lung physiology, CO pretreatment corrected the LPS-induced changes in resistance and compliance of the lung and improved the derangement in pulmonary gas exchange in pigs after LPS challenge (69).

CO and the septic heart

The heart plays a pivotal role in sepsis. Because of a decreased total vascular resistance, cardiac output increases at an

Table 3. Effect of Carbon Monoxide (CO) Treatment in the Lung and in the Heart

Organ	*Model*	*Species*	*CO dose (ppm)*	*Effect*	*Year*	*Ref.*
Lung	Acute lung injury, hyperoxia induced	Mouse	250	Lung injury ↓ Survival ↑	2003	90
	Acute lung injury, hyperoxia induced	Rat	50–500	No effect	2001	17
	Acute lung injury, hyperoxia induced	Rat	250	Lung injury ↓ Inflammatory response ↓	1999	89
	Bronchoconstriction	Mouse	250–10,000	Bronchodilatation	2003	3
	Ischemia/reperfusion	Mouse	500	Apoptosis ↓	2003	
	Ischemia/reperfusion	Mouse	500	Apoptosis ↓	2003	
	Ischemia/reperfusion	Mouse	1,000	Fibrin accumulation ↓ Survival ↑	2001	31
	Pneumonia, endotoxin-induced	Pig	250	Compliance ↑ Extravascular lung water ↓	2004	68
	Lung transplantation	Rat	500	Inflammation ↓ Apoptosis ↓ Organ function ↑ Survival ↑	2003	120
Heart	CABG	Pig	250	Edema ↓, Apoptosis of myocytes ↓	2004	56
	Heart transplantation	Rat	20 (rec)	No effect	2005	84
	Heart transplantation	Rat	20 (rec) +/BV, i.v. (don + rec)	Organ function ↑ Survival ↑	2995	84
	Heart transplantation	Rat	400 (don + org)	Apoptosis ↑ Time of ischemia ↑ Organ function ↑	2004	2
	Heart transplantation	Rat	250–400 (don + rec)	Neutrophil infiltration ↓ Thrombosis ↓ Infarction ↓	2001	111
	Myocardial infarction	Rat	500	Infarction area ↑	2005	71
	Myocardial infarction	Mouse	CORM, i.v.	Infarction area ↓	2005	123
	Myocardial infarction	Rat	250–500	No effect	2004	30
	Myocardial infarction	Rat	1,000	Infarction area ↓	2004	30

CO application was to the organ donor (don), to the organ recipient (rec), or to the organ itself (org).

early stage of sepsis (hyperdynamic state). This subsequently changes to a hypodynamic state in which increased vascular resistance, reduced microvascular flow, and decreased cardiac output are hallmarks (58). The cardiac dysfunction during sepsis is characterized by biventricular dilation, a decreased ejection fraction, and a decreased response to fluid resuscitation. The myocardial depression typically persists in septic shock patients until death or recovery (20). The exact mechanism of septic cardiomyopathy remains elusive. The postulated causative mechanisms of myocardial dysfunction include myocardial ischemia, myocardial edema, and release of myocardial depressant substances (64). In the heart, CO synthesis seems to be locally regulated. In right-heart failure, for instance, CO synthesis has been observed in the right but not in the left ventricle (101). In patients with coronary heart disease, an increased production of CO in peripheral monocytes was observed. This increase correlated with the severity of the disease state (15). In this regard, McFaul and McGrath (70) provided evidence that CO increases coronary blood flow. Recently, Stein *et al.* (123) demonstrated that application of CORMs reduced the myocardial infarction area after transient occlusion of the coronary vessels in mice (123). In this model, the protective effect of inhaled CO was less remarkable than intravenous injection of CORMs (30). These findings might render CO a protective substance increasing coronary blood flow and reducing myocardial ischemia. Whereas in the adult septic patient, myocardial dysfunction does not appear to be due to myocardial ischemia (20, 55), septic children who are tachycardic have an increased demand of oxygen, and myocardial perfusion is compromised by tachycardia (64).

As reviewed elsewhere, the systemic release of myocardial suppressant substances seems to contribute to septic cardiomyopathy (20, 58). Here, complement activation, induced NO synthesis, and the release of pro-inflammatory cytokines play a critical role. TNF-α, IL-1β, IL-2, and IL-6 have been implicated especially as myocardial suppressant substances responsible for myocardial dysfunction (39). As mentioned earlier, exogenous application of CO diminishes the release of pro-inflammatory cytokines in various sepsis models. Therefore, it would be not surprising if CO were to protect the heart in this context.

Regarding CO as potential therapeutic antiinflammatory sub-

Table 4. Effect of Carbon Monoxide (CO) Treatment in the Liver, Kidney, and Gut

Organ	*Model*	*Species*	*CO dose (ppm)*	*Effect*	*Year*	*Ref.*
Liver	Acute liver failure	Mouse	250	Liver cell injury ↓	2004	109
	Acute liver failure	Mouse	250+ BV, i.v.	Liver cell injury ↓ Survival ↑	2004	109
	Acute liver failure	Mouse	250	Apoptosis ↓ Liver function ↑	2003	144
	Liver transplantation	Rat	100 (rec)	Inflammation ↓ Liver cell injury ↓	2005	48
	Liver transplantation	Rat	MC, p.o. (rec)	Apoptosis ↓ Organ function ↑ Survival ↑	2002	49
Kidney	Endotoxemia, LPS-induced	Pig	250	Susceptibility to ARF ↓ Serum creatinine ↓	2005	69
	Ischemic acute renal failure	Rat	1,000	Tissue injury ↓ Apoptosis ↓ Serum creatinine and urea ↓	2006	9
	Ischemia/reperfusion	Mouse	CORM, i.v.	Renal function ↑	2005	132
	Kidney transplantation	Rat	MC, p.o. (rec)	Arteriosclerosis ↓ Fibrosis ↓ Glomerulosclerosis ↓ Inflammation ↓	2005	66
	Kidney transplantation	Rat	20 (rec)	No effect	2005	84
	Kidney transplantation	Rat	20 (rec) +BV, i.v. (don + rec)	Organ function ↑ Survival ↑	2005	84
	Kidney transplantation	Rat	250 (rec)	Apoptosis ↓ Inflammation ↓ Organ function ↑ Survival ↑	2004	85
Gut	Colitis	Mouse	250	No effect	2005	8
	Colitis, chronic	Mouse	250	Inflammation ↓	2005	38
	Necrotizing enterocolitis	Rat	250	Inflammation ↓	2005	146
	Postoperative ileus	Rat	75	Intestinal motility ↑	2005	73
	Postoperative ileus	Pig	250	Intestinal motility ↑	2005	73
	Postoperative ileus	Mouse	250	Inflammation ↓ Intestinal motility ↑	2003	72

CO application to the organ donor (don) or recipient (rec). Biliverdin application was intravenous (BV, i.v.). Methylene chloride application *per os* (MC, p.o.).

stance in sepsis, it must be considered that CO also potentially acts as a vasodilator. Although not as potent as NO, CO can cause regional and systemic vasodilatation. Hypothetically, during a hyperdynamic stage, exogenous CO could potentially aggravate peripheral vasoplegia (102). Conversely, CO reduces hypoxic pulmonary vasoconstriction (81, 131), and if administered over the long term, even pulmonary hypertension (25). Therefore, when sepsis leads to ALI/ARDS with increased pulmonary artery pressure, CO might possibly reduce the afterload for the right heart. However, no study confirms this hypothesis, and experiments indicating whether CO protects the septic heart are lacking.

CO and the septic liver

The liver plays a tremendous role in the removal of endotoxins, bacteria, neutrophils, and cytokines from the systemic circulation. Liver injury and consequently liver failure in sepsis is based on its inflammatory response and on a disturbed micro- and macrocirculation, especially in the later, hypodynamic state of sepsis (22, 125).

CO is a major factor maintaining regional perfusion in the liver. This is especially true under pathologic circumstances (93). Application of CO increases hepatic perfusion after ischemia/reperfusion, portal hypertension, and sepsis (27, 92, 124). As a result, low-dose CO reduces cellular hypoxia (4, 145). In murine sepsis models, inhaled CO diminished liver cell injury (109, 110, 144) and increased survival when combined with biliverdin, another byproduct of HO metabolism (109). These findings were confirmed by a study in which liver dysfunction in pigs was ameliorated by inhalation of CO (69). In that context, CO seems to protect liver integrity at an early stage of sepsis. The liver dysfunction in a SIRS model after limb ischemia/reperfusion, for instance, could be prevented by CO inhalation (87, 137). Hepatic protection in sepsis models was accompanied by decreased apoptosis. In contrast to the lung, in

the liver, CO augmented iNOS induction in response to LPS challenge (108, 110). Thus, the relative role of iNOS in CO-mediated cytoprotection remains unclear and appears to play tissue-specific roles. It is still not clear whether the antiinflammatory, antiapoptotic, vasodilative effect, or a combination of all three properties of CO, is responsible for the observed hepatic protection during septic models.

CO and the septic kidney

Sepsis is often accompanied by acute renal failure (ARF), such that patients with the combination of the two diseases have a higher mortality rate than those with sepsis only. The pathologic changes observed in sepsis are identical to prerenal ARF or postischemic acute tubular necrosis (ATN) or both, seen in other forms of injury to the kidney. The major pathologic features of ARF include endothelial and tubular cell injury, intratubular obstruction, and enhanced inflammatory processes. Evidence suggests that multiple factors contribute to the acute deterioration of renal function, which includes both systemic and organ-specific effects. Systemic hypotension and compensatory renal vasoconstriction results in hypoxic/ischemic injury, which is mostly observed in the proximal tubule cells and the outer portion of the medulla. Because of the relative hypoxia occurring here, proximal tubule cells are more susceptible to a further decrease of blood-oxygen content, as seen in sepsis. Conversely, neutrophil activation and direct endotoxin injury to the kidney also have a major, but incompletely understood role in the development of ARF (104). Endotoxin injury may further perpetuate renal vasoconstriction by activating the renin–angiotensin–aldosterone system and endothelin-1 (ET-1), by inducing iNOS, and by stimulating the release of cytokines (*e.g*., TNF-α, IL-1) and chemokines.

Furthermore, the enhanced production of ROS, and the activation of neutrophils by endotoxin and bacterial chemotactic peptides, may also lead to renal injury. Therapeutic interventions have failed in the past to ameliorate kidney function, and thus ARF is a major contributor to sepsis-related mortality (114).

The effects of increased HO-1 and associated production of CO have been investigated in endotoxemia and hypoxic/ischemic injury models of ARF. Studies by Agrawal (1) and Haugen (36) demonstrated increased HO-1 protein expression in rodent models of ARF. The vasodilatory role of CO also was noted by Johnson (47). Shimizu *et al*. (117) found increased tubular necrosis in animals treated with an HO-1 inhibitor (tin-mesoporphyrin) in a rat model of renal ischemia. By using HO-1 knockout mice, Wiesel *et al*. (135) observed enhanced renovascular hypertension and ARF after ischemic injury to the kidney. They suggested that in their model, the lack of HO-1 and HO-derived CO resulted in increased ET-1 levels and inflammation after injury. In contrast, in an endotoxin-induced model of ARF, HO-inhibitor (zinc protoporphyrin)-treated animals responded with improved renal function and hemodynamics when compared with vehicle-treated mice (97). These findings emphasize the complex effect of CO on hemodynamics. A high concentration of CO can inhibit NO effects via blocking NOS, whereas low doses can induce NO release and lead to vasodilation. Another possible interpretation, given by the authors, is that the robust vasodilatory effects masked the antiinflammatory effects of CO.

Direct protective effects of inhaled (250 ppm) CO were observed after ischemia/reperfusion injury in transplanted kidneys (85). Kidney grafts showed improved vascularization, blood flow, and prolonged survival if recipient rats received CO for 1 h before and for 24 h after surgery. Direct and indirect markers of inflammation, including neutrophil and macrophage cell counts, IL-6, TNF-α, and IL-1β levels, also were reduced in these animals. Injury scoring for ATN showed a marked decrease along with a reduction in the number of apoptotic tubular cells. The authors pointed out that CO was incapable of completely preventing ischemic kidney injury and only ameliorated glomerular filtration and animal survival. In a pig model of endotoxemia, CO pretreatment prevented the acute elevation of serum creatinine levels after LPS injection (69). Recently, Berhardt *et al*. (9) used 1,000-ppm CO for 10 h to pretreat rats before hypoxic/ischemic renal injury. In this model, CO treatment led to ameliorated renal function and reduced tissue damage when compared with air-treated controls.

In conclusion, ARF often complicates sepsis, and the treatment options are very limited. Increased HO-1 levels were detected in animal models of ARF. HO-1-derived CO and exogenous CO treatment reduces inflammation, prevents tubular necrosis, and prolongs survival after injury to the kidney. The effect of CO on renal hemodynamics remains controversial.

CO and the septic gut

In the pathology of sepsis, the gastrointestinal system can have at least a dual role, by either initiating systemic inflammatory processes or perpetuating the existing sepsis, leading to multiple organ failure (MOF). Intestinal hypoperfusion and reperfusion plays a central role in either scenario. Hypoperfusion depresses the gut barrier function, leading to bacterial and endotoxin translocation into the circulation *via* the lymphatic vessels. Conversely, reperfusion of the intestines results in the release of proinflammatory cytokines, chemokines, and the expression of adhesion molecules on vascular cell surfaces. Neutrophil recruitment to the intestine results in inflammation. Resident and recruited leukocytes represent a major source of iNOS and cycloxygenase-2 (COX-2) activities. NO and prostaglandins exhibit inhibitory effects on bowel mobility, resulting in ileus. The hypoperfused gut is also prone to ileus in the stomach and the small bowel, which is a source of toxins and bacteria to cause a late sepsis-related MOF. MOF will eventually worsen circulation and result in further deterioration of bowel function. Finally, in the critically ill with sepsis, when intubated and lacking food intake, bacterial overgrowth in the upper gastrointestinal system can cause aspiration, and consequent nosocomial pneumonia (35).

In the cellular mechanism of sepsis, disarranged apoptosis was described to have an important role, especially in the intestinal system, in which increased apoptosis is observed in the gut epithelium (45).

One of the common clinical aspects of gastrointestinal involvement in the pathogenesis of sepsis is small bowel dysmotility. This is a major complication after abdominal surgery, which may lead to ileus, enhanced regional inflammation, and if not treated, contribute to MOF. Antiinflammatory therapy is limited at present to prevent such complications. Experiments with small animals showed increased HO-1 and IL-10 expression after small intestinal manipulation (72). Interestingly, when

control animals (no surgical procedure) were treated with 250-ppm CO, the enhanced expression of HO-1 and IL-10 was detected. If CO treatment preceded intestinal manipulation, proinflammatory cytokine levels, as well as iNOS and COX-2 gene expression were reduced in the *muscularis externa*. Functional studies showed a significant improvement in intestinal transit in CO-pretreated animals. The authors concluded that IL-10 and HO-1 may act in concert to downregulate proinflammatory mediators. Their early induction with pretreatment could contribute to the diminished inflammatory response that was observed after surgery. In a different model, after small intestinal transplantation in rats, CO treatment also resulted in decreased inflammation and increased motility in the bowel graft (83). Probably the most striking finding of this study is that spontaneous jejunal smooth muscle contractility, which is decreased in the graft after transplant, could be preserved if the recipient animals received CO treatment before and after surgery. Recently, the same laboratories tested the effect of inhaled CO in a rat and a porcine postoperative ileus model (73). The rat model showed similar positive effects on gastrointestinal transit time with 75 ppm as seen earlier with 250 ppm CO. Lower doses were not beneficial. Pretreatment time was extended to 3 h and proved to be more effective than 1-h pretreatment. CO treatment after surgery did not modify the study outcome. Results from the small-animal study were confirmed in the porcine experiments. Animals that received a 3-h pretreatment of 250-ppm CO before surgery had improved gastrointestinal motility, less small bowel dilation, and less neutrophil recruitment to the intestine than did their air-treated peers. Pigs have intestinal systems similar to those of humans, and this is one of the first studies in which surgery, followed by postoperative care, including opioid analgesia, mimicked settings applied in hospitals for humans. Another interesting aspect of CO therapy is its effect on mortality. This was assessed in a model of necrotizing enterocolitis (NEC). Experimental NEC was induced with formula feeding and hypoxia in neonatal rats. Histology results show that 1-h 250-ppm CO treatment protected animals from developing morphologic features of NEC, such as pneumatosis interstitialis and necrosis in the terminal ileum. Mortality on the fourth day of life dropped from 40% to 21.4% when air-treated and CO-treated animals were compared (146). Hegazzi and colleagues (38) described CO effects in Th1-cell–mediated inflammatory bowel disease in a mouse model. After injury, 4 weeks of CO inhalation significantly ameliorated histology outcomes, suggesting that CO also affects IFN-γ signaling in macrophages (38). Besides antiinflammatory and antiapoptotic effects in the gut, CO could contribute to the voltage gradient in the smooth muscle layer of the gastrointestinal system (26). The gradient in resting membrane potential is responsible for regulating how much of the thickness of the smooth muscle layer is contracted during the propulsive movements. Farrugia and co-workers (26) showed that the loss of HO-2 enzyme leads to the loss of gradient, membrane depolarization, and sequential contraction in the entire muscular layer. In the intestinal system, smooth muscle cells do not immunostain for HO-2. However, interstitial cells of Cajal contain large amounts of HO-2 and subsequently produce CO. These cells are specialized mesenchymal cells that generate electric signals and modulate neurotransmission for smooth muscle contraction. The authors suggested that the other role of these cells is to produce CO, to maintain a hyperpolarized cellular membrane, and to help set the interstitial smooth membrane potential gradient.

The gastrointestinal system has a key role in inciting and perpetuating sepsis. Intestinal dysmotility and consequent inflammatory mediator spread to the circulation are hallmarks of the pathologic process. In animal models, CO treatment has been shown to ameliorate both aspects. Promising porcine experiments are a step forward to human application; however, the necessity to pretreat patients with CO may limit its application to scheduled interventions.

PERSPECTIVE

In an initial attempt to translate the antiinflammatory effects of CO observed in rodents to humans, a preclinical trial was recently performed. Humans were exposed to 500-ppm CO for 1 h by inhalation, a dose that increased CO-Hb levels to 7%, followed by LPS injection. As a result from LPS challenge, IL-1α, IL-1β, IL-6, and IL-8 were reported to be transiently increased in these subjects. In this study, CO inhalation did not affect the cytokine response to endotoxin treatment in humans (67). However, given the protective characteristics of CO application in the vast majority of sepsis models so far, it becomes essential to elucidate further the potential of CO for reducing inflammation in septic patients. The antiinflammatory, antiapoptotic, antioxidative (or a combination of these) properties, renders CO an interesting tool for potentially treating sepsis. The authors are aware of at least five ongoing clinical trials with inhaled CO. It will be very interesting to see the results in a few years.

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ABBREVIATIONS

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ARF, acute renal failure; ATN, acute tubular necrosis; CO, carbon monoxide; CO-Hb, carboxyhemoglobin; CORM, carbon monoxide–releasing molecule; ERK1/2, extracellular regulated kinase-1/2; ET-1, endothelin-1; GM-CSF, granulocyte–macrophage colony-stimulating factor; Hb, hemoglobin; HO-1/2, heme oxygenase-1/-2; IL, interleukin; IFN-γ interferon-gamma; JNK, c-Jun NH_2-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MODS, multiple organ dysfunction syndrome; MOF, multiple organ failure; NEC, necrotizing enterocolitis; NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; p38 MAPK, p38 mito-

gen-activated protein kinase; ppm, parts per million; SIRS, systemic inflammatory response syndrome; TNF-α tumor necrosis factor alpha.

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