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Carbon monoxide releasing molecules: New insights for anticoagulation strategy in sepsis

B.W. Sun* **and X. Chen**

Department of Burn and Plastic Surgery, Affiliated Hospital, Jiangsu University, Zhenjiang 212001, Jiangsu Province (China), Fax: 0511-85029089, e-mail: sunbinwe@hotmail.com

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Abstract. Sepsis is a common and serious medical condition caused by hemorrhage, trauma, or abdominal surgery. Despite new understanding and much progress in therapies that specifically interfere with an interesting target, sepsis remains the leading causes of death in critically ill patients. Various therapies have been studied, but the majority of these treatments fail in clinical trials. It is clear that all septic patients exhibit coagulation abnormalities. These abnormalities range from subtle to marked activation of coagulation system, and finally to fulminant DIC. Studies confirmed that carbon monoxide has important cytoprotective function and anti-inflammatory properties. Until now, the question of whether CO plays a critical role in improving the coagulation system and then decreasing mortality during sepsis has not yet been definitely answered. Attempts to confirm this strategy may lead to new directions in the study of treatment of sepsis and the development of a novel agent for this disorder.

Keywords. Carbon monoxide, sepsis, inflammatory response, coagulation, strategy.

Introduction

Sepsis is a common and serious medical condition caused by a severe systemic infection leading to a systemic inflammatory response, which frequently occurs after hemorrhage, trauma, burn, or abdominal surgery. It is a leading cause of morbidity and mortality in critically ill patients. Crude mortality rates of 45 % are constant and translate to approximately 90 000 deaths each year in the United States [1]. It has been shown that the increase of vascular permeability and the promotion of tissue edema by vasorelaxation may lead to the hemodynamic changes during sepsis. A variety of cytokines are released into the microcirculation by neutrophils, endothelial cells, and monocytes during phases of hypoxia and reperfusion [2, 3]. In addition, in about 30–70 % of patients with sepsis, disseminated intravascular coagulation (DIC) occurs. Therefore, it should be important to address the question whether anticoagulant therapy produces any beneficial effects in patients with sepsis [4]. Actually, various therapies including anti-tumor necrosis factor (TNF) therapies, antibodies against endotoxin, and anti-interleukin (IL)-1 have been studied; however, the majority of these treatments failed in clinical trials [5]. Many agents/approaches still only improve septic survival by 10 % [6], and their modes of action are poorly understood. Another limitation of anti-inflammatory agent studies is that much of the preclinical data was often based on lethal bacterial-toxin-based studies, mono-specific intravenous (i. v.) microbial challenge, or pretreatment ap-

* Corresponding author.

proaches, which did not replicate the septic patients' status adequately [5].

Thus, it is urgent to identify new therapeutic tools for the treatment of sepsis.

Normal hemostasis exists as a finely tuned balance where the blood typically remains liquid to allow free flow within the vessels yet clots appropriately to control bleeding. During inflammatory situations such as SIRS (systemic inflammatory response syndrome) or sepsis, however, significant alterations occur at multiple levels within both the coagulation system and the cells that regulate this system, such as changes in coagulability, endothelial cell injury, and abnormal blood flow [7]. In septic patients, all three of the above classic alterations are present and culminate in reduced blood flow to vital organs, resulting in tissue hypoxia and, ultimately, in the development of organ failure. Therefore, new therapies targeting abnormalities in the coagulation system resulting from systemic illnesses are necessary, even urgent.

CO, commonly viewed as a silent killer, is a colorless, tasteless, and odorless gas. CO avidly binds to hemoglobin and forms carboxy hemoglobin (COHb) with an affinity 240 times higher than that of oxygen, possibly causing an interference with the oxygen carrying capacity of the blood and consequent tissue hypoxia. Increasing levels of COHb can result in a wide range of symptoms from mild cognitive impairment including reduction in visual perception and driving performance to more severe effects like headache, weakness, gastrointestinal symptoms, and finally progressive confusion, collapse, and coma [8]. On the other hand, small amounts of CO are continuously produced in mammals, and the intracellular levels of this gaseous molecule can markedly increase under stressful conditions following induction of HO-1 (haem oxygenase-1), a ubiquitous enzyme responsible for the catabolism of haem. Activation of the HO-1 pathway is part of a complex homoeostatic adaptation of cells to the redox imbalance inflicted by stressful stimuli. Although the toxicity of CO has been extensively studied, it is now also being explored for its physiological effects and potential therapeutic benefits. Since the realization that the poisonous gas, nitric oxide (NO), has a significant biological role in physiology and pathophysiology, CO, which is a structurally similar gas, has gained significant attention as a molecule with many analogous chemical and biological properties [9]. Abundant research has suggested that this endogenous CO, a by-product of HO-1, can modulate inflammation. Indeed, CO is an important signalling mediator possessing vasodilatory properties, which are achieved by activation of the guanylate cyclase–cGMP pathway as well as large-conductance potassium channels. In addition, some experiments have determined that the administration of exogenous CO inhibits lipopolysaccharide(LPS)-induced production of cytokines both *in vivo* and *in vitro*, and consequently exhibits important cytoprotective function and anti-inflammatory properties that are beneficial for the resolution of acute inflammation [10, 11]. Since CO is a gaseous molecule, CO inhalation at a low concentration would be a straight forward delivery method to utilize CO as a therapeutic tool. CO inhalation therapy could be a clinical strategy because of its simple method of application. However, as CO at high concentrations is known to be toxic, the secure and optimal delivery of gaseous CO needs to be carefully conducted. Adjustment of inhaled CO dose by blood COHb monitoring is complicated, even difficult. Recently, transitional metal carbonyls have been identified as potential CO-releasing molecules (CORMs) with the potential to facilitate the pharmaceutical use of CO by delivering it to tissues and organs [12, 13], and could also be helpful to avoid problems of CO-poisoning. CORM-1 and CORM-2 are soluble in organic solvents such as DMSO. The first water-soluble ruthenium-based carbonyl, CORM-3, was synthesized by co-ordination of the amino acid glycine to the metal centre [Ru(CO)3Cl-glycinato]. CORM-3 is a relatively stable compound in water but promptly releases CO in the presence of myoglobin [half-life ($t^1/_2$) $\leq$ 1 min]. More recently a boron-based CO-RM (CORM-A1) which does not contain a transition metal has been identified, releasing CO at a slower rate [half-life ($t^1/_2$) = 21 min] compared with transition metal-containing CO-RMs [14]. CORMs have been shown to act pharmacologically in rat aortic and cardiac tissue where liberation of CO produced vasorelaxant effects [15, 16] and decreased myocardial ischemia/reperfusion damage, respectively. Our previous studies [17, 18] have also shown that CORM-2, one of the novel CORMs, inhibited overexpression of adhesion molecules (such as intercellular adhesion molecule-1, ICAM-1, vascular adhesion molecule-1, VCAM-1), attenuated leukocytes sequestration in organs of cecal ligation and puncture (CLP)- or burn-induced mice by interfering with nuclear factor kappa B (NF-κB) activation.

It is clear that strategies aimed at amplifying the action of CO could lead to the development of pharmacological and therapeutic approaches in sepsis. This short review will briefly focus on anti-inflammatory and cytoprotective actions of CORMs that have been discovered so far, and discuss whether CO, endogenous or exogenous, plays a critical role in improving the coagulation system and then decreasing mortality during sepsis.

Effects of CORMs on decrease of intracellular oxidative stress and NO production in sepsis

Based on current research findings, oxidative stress is believed to be the major cause of organ damage. Nitrosative stress initiates an inflammatory cascade that includes acute phase protein synthesis, upregulation of inflammatory adhesion molecules, and proinflammatory cytokine release [19, 20]. Severe injury (e.g. thermal injury) is associated with lipid peroxidation mediated by reactive oxygen species (ROS) and nitric oxide (NO), and is believed to be an important cause of oxidative damage to cellular membranes and, eventually, cell death [21]. In our studies, we found that the protein expression of inducible nitric oxide synthase (iNOS) and the overabundance of NO were significantly decreased in thermal injury-induced septic mice after *in vivo* administration of CORM-2. In addition, both the production of ROS and NO were markedly decreased in LPS-stimulated human umbilical vein endothelial cell (HUVEC) with co-incubation of CORM-2. In addition, findings in recent years strongly suggest that the stress-inducible gene heme oxygenase (HO)-1 plays an important role in protection against oxidative stress. Our laboratory [22] and others [23, 24] have shown that induction of HO-1 provides protection both *in vivo* and *in vitro* against oxidative stress. We found that HO-1 is significantly up-regulated in HUVECs by LPS stimulation. Of interest is that the expression of HO-1 in LPS-stimulated HUVECs, both with preconditioning and coincubation of CORM-2, increased more significantly as compared to the LPS group. This result indicated that not only might LPS significantly induce the expression of HO-1, but also that an increase in HO-1 expression can be further enhanced by the administration of CORM-2. Therefore, CORM-2 ultimately leads to potent cytoprotection and inhibition of oxidative stress during sepsis [22].

Effects of CORMs on downregulation of cytokine levels in sepsis

The increase in production of pro-inflammatory mediators such as TNF-α and IL-1β in sepsis is closely associated with activation of leukocytes and macrophages which were sequestrated in the tissue [25]. There is evidence that CLP or thermal injury significantly upregulated cytokine levels (TNF-α and IL-1β) in serum, tissue homogenates and bronchoalveolar lavage (BAL). Similarly, elevated plasma concentrations of a variety of cytokines and chemokines such as TNF-α, IL-1, and IL-6 have also been described in septic patients [26]. There are well described conclusions that, through enhancing the expression of HO-1, the low cytokine levels could result from any direct or indirect action of endogenous CO that improves the pathophysiology of sepsis [27]. In parallel, in our mouse model, we have demonstrated that after application of CORM-2 as a exogenous CO, the elevation of cytokines in serum, tissue homogenates and BAL was effectively abolished, suggesting that an alternative possibility is that the anti-inflammatory effects of CORM-2 are due to its potential inhibition of the production and secretion of cytokines, especially TNF-α. [17, 18, 28, 29].

Effects of CORMs on inhibition of adhesion molecules expression and nuclear factor-κB activation in sepsis

The direct cause of leukocyte sequestration in sepsis is considered to be the increased expression of adhesion molecules. ICAM-1 activates leukocytes and endothelial cells (ECs), which in turn prompt the release of various inflammatory mediators, resulting in systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), which may develop further into progressive multiorgan failure (MOF) and death [30, 31]. NF-κB system, one of the major signaling pathways, is responsible for the expression of adhesion molecules in response to cytokines or LPS during sepsis [32]. Recent studies supported that CORM-2 administration in CLP-induced septic mice had protective effects on organ functions by inhibiting the expression of ICAM-1 and VCAM-1 [17, 18]. In parallel, under the intravital microscopy, the number of stationary leukocytes and adhesion leukocytes within both liver sinusoids and postsinusoidal venules was significantly reduced after *in vivo* application of CORM-2 in septic animals. Particularly, our studies have documented that CORM-2 plays an important role in inhibition of NF-κB activity [17, 18]. These findings here strongly indicated that CORM-2 appears to modulate inflammation by interfering with NF-κB activation, suppressing endothelial cell pro-adhesive phenotype and therefore attenuating leukocytes sequestration in organs of septic animal.

Current strategies targeting the coagulation system in sepsis

During inflammatory situations such as sepsis, significant alterations occur at multiple levels within both the coagulation system and the cells that regulate this

system [33]. Virtually all septic patients exhibit coagulation abnormalities. These abnormalities range from subtle activation of coagulation, which can only be detected by sensitive markers of coagulation factor activation, to more marked activation, which may be detectable based on a small decrease in platelet count and subclinical prolongation of global clotting times – and finally to fulminant DIC, which is characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites [34]. During the pathogenesis of sepsis, alterations in expression of coagulation-involved factors (e.g. tissue factor, TF) occur [35]. It has been observed that the use of glycoprotein IIb/IIa inhibitor can attenuate endotoxin-induced monocyte TF expression, leading to a marked reduction in endothelial injury, increased endothelium-dependent relaxations, and improved survival rates in the treated animals [36].

The successful clinical trials with activated protein C (APC) for the treatment of sepsis were initiated following studies in the baboon model of *Escherichia coli* sepsis [37]. Approval of APC for the treatment of septic patients clearly demonstrates that alterations in the coagulation system are important in sepsis mortality [38]. However, analysis of related data showed that the most beneficial effects were observed in patients with the worst prognosis, who even had a significantly increased risk of bleeding if treated with APC [39]. Up to now, the question of whether CO, endogenous or exogenous, plays a critical role in improving the coagulation system and then decreasing mortality during sepsis has not yet been definitively answered.

Carbon monoxide releasing molecules: new insights for anticoagulation strategy in sepsis

Taken together, evidences mentioned above have led us to understand that CORMs, the novel exogenous CO releasing molecules, play an important role in inhibiting leukocyte sequestration and downregulating inflammatory responses both in *in vivo* or *in vitro* experimental models. However, no studies have assessed the effects of CORMs on its regulation in activation of the coagulation cascade, the procoagulant-fibrinolytic balance, and interactions between inflammation and coagulation in sepsis. With the understanding of CORMs mentioned above, we believe that it will be more noteworthy to confirm whether CORMs can regulate the coagulation system with the potential to facilitate the pharmaceutical use of CO by delivering it to tissues and organs during sepsis. Moreover, it would be of interest to gain insight into the molecular mechanisms involved in anticoagulant treatments, improvement in the hypercoagulable state, and subsequent prevention of the coagulation abnormalities that protect the host from organ failure or improve overall survival. Obviously, this strategy not only targets specific anticoagulation associated with CORMs, but also targets the interaction between inflammatory response and coagulation system, and will generate potentially relevant clinical and therapeutic implications for the regulation and treatment of dyscoagulation in sepsis.

In light of the personal and social burden of the treatment of sepsis, we think that further studies on exploring new therapies are necessary, even urgent. Research on the role of CORMs might allow advancements in this field. Studies assessing the regulation effects of CORMs on the procoagulant-fibrinolytic balance in sepsis as well as investigations into the potential molecular mechanisms involved in this new therapy are welcome. This may have important clinical and therapeutic implications, allowing a more specific management and, ultimately, a better result for patients with both sepsis and SIRS.

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