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The Social Network of Carbon Monoxide in Medicine

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Abstract

Networking between cells is critical for proper functioning of the cellular milieu and is mediated by cascades of highly regulated and overlapping signaling molecules. The enzyme heme oxygenase-1 (HO-1) generates three separate signaling molecules through the catalysis of heme – carbon monoxide (CO), biliverdin, and iron – each of which acts via distinct molecular targets to influence cell function, both proximally and distally. This review focuses on state-of-the art developments and insights into the impact of HO-1 and CO on the innate immune response, the effects of which are responsible for an ensemble of functions that help regulate complex immunologic responses to bacterial sepsis and ischemia/reperfusion injury. HO-1 exemplifies an evolutionarily conserved system necessary for the cellular milieu to adapt appropriately, function properly, and ensure survival of the organism.

Keywords

heme oxygenase-1; gasotransmitter; CO-releasing molecule; innate immunity; hemoprotein; ischemia reperfusion injury; organ transplantation; sepsis

Heme oxygenase as a Protective Immunological Web

All cells catabolize heme using one of two isoforms of heme oxygenase (HO), HO-1 or HO-2, which are the inducible and constitutive isoforms, respectively [1]. In addition to carbon monoxide (CO), free iron and biliverdin are also formed during heme degradation. Bilirubin (BR) is generated from biliverdin (BV) by biliverdin reductase, while the iron is rapidly sequestered into a ferritin [2]. Each product independently influences cellular functions in unique and succinct immunological modalities (see Glossary) that when summed together optimize the ability of the cell to respond to the needs of the tissue (Box 1).

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Figure 1.
Catabolism of Heme by HO-1

Box 1

Heme degradation pathway and roles of the HO products

***Biliverdin and Bilirubin:** are powerful serum antioxidants. They impart additional anti-inflammatory action through BVR to inhibit TLR4 expression [16] and activate an Akt–IL-10 signaling axis [12]. Effective therapeutic in pathophysiological instances and can influence inflammation and cell death.

***Ferrous Iron:** can potentially interact with H_2O_2 to generate hydroxyl anions. Fe^{2+} is rapidly sequestered into ferritin and overexpression of ferritin allows for the protective signaling in models of ischemia-reperfusion injury.

***Carbon Monoxide:** is an inert, non-reactive gas molecule. Likely targets are divalent cations typically located within enzyme complexes. The heme moiety is the most well defined cellular CO target present in numerous cellular proteins. As such, CO modulates the innate immune response, cell growth and proliferation, and cell survival acting therapeutically in pathophysiological instances influencing inflammation, abnormal growth and cell death. An optimized dosing regimen is still unclear which will likely differ depending on the therapeutic requirements.

HO-1 is activated within hours of exposure to a host of cellular stressors including oxidants, pathogens, chemokine mediators, and growth factors [3]. CO generated by HO-1 regulates, either positively or negatively, the inter- and intra-cellular networking and communication

within the tissues and organ systems, allowing them to respond appropriately to the stressor or change in environmental cues. Metabolic shifts in the environment, such as fluctuations in oxygen or glucose bioavailability, or the onset of cytokine and growth factor release rapidly induces stress response genes such as HO-1. The absence of HO-1 in mice or humans leads to chronic, multi-organ systemic inflammation, emphasizing the crucial importance of this enzyme in regulating fundamental physiology and the requisite stress and immune response [4, 5]. One conundrum that has besieged the field is how HO-1, as a heme catabolizing enzyme, mediates such potent and broad-reaching salutary effects in contexts ranging from cancer to cerebral malaria [6].

Cells must exist in a highly plastic or dynamic state so as to adjust rapidly and specifically to stimulatory input. When the degree of stimulation from the environment reaches a threshold and the protective cellular mechanisms are breached, the immune response becomes dysfunctional, leading to cell death and organ failure. At this point, the expression of genes classed as 'protective', such as HO-1, is increased as the cell attempts to preserve its integrity and induce corrective measures that provide the greatest probability of continued survival. Prior induction of HO-1 or administration of heme degradation products in expectation of overwhelming stress circumstances is potentially salutary [7–12].

The importance of HO-1 during an innate immune response is perhaps best exemplified by observations of the HO-1 deficient mouse, as well as by reviewing the two cases of HO-1-deficiency in man [4, 6, 13] and the use of well-characterized pharmacological tools. The magnitude of the benefits afforded by HO-1 has been clearly substantiated in the literature over the past 20 years by observations in a broad spectrum of disease models, particularly those related to the innate immune response [14]. In most instances, the exogenous addition of CO or BV can substitute for HO-1 deficiency [10, 15]. Importantly, although both products limit cell and tissue damage, the protective mechanisms of CO and BV do not overlap. In addition, after BV activates BVR, it acts as a kinase and transcriptional regulator of genes including HO-1 and TLR4 [12, 16, 17]. Both BV and CO preserve cellular and tissue survival *in vivo*, likely reflecting the participation of disparate signaling pathways in regulating inflammatory sequelae. It may also be that administration of each product can influence the regulation of HO-1 expression [18]. Although suboptimal doses of CO or BV each improve allograft tolerance following transplantation, ample evidence demonstrates the additive effect of co-administering CO and BV in addition to recapitulating the effects observed with HO-1 induction [19].

Demonstrating that the effects of exogenous CO or BV are equivalent to those induced by a similar amount of metabolic products generated by HO-1 remains challenging. D'Amico *et al.* clearly demonstrated that CO originates from HO-1 modulated cellular respiration and that this enzyme-mediated effect was equivalent to that observed when CO was administered exogenously [20]. These findings provided critical information and, for the first time, linked HO-1-derived CO with studies of exogenously administered CO. Similar studies have not been performed with BV. Rescue experiments with exogenous CO in *Hmox1*^{-/-} mice also support the proposal that CO is, in large part, the cellular mechanism underlying the effects observed with HO-1 induction [21, 22].

Given the above, the comparisons become more complicated when considering animal studies because *in vivo* elevations in HO-1 levels do not generate detectable carboxyhemoglobin (COHb) levels systemically. By contrast, inhaled CO requires an increase in COHb levels of 5–15% to replicate the functional consequences elicited with HO-1 induction. Perhaps the congruency in the functional effects can be explained by the amount bound to hemoglobin in the circulation versus the concentrations present at the cell and tissue level. CO levels that are generated by HO-1 locally by the cell are equivalent to

amounts of CO offloaded when delivered via hemoglobin. Administration of a CO releasing molecule (CO-RM) in many cases does not lead to an increase in COHb yet can mimic HO-1 protection and may reflect offloading of CO at or within the cell. Very small amounts of CO do access the tissue compartment over time [23, 24], however, very few studies have assessed a dose-response of CO in the tissue compartments.

The Pharmacology of Carbon Monoxide as a “Homeodynamic” Gas

In the remainder of this review, we focus on CO for two principal reasons. First, CO is the most extensively studied of the three HO-1 products. Second, and perhaps more importantly, CO is the one product that thus far is being translated to clinical use. We focus on two examples of inflammatory sequelae, septic shock and ischemia-reperfusion injury (IRI), and integrate the known molecular mechanisms of CO action with the latest insight into the translational potential that has led to the initiation of a clinical trial for kidney transplant patients.

Despite mounting evidence that has accrued over the past decade in support of the beneficial effects of CO, it continues to be demonized as a toxic byproduct of fossil fuel combustion or as byproduct waste of heme degradation. High doses of CO (>10 000 ppm) are certainly toxic because it inhibits cellular respiration and interacts tightly with hemoglobin, limiting oxygen delivery [25]. The FDA has set guidelines for human use that prevent levels in excess of 12–14% COHb, which was based on rigorous Phase I human and animal studies. At these COHb concentrations, no untoward effects or adverse events were observed in healthy volunteers (data reported at the 6th International Heme Oxygenase Congress held in Miami Beach, USA 2009). Inhaled CO is currently being developed in at least four clinical trials in North America in the context of paralytic ileus, pulmonary fibrosis, pulmonary hypertension, and organ transplantation (www.clinicaltrials.gov).

Although inhaled CO is the simplest and least complicated mode of therapeutic administration, the field now has two additional ways of administering CO, including the CORMs and a hemoglobin-based CO carrier (HBCOC) [26, 27]. The foundation upon which CORM and HBCOC technology rest is based upon administering CO as cargo to be delivered systemically and with tissue specificity. Both CO-RMs and HBCOC, in addition to inhaled gas, hold great potential as innovative therapeutics and show very similar or overlapping effects in preclinical animal models. The reader is referred to references [28, 29] and Table 1 for further information on these modes of CO exposure. Briefly, the delivery molecules offer intriguing possibilities for tissue specificity. CO-RMs can be created with a variety of design attributes that make them very attractive modes of delivery: they can be engineered to deliver their CO cargo at the site of inflammation, where CO is released in response to elevated reactive oxygen species; designed to release CO in response to ultrasound waves or light; or carried in conjunction with proteins that recognize specific tissue receptors, such as the asialoglycoproteins on liver cells.

In our studies, we re-define CO as homeodynamic, replacing the traditional term, homeostatic, because we view CO as a molecule that regulates cellular function continuously as dictated by the environment and cellular milieu, and not necessarily to simply restore quiescence as homeostasis would imply. CO is not simply anti-inflammatory, antiapoptotic, or anti-proliferative, but under certain conditions CO exhibits proinflammatory, proapoptotic, and pro-proliferative effects within the same animal at various locations and with differing kinetics. Homeodynamic encompasses a degree of flexible attributes to the molecule as it responds to the needs of the cell, tissue, and organ system by orchestrating the host responses in a manner that optimizes survival. The behavior of CO is, therefore, not one single event with one molecular target such as phosphorylation

of a single kinase, but rather propagates and potentiates a network of physiologic events (Box 1). As depicted in Figure 1, CO can exert numerous pleiotropic effects on the cell. In essence, CO interconnects various signaling pathways both intra- and intercellularly, depicted in the figure as an umbrella under which CO enables the appropriate response of the cell and organism to distinct environmental input.

CO functions similarly to other signaling molecules, such as nitric oxide and hydrogen sulfide, by amplifying a signal intracellularly that leads to changes in cellular function locally. However, CO is unlike other signaling molecules because it propagates effects pericellularly as well as distally to remote cells and tissues through its high diffusivity, its portability (via hemoglobin), and its biochemical stability (integrity). As perhaps the smallest signaling molecule, it can easily traverse through lipid bilayers and bind to its targets, much like oxygen and carbon dioxide as they are mobilized throughout the body. Perhaps the best example of remote effects is that of a transplanted heart allograft where inhaled CO prevented rejection of a second donor heart transplanted into the abdomen of a recipient mouse [22]. With such characteristics, CO facilitates the behavior of a matrix of interactions among cell types that, in turn, facilitates rapid responses both to stressors and danger signals. These effects are met with cell-specific responses, as exemplified by being pro-proliferative for endothelial cells and anti-proliferative for vascular smooth muscle cells in the same environment, likely reflecting different primary targets available in each cell type [30–34]. For example, inducible hemoproteins such as nitric oxide synthase II (NOS2/iNOS) are elevated in activated macrophages versus a naïve resting macrophage, where NOS2 has very low expression (Figure 2), whereas NOS3 (eNOS), primarily found in endothelial cells, is constitutively present. Therefore molecular targets for CO are constantly changing depending on the overall physiologic state of the cell, i.e., active or quiescent (**Box 3**). The binding of CO to the heme in proteins such as NOS2 modulates their function either positively or negatively. CO, being non-reactive unlike its sister gas nitric oxide (NO), constrains its cellular targets to primarily metal complexes such as the hemoproteins found in abundance in all cells. Importantly, hemoprotein expression levels change depending on the activation status of the cell. CO in binding to the heme moiety of a protein induces a conformational change that can alter the activity of the protein. One of the best examples is soluble guanylate cyclase where CO when bound to the heme of this enzyme augments its activity, resulting in increased generation of cGMP. The reactivity of CO to hemoproteins is completely reversible with clear pharmacodynamics. The communicative interrelationships between CO and NO have been well described, particularly in the context of vascular proliferative disease and tissue regeneration and repair [18, 31, 35, 36].

Effects of HO-1 and Carbon Monoxide on the Innate Immune Response

The potential of HO-1 and CO as therapies has been extensively studied in numerous preclinical models ranging from autoimmune disease to vascular injury [8][37], and although well-described pleiotropic effects are observed in most immune cell types (Table 2), no unified mechanism has yet been identified. Below we provide two examples that perhaps best exemplify the astonishing roles HO-1 and CO play in dictating an appropriate immunologic response to tissue stress, and parallel that observed in other model systems and cell types.

Sepsis

In addition to endotoxin and bacterial infection, *Hmox1*^{−/−} mice exhibit exaggerated symptoms of experimental autoimmune encephalomyelitis [8, 38, 39]. The first anti-inflammatory effects of HO-1 were observed in a rat model of rhabdomyolysis, and this was followed by evidence of protection in a model of lethal endotoxic shock where HO-1 expression was induced by the administration of heme [40, 41]. Similarly, low

concentrations of CO were equally protective in rats and mice, mimicking HO-1 activity and ushering in the application of CO, beyond what was historically recognized as toxic [7, 42]. In these studies, pretreating mice with CO before shock simultaneously decreased proinflammatory and increased anti-inflammatory cytokine expression, resulting in reduced organ injury and death [7, 43]. Inhaled CO or the use of a CO-RM enhances bacterial clearance as well as mitochondrial energy metabolism, rescuing mice from lethal cecal ligation and bowel perforation-induced sepsis [10, 44]. Important here is that the innate immune response to CO may vary depending on whether CO is applied before or after inflammation/infection is established and whether the challenge is bacterial endotoxin alone or a live infection (Figure 2). Pretreatment with CO boosts anti-inflammatory measures and inhibits proinflammatory sequelae; these effects are driven primarily by the activity of CO on the redox status of the cell such that a subsequent challenge with endotoxin results in tolerance. By contrast, pretreatment with CO prior to an inoculum of live bacteria impedes the inflammatory response necessary for appropriate bacterial clearance, whereas administration of CO after inoculation evokes an enhanced inflammatory response, augmented bacterial clearance mediated in part by TLR4 (Figure 2)[15], and a more rapid resolution of infection. Explanations for many, if not all, of the effects of CO *in vitro* may ultimately be linked to mitochondria and oxygen consumption [45]. Indeed, seminal work showing the ability of CO to increase mitochondrial biogenesis may underscore many of the reported observations of the innate response of the cell to CO [46]. *In vivo*, the mode of action becomes more complex in sepsis as there are reports that CO interferes with heme release [38], thereby preventing the ability of heme to contribute to reactive oxygen species generation and tissue damage. Counterintuitively, administration of heme, which potently induces HO-1, interferes with appropriate bacterial clearance [38]. Additional modes of action do not involve biogenesis or heme bioavailability, but rather the ability of CO to directly modulate signaling pathways in the cell that influence gene regulation and functioning of the immune response [47, 48]. This ensemble of reactions is a crucial consideration in situations of acute endotoxic shock, T cell activation, or sterile inflammation (such as acetaminophen poisoning or hemorrhagic shock/resuscitation) where unfettered inflammation can be lethal. By contrast, inhibiting requisite proinflammatory pathways during an ongoing infection would be detrimental to appropriate pathogen elimination [10, 15].

Ischemia Reperfusion Injury (IRI)

Our understanding of the beneficial effects of HO-1 and CO has been defined by their role in organ transplantation, which includes IRI. Induction of HO-1 prior to organ harvest and transplant in donors, recipients, and grafts improves outcome, which is in stark contrast to the enhanced rejection exhibited by *Hmox1*^{-/-} mice [49–52]. HO-1 induction blocks acute and chronic rejection in allogeneic and xenotransplant models and significantly reduces IRI [22, 52–54]. The mode of action of HO-1 is broad with one commonality being less inflammation. Many of the salutary effects observed with HO-1 were rescued in *Hmox1*^{-/-} mice by treatment with CO [21, 22, 55].

In IRI observed in a large animal model of kidney transplantation, CO synchronously blocks proinflammatory cytokine production, suppresses apoptosis of endothelial and epithelial cells, and enhances proliferation of kidney tubular cells [56–60]. Inhibition of Th1 type cytokines [interleukin (IL)-2, INF γ] and proinflammatory mediators (TNF α , IL-1 β , IL-6, COX-2) are critical targets for CO in protecting heart allografts in rats [61]. Furthermore, CO ameliorates IRI associated with cold storage of liver grafts [62, 63] and attenuates IRI in the liver [64], lung [65], heart, and small intestine[66], providing important insight into how HO-1 and CO reduce acute and chronic rejection [67].

Like macrophages, dendritic cells (DCs) play an important role in the early initiation and recognition of the immune response. Although HO-1 is a well-described immunomodulator of DC development and function [68, 69], studies on the effects of CO on DC are not as comprehensive as those described for macrophages. Administration of CO inhibits DC maturation, secretion of proinflammatory cytokines, and alloreactive T cell proliferation while increasing IL-10 production to induce a tolerogenic DC phenotype [70]. *In vivo*, DC-mediated autoimmune diabetes is ablated if DCs are pretreated with CO prior to adoptive transfer into recipients [70]. Administration of CO to donors prior to transplant prevents transplant-associated vasculopathy mediated, in part, by inhibiting alloaggressive T cell proliferation in recipients. CD11c⁺ DCs exposed to CO downregulated CIITA mRNA expression, and CO treatment of bone marrow-derived dendritic cells (BMDCs) decreased MHCII levels after activation, thus preventing the development of intimal hyperplasia and graft failure [8, 71]. Importantly, the development and maintenance of CD4⁺CD25⁺ regulatory T cells (Treg) depends on CO generation by antigen presenting cells [68, 72]; however, these findings are not consistent with the normal development and function of Treg in *Hmox1*^{-/-} mice [73]. Moreover, CO seems to codify strong anti-proliferative effects in T effector cells promoting Fas/CD95-induced apoptosis [74, 75].

Inhaled CO entered clinical trials in 2007 for treating kidney transplant recipients intraoperatively for 1 hr during the transplant surgery. The Phase II human trial was based upon large animal data showing that the nontoxic concentration of inhaled CO to recipients intraoperatively improved postoperative kidney function [76]. CO accelerated recovery of a severely compromised kidney with a more rapid return of serum creatinine to baseline (as a measure of kidney damage), less inflammation, and enhanced repair resulting from the IRI [76]. Kidneys stored in preservation solution containing dissolved CO demonstrate a similar pattern of early injury and more rapid recovery [77], reinforcing the idea that donor or graft pre-treatment might provide additional benefits. The clinical trial and pig study described above utilized a unique CO delivery device where administration of inhaled CO gas was based on body weight and the device adjusted the volume of gas delivered based upon respiratory rates so as to deliver highly precise amounts of CO with each breath resulting in very predictable COHb levels [78].

Concluding remarks

There is tremendous potential for the therapeutic application of the HO-1 system in the clinic, either by inducing HO-1 activity or expression, or by administering one or more of its products. CO is in clinical trials, primarily as a gas, although CO-RM treatment paradigms as well as CO-saturated hemoglobin solutions are also emerging and entering clinical trials (Table 1). Inhaled CO is furthest along in terms of clinical evaluation with studies planned in the context of lung fibrosis, pulmonary hypertension, paralytic ileus, and vascular injury. The results of these trials are still pending, but are based upon sound preclinical data sets. CO-saturated hemoglobins are being tested as therapy for sickle cell disease to reduce sickling and the severity of crises. Completed physician-initiated studies with inhaled CO in human volunteers were performed in acute lung inflammation and COPD where anti-inflammatory effects were observed [79–81], but these studies did not evaluate careful dose ranging. Clearly, there is a great deal of work yet to be done to understand the therapeutic potential of HO-1 and CO in a clinically useful modality, particularly because there are a handful of reports arguing that increased inhibition of HO-1 can also be beneficial in certain cancers [82–84].

HO-1 can be viewed as “social” because it participates in an extremely complex, but elegantly interlinked, communication network facilitated by its three bioactive products that are active in and among cells and tissues, which must act in conjunction to regulate cellular

function. The sheer complexities of HO-1 regulation by oxidant responsive transcription factor nrf2 and the network of molecular mechanisms that emerge as targets for the HO-1 products as the cell transitions from relative quiescence to an activated state are immense. The matrix of functions subserved by HO-1 cannot be fruitfully explicated outside the context of CO, BV, and Fe²⁺. The intricate web of functional interrelationships among these products are not unlike other well-defined signaling cascades in that they can be amplified, are highly regulated, and are in many cases sufficiently flexible to be cell and tissue-type specific. CO, perhaps by the sheer enormity of published reports and emergence of specific targets (**Box 3**), best represents HO-1 activity, clearly acting as a homeodynamic physiologic mediator in the cardiovascular, immune, and nervous systems, primarily by being a highly diffusible, reactive, and highly potent short-term “information engineer” [85–87]. It is clear, in short, that the role of the reticulated HO-1 products towards cellular function is multiform, rigorously orchestrated, and cohesive as an irreducible, executive gestalt.

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Glossary

Modalities	refers to the ways by which changes occur
Hemoproteins	proteins that contain a molecule of heme as a part of their structure, which is necessary for the function of the protein. Hemoproteins can serve many functions in the cell, including oxygen transport (e.g., hemoglobin, myoglobin, neuroglobin), as enzymes (e.g., cytochrome p450, peroxidase, catalase), and as part of the electron transport chain
Carboxyhemoglobin	hemoglobin that has carbon monoxide competitively bound to the heme moiety, in the place of oxygen. CO has a binding affinity approximately 2 orders of magnitude greater than that of oxygen, and the presence of CO effectively decreases the amount of hemoglobin available for oxygen transport
Ischemia-Reperfusion Injury (IRI)	a situation where blood flow to an organ or tissue is reduced or stopped for a period of time and then flow is reestablished. This halting of flow and then reestablishing flow leads to an acute inflammatory response and injury
Rhabdomyolysis	the rapid breakdown of skeletal muscle, which releases large quantities of cellular components into the bloodstream. These cellular components can damage the kidneys, leading to acute kidney injury and the upregulation of HO-1 expression
Cecal Ligation	closing with suture of the cecum, a portion of the gastrointestinal tract between the small and large intestines that contains a large amount of bacteria. When ligation is followed by a puncture, bacteria are released and eventually make their way to the bloodstream, leading to septicemia and sepsis

Alloreactive	reactivity of a T cell to an alloantigen, or an antigen derived from a genetically non-identical source. A patient is exposed to alloantigens in, for example, the case where a transplant occurs between two individuals who are not identical twins
Alloaggressive	an attack by T cells on a foreign cell
CIITA	a human gene that encodes the class II major histocompatibility complex
Intimal Hyperplasia	uncontrolled growth of the smooth muscle cells of a blood vessel. The intimal layer of a blood vessel consists primarily of vascular smooth muscle cells, and the growth of these cells, including deposition of extracellular matrix, is part of the normal blood vessel response to injury
Gestalt	a configuration of elements into a pattern or composition that as a whole cannot be explained as the sum of its parts

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Figure 1. Social Networking of Heme Oxygenase-1 and Carbon Monoxide in Innate Immunity
Carbon monoxide (CO), generated by HO-1 or provided as an exogenous gas, is depicted here as an umbrella representing the very broad effects of CO as it regulates many aspects of the innate immune response to stressors. Inhaled CO imparts potent salutary effects against both pathogenic (bacterial sepsis) and sterile inflammatory (chemical-induced, e.g., acetaminophen-induced liver injury) sequelae and functions homeodynamically to restore basal cellular function. CO functions in a manner that befits the need of the cell to ensure the optimal probability for survival.

Figure 2. Signaling Pathways of Carbon Monoxide in the Cells of the Innate Immune System
Comparativeresponse of a cell to stress or injury is dependent on the time of CO application administered pre or post insult. Signaling molecules and pathways that are induced by pretreatment with CO lead to the establishment of a protective barrier to prevent an inflammatory response (left panels). Essentially, CO conditions the cell to be tolerant of subsequent stressors. If, however, the cell is exposed to the stress prior to CO exposure (right panels), CO amplifies the response in a highly controlled fashion to more rapidly restore homeostasis and ensure survival. ROS, Reactive Oxygen Species; MAPK, Mitogen Activated Protein Kinase; NOS, Nitric Oxide Synthase; HIF1 α , Hypoxia Inducible Factor 1 α ; PPAR γ , Peroxisome Proliferating-Activated Receptor- γ .

Table 1

Characteristics of CO Delivery Modalities

Molecule	Pros	Cons
Carbon Monoxide Gas	<ul style="list-style-type: none"> • Inert, pure, non-metabolized. • Safety, pharmacology, and toxicology are extremely well understood. • Delivery device is FDA- approved. • Phase II trials have begun • Efficacy proof-of-principle for human trials is supported by both large and small animal models. 	<ul style="list-style-type: none"> • Gaseous therapeutic that requires a specific delivery device, • Negative view by public, • Inhaled route of administration using elevated COHb as measure of exposure. • Currently only designed for hospital-based use.
CO-RM^a	<ul style="list-style-type: none"> • Small, novel molecules allow creative medicinal chemistry • Potential modulation of bioavailability and tissue specificity • Multiple routes of administration, allowing for hospital or home use • Minimal effects on COHb • Proof of concept, primarily in small animals. 	<ul style="list-style-type: none"> • Safety and toxicology are well • Similar pharmacology to CO delivery as inhaled gas. • Stability reported to be greater than 1 year. • In Phase I trials for sickle cell anemia. • Added potential of hemoglobin-induced HO-1.

Molecule	Pros	Cons
HBCOC^b	<ul style="list-style-type: none"> • Early in preclinical development • Backbone of the molecule has unclear safety and toxicology as well as unclear stability and pharmacology. • Stability of compounds is unknown. 	<ul style="list-style-type: none"> • Cell free hemoglobin may modulate vasomotor tone due to its ability to scavenge nitric oxide. • Unclear pharmacology of polyethylene glycol (PEG). • Hospital-based, intravenous delivery only.

^a black=carbon, red=oxygen, gray = metal, other colors=ancillary ligands.

^b red = hemoglobin, blue=poly(ethylene) glycol.

Table 2

Effects of CO on Cells of the Immune System

Cell type ^a		Effects of CO
Innate Immunity	MΦ	<ul style="list-style-type: none"> • Anti-inflammatory effects when applied prior to stimuli; acting via MAPK, PPARγ, HIF1α and heme-binding proteins • Proinflammatory effects when applied after bacterial challenge by boosting phagocytosis, bacteria killing, and reducing heme bioavailability.
	DC	<ul style="list-style-type: none"> • Blocks maturation and modulates secretion of cytokines to induce a tolerogenic phenotype of DC; acting via interferon regulatory factor 3 signaling pathway.
	PMN	<ul style="list-style-type: none"> • Inhibits migration of PMNs and blocks their proinflammatory phenotype.
	EC	<ul style="list-style-type: none"> • Enhances proliferation and mobilization in response to wounding. • Blocks adhesion molecules expression. • Inhibits apoptosis via p38 MAPK and STAT3.
Adaptive Immunity	T cells	<ul style="list-style-type: none"> • Inhibits proliferation and activation of T effector cells, while preserving and inducing expansion of T regulatory cells • CO promotes Fas/CD95 induced apoptosis in Jurkat cells.
	Mast cells	<ul style="list-style-type: none"> • Inhibits PMN-induced mast cells activation. • Blocks release of histamine via cGMP signaling.
	Basophils	<ul style="list-style-type: none"> • Inhibits activation and histamine release via a cGMP and calcium dependent pathway.
	B cells, NK cells	<ul style="list-style-type: none"> • Unknown effects

^a**Abbreviations:** MΦ: Macrophage; DC: Dendritic Cell; PMN: Polymorphonuclear leukocyte; EC: Endothelial Cell; NK: Natural Killer; cGMP: cyclic guanosine monophosphate