Carbon monoxide-releasing molecules (CO-RMs): vasodilatory, anti-ischaemic and anti-inflammatory activities

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Abstract
The well-known adverse effects of CO (carbon monoxide) intoxication are counterbalanced by its positive actions when small amounts are produced intracellularly by the cytoprotective enzyme HO-1 (haem oxygenase-1). As compelling scientific evidence accumulated to sustain that HO-1 plays a fundamental role in counteracting vascular and inflammatory disorders, we began to appreciate that a controlled delivery of CO to mammals may provide therapeutic benefits in a number of pathological states. This is the rationale for the recent development of CO-RMs (CO-releasing molecules), a group of compounds capable of carrying and releasing controlled quantities of CO in cellular systems, which offer a plausible tool for studying the pharmacological effects of this gas and identifying its mechanism(s) of action. The present review will highlight the encouraging results obtained so far on the vasodilatory, anti-ischaemic and anti-inflammatory effects elicited by CO-RMs in in vitro and in vivo models with an emphasis on the prospect of converting chemical CO carriers into CO-based pharmaceuticals.

Introduction
The strong affinity of CO (carbon monoxide) for the reduced iron-haem in Hb, which is approx. 220-fold higher than the affinity of Hb for molecular oxygen, typifies the inherent dangerous properties of CO, since the formation of high levels of HbCO (carbon monoxo-Hb) in blood would consequently compromise oxygen supply to tissues, leading to hypoxia and CO poisoning [1]. 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, and it is becoming evident that increased CO production reflects a dynamic and active involvement of this by-product in the cytoprotective response [2]. 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. Moreover, comprehensive studies published in the last decade corroborate the anti-ischaemic, antioxidant and anti-inflammatory properties of the endogenous HO-1/CO system in a number of experimental models [2]. Intriguingly, several reports reveal that exogenous administration of appropriate doses of CO gas to cells, organs or whole animals exert a beneficial effect that, to some extent, mimics the cytoprotective action elicited by HO-1 stimulation [2]. Thus, based on the crucial contribution of the HO-1/CO pathway in the maintenance of vascular activities, protection against ischaemic injury and resolution of inflammatory conditions, it is clear that strategies aimed at amplifying the action of CO could lead to the development of pharmacological and therapeutic approaches. Despite recent findings emphasizing the potential of using CO gas as a therapeutic agent in pathophysiological states, there is still a lack of consensus on the specific cellular target(s) responsive to CO that mediates its multiple cytoprotective actions. Moreover, as pointed out at the beginning, the undesired effect of CO gas inhalation on the oxygen-carrying capacity of Hb is a crucial aspect that needs to be meticulously addressed before CO gas can be approved as a therapeutic agent. The danger associated with the use of CO gas for medical applications has hindered considerably the progression of studies in humans, although few cautious pilot studies assessing CO function in normal subjects have been conducted [3]. The recent development of a technology [CO-RMs (CO-releasing molecules)] that controls the delivery and action of CO under different pathological conditions [4,5] represents a major step forward in the implementation of CO-based pharmaceuticals for prophylactic and therapeutic applications. This short review will briefly focus on how CO-RMs were identified, discuss their chemical features and recapitulate on their versatile pharmacological activities that have been discovered so far.

Discovery and characterization of CO-RMs
The idea of developing compounds able to carry and deliver CO to biological systems originated in the late 1990s
when emerging evidence was pointing to the HO-1/CO pathway as a crucial player in the resolution of several pathological states [6]. As CO is known to bind strongly to transition metals in organic solvents to form stable ‘carbonyl complexes’ [7], I thought that a reversible process could be achieved by specifically triggering the release of the gas from these archetypical ‘solid forms’ of CO. The early finding showing that, under appropriate conditions, Mn₂C₅O₁₀ (manganese decacarbonyl) and Ru(CO)₃Cl₂ dimer [tricarbonyldichlororuthenium(II) dimer] were able to release CO in association with typical CO-mediated pharmacological effects such as vasodilatation and hypotension convinced us that these two transition metal complexes could form the basis for designing a new class of compounds (CO-RMs) aimed at delivering controlled amounts of CO to tissues and organs. Once the CO-releasing properties of both Mn₂C₅O₁₀ (CORM-1) and Ru(CO)₃Cl₂ dimer (CORM-2) were characterized and their biological action confirmed to be directly linked to the CO liberated [8], a further important step was soon taken to improve the compatibility of these chemicals with the biological system. As opposed to CORM-1 and CORM-2, which are soluble in organic solvents such as DMSO, the first water-soluble ruthenium-based carbonyl was synthesized by co-ordination of the amino acid glycine to the metal centre [Ru(CO)₃Cl-glycinato]. The new CORM-3, which is a relatively stable compound in water but promptly releases CO in the presence of myoglobin [half-life (t½) = <1 min] or other biological stimuli that directly interact with the ruthenium metal, proved to possess vasodilatory and anti-hypertensive properties [4,9]. In parallel with the development of transition metal carbonyl complexes as CO-RMs, a second class of chemicals was soon identified to possess the ability to generate CO in aqueous solutions. Sodium boranocarbonate (CORM-A1), which does not contain a transition metal carbonyl but a carboxylic group that is converted into CO through hydrolysis, can slowly liberate CO in the presence of myoglobin [half-life (t½) = 21 min] under physiological conditions, consequently eliciting vasodilatory properties that reflect the rate of CO release [10]. Thus, based on the notion that chemical agents can be utilized to liberate or generate controlled amounts of CO, we and other groups have begun to study the biochemical and pharmacological behaviour of CO-RMs in vitro, ex-vivo and in vivo experimental models. The chemical structures of the first four prototypic CO-RMs identified are represented in Figure 1 and their biological activities are summarized below.

**CORM-1 and CORM-2: bioactivity of lipid-soluble CO carriers**

CORM-1 and CORM-2 are transition metal carbonyls whose solubility is restricted to organic solvents; consequently, DMSO has been the classic vehicle used to test these compounds in vitro and in vivo. Moreover, while CORM-1 requires light to liberate CO, CORM-2 spontaneously releases CO once in contact with myoglobin or other haem-dependent proteins that trigger the dissociation of CO from the metal [4,5,11]. Following our report on the vasodilatory effects caused by CORM-1 and CORM-2 both in isolated vessels and hearts as well as in rats in vivo [8], scientists started to use these compounds as a valuable tool to: (i) mimic the action of the HO-1 pathway; (ii) investigate the mechanism underlying the pharmacological action of CO gas; and (iii) assess the effectiveness of CO liberation in mediating protective responses under pathological conditions. Xi et al. [12] reported that CORM-1, upon appropriate stimulation with light, activates large-conductance Ca²⁺-dependent potassium channels in neonatal porcine cerebral arteriole smooth-muscle cells; this effect can be reproduced by CO gas but is lost when CORM-1 is not stimulated by light. These results could explain the vasodilatory action of CO in cerebral circulation since the effect of CORM-1 on potassium channel activation in these cells can be prevented by blockade of cGMP production with ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinolin-1-one) [12], an inhibitor of guanylate cyclase. However, the scenario may be more complicated in vivo since a permissive role of NO (nitric oxide) in CORM-1-mediated cerebral vasodilatation has been suggested in piglets [13,14]. In a similar fashion, Stanford et al. [15] showed in pulmonary artery smooth-muscle cells that CORM-2 inhibits the production of endothelin, a potent vasoconstrictor implicated in the progression of pulmonary hypertension [15]. Apart from its vasodilatory action, HO-1-derived CO has been shown in both arterial and airways smooth-muscle cells as well as human Jurkat T-cells to be anti-proliferative, an effect that could be simulated by CORM-2 [16,17]. Arregui et al. [18] reported that liberation of CO from CORM-1 has a positive effect on the renal circulation, as treatment of rats with this CO carrier leads to a significant increase in renal blood flow, glomerular filtration rate and urinary cGMP excretion both under basal conditions and after inhibition of haem oxygenase activity [18]. CO-mediated guanylate cyclase and potassium channel activation by CORM-1 and CORM-2 have also

![Figure 1 | Chemical structure of lipid-soluble and water-soluble CORMs](image-url)
been implicated in inhibition of afferent arteriole constriction in the kidney [19], anal sphincter relaxation [20], non-adrenergic non-cholinergic inhibitory neurotransmission in porcine jejunum [21], immunophotoprotection against UV radiation in rat skin [22], modulation of ion transport in human intestinal epithelial cells [23] and vasorelaxation in hypertensive animals following exercise training [24].

The DMSO-soluble CO-RMs appear to have important anti-inflammatory properties in vitro. The ability of CORM-2 to significantly reduce lipopolysaccharide-induced NO and cytokines production in murine macrophages has been confirmed independently in several studies [25–29]. The effect of CORM-2 is strictly dependent on the amount of CO liberated by the compound since the inactive counterpart [iCORM-2 (inactive CORM-2)], which contains ruthenium but does not release CO, is incapable of preventing the inflammatory response [25]. The anti-inflammatory action of CORM-2 has been extended to microglia [30] where IFN-γ (interferon-γ)-induced neuroinflammatory response is reduced by the presence of the compound. Similarly, in intestinal epithelial cells [31], activation of NO production by cytokines is significantly decreased by CORM-2 alongside a reduction in IL (interleukin)-8, IL-6 and matrix metalloproteinase-7 mRNA expression. These results, which are in agreement with the finding showing that CORM-2 reduces the expression and activity of matrix metalloproteinase-1 and -2 in alveolar epithelial cells [32], suggest that inhibition of NF-κB (nuclear factor κB) and MAPks (mitogen-activated protein kinases) activation by CO contribute to its anti-inflammatory activity, although the exact molecular mechanism remains to be fully elucidated. CORM-1 and CORM-2 have also been demonstrated to act as anti-inflammatory agents in vivo. In a model of carrageenan-induced inflammation in the mesenteric microcirculation, administration of CORM-1 significantly reduced the migration, rolling and adhesion of neutrophils to the endothelium in the inflammatory site [33]. In the same study, neutrophil recruitment was exacerbated by an inhibitor of HO-1 activity, and the anti-inflammatory effect of CORM-1 was abolished by the inhibitor of guanylate cyclase, ODQ. Similarly, using an experimental model of cutaneous burn injury, Sun et al. [34] reported that intravenous administration of CORM-2 in mice attenuated the accumulation and adhesion of polymorphonuclear leukocytes to sinusoidal endothelial cells in the liver, an effect that appeared to be mediated by inhibition of NF-κB and reduction in the expression of adhesion molecules [ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1)] [34]. A similar anti-inflammatory action by CORM-2 on leukocyte sequestration has been recently confirmed in the lung of thermally injured mice [35].

**CORM-3 and CORM-A1: bioactivity of water-soluble CO releasers**

CORM-3 and CORM-A1 represent the first examples of water-soluble CO releasers. As anticipated in the Introduction, the two compounds are fundamentally different in terms of chemical structure and rate of CO liberation: CORM-3 is a ruthenium-based tricarbonyl complex with a fast CO release ($t_{1/2} < 1\text{ min}$), whereas CORM-A1 is a boron-containing carboxylic acid that under physiological conditions releases CO with a slow kinetic ($t_{1/2} = 21\text{ min}$) [4,9,10,36]. This chemical difference dictates the way CO causes vasorelaxation and hypotension since CORM-3 elicits a prompt and rapid vasodilatory effect in vitro and in vivo, whereas CORM-A1 promotes mild vasorelaxation and hypotension. In addition, CORM-3-induced vasorelaxation in aortas appears to be primarily cGMP- and endothelium-dependent [37]; in contrast, the vasodilatory effect mediated by CO slowly liberated from CORM-A1 involves guanylate cyclase and potassium channel activation but appears to be endothelium-independent [10]. The mechanism underlying these physiological effects has been confirmed more recently in other experimental models. In fact, CORM-3 has been shown to contribute to mesenteric vasodilatation in cirrhotic rats via stimulation of large-conductance calcium-activated potassium channels [38], while in mouse kidney CORM-A1 causes an increase in renal blood flow combined with a decrease in vascular resistance through activation of guanylate cyclase and opening of potassium channels [39].

The vasodilatory properties of CORM-A1 and CORM-3 are associated with their positive inotropic and anti-ischaemic effects, which have been observed primarily in cardiac and renal tissues [40,41]. In fact, in vitro experiments demonstrated that CORM-3 prevents hypoxia-reoxygenation damage in rat cardiomyocytes and protects against ischaemia/reperfusion injury in isolated hearts, an effect involving the activation of mitochondrial ATP-dependent potassium channels [9,42]. In mice, administration of CORM-3 reduces infarct size and prolongs the viability of cardiac allografts following transplantation [9,43]. The reno-protective effects of CORM-3 have been reported in mice following ischaemia-induced renal failure [44] and in a model of cisplatin-induced nephrotoxicity in rats [45]. Both CORM-3 and CORM-A1 also improved renal function following cold ischaemia in isolated rabbit kidneys and this effect was associated with increased vascular perfusion, glomerular filtration rate and mitochondrial respiration [41]. It is intriguing that mitochondria are strongly emerging as a possible preferential target for the therapeutic and beneficial actions of CO gas [46,47] as well as CORMs [17,48,49], in contrast with the classical view that CO is poisonous to cells because of its high affinity for the haem of cytochrome oxidase, the transmembrane protein complex that acts as a crucial electron acceptor in the synthesis of ATP. As in the case of the DMSO-soluble metal carbonyls CORM-1 and CORM-2, CORM-3 proved also to be an effective anti-inflammatory agent as recent studies revealed that this water-soluble agent: (i) inhibits NO and TNFα (tumour necrosis factor α) production in macrophages and microglia stimulated with pro-inflammatory mediators [25,50,51]; (ii) attenuates the adhesion of polymorphonuclear neutrophils to endothelial cells in vitro and in vivo [52]; (iii) down-regulates the responsiveness of human neutrophils...
to inflammatory stimuli by decreasing superoxide production and CD-11 expression [53]; (iv) reduces neutrophil infiltration in rat kidneys exposed to ischaemia/reperfusion injury (R. Motterlini, unpublished work).

**Conclusions and future perspectives**

The results generated in the last few years indicate that CO-RMs possess effective vasodilatory, anti-ischaemic and anti-inflammatory activities; however, their pharmacological action may be associated with other important cytotoxic effects provided by the small amounts of CO released as these compounds protect hepatocytes from glucose-deprivation-induced cytotoxicity [54], inhibit hypertrophy in cardiac myocytes [55], alleviate ischaemia-induced renal failure [44] and protect against peroxynitrite-induced apoptosis in PC12 cells (pheochromocytoma cells) [56]. Clearly, more work needs to be conducted to decipher the precise mechanism(s) of actions by which CO-RMs exert their beneficial effects.

Apart from the activation of typical targets (i.e. guanylate cyclase and haem-containing potassium channels), recent evidence indicates that a more ‘physiologically controlled release of CO’ from CO-RMs may affect the activity and function of several haem- and metal-dependent proteins that are crucially involved in processes controlling cell signalling and oxidative stress [49]. The reaction of reactive oxygen species from NADPH oxidase and mitochondria is most likely among these targets [17,27,47,48] but other, so far unidentified, haem-dependent enzymes may act as preferential sensors to the signals exerted by CO [49]. Nonetheless, the results on CO-RMs’ biology and chemistry undoubtedly indicate that these compounds are good candidates for the implementation of CO-releasing drugs [5,57]. As more and diversified new CO-RMs are being developed [57–59], we envision that this class of agents will be transformed into pharmaceuticals for the treatment of vascular and inflammatory disease states.

I thank Dr Roberta Foresti for helpful discussions and a critical reading of this paper. This work was partially supported by the Henry Smith Charity.

**References**


Received 21 June 2007
doi:10.1042/BST0351142